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

by

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Declaration

I, Mahlape Patricia Letsoela, 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.

……

Mahlape Patricia Letsoela

2024

I certify that the above statement is correct.

…….....

Doctor Leana Esterhuizen (Supervisor)

……

Professor Annabel Fossey (Co-supervisor)

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Abbreviations

ASPT:Average Score Per Taxon

ANOVA:Analyses of variance

CCME: Canadian Council of Ministers of the Environment

CFU:Colony forming unit

COD:Chemical Oxygen Demand

DO:Dissolved Oxygen

DWAF:Department of Water Affairs and Forestry

DWS:Department of Water and Sanitation

EC:Electrical Conductivity

ECs:Emerging Contaminants

EDC:Endocrine Disrupting Coumpounds

EPA:Environmental Protection Agenc

FAO:Food and Agricultural Organisation

FC:Faecal coliform

GSM:Gravel, sand and mud

IHAS:Integrated Habitat Assessment System

IHI:Index of Habitat Integrity

MDGs:Millennium Development Goals

MMM:Mangaung Metropolitan Municipality

MPN:Most probable number

NO_3:Nitrates

NTU:Nephelometric turbidity unit

PCPs:Personal Care Products

PhACs:Pharmaceuticals

PO_4:Phosphates

SADC:South African Development Community

SANS:South African National Standards

SASS: South African Scoring System

SASS^4:South African Scoring System Version 4

SASS^5:South African Scoring System Version 5

SAWQG:South African Water Quality Guidelines

SDGs:Sustainable Development Goals

SIC:Stones in current

SOOC:Stones out of current

UN:United Nations

UNDP:United Nations Development Programme

USEPA:United States Environmental Protection Agency

UV:Ultraviolet light

WHO:World Health Organisation

WQI:Water Quality Index

WWTP:Waste Water Treament Plant

Abstract

Introduction: The streams in the Free State play a crucial role in providing water for agricultural, industrial and recreational activities as well as in domestic households for sanitation purposes. These streams also provide a healthy home for numerous aquatic animals and plants. However, in recent years, there has been an upsurge in stream water pollution by anthropogenic activities. These include domestic, agricultural and industrial activities, such as waste water treatment plant (WWTP) that dispose of effluent containing high concentrations of emerging contaminants into the streams. The polluted water may cause illness in and deaths of humans and animals as well as kill less tolerant aquatic organisms resulting in a declined biological diversity of the stream. Therefore, water quality of the Bloemspruit and its tributaries, the Fonteinspruit and the Renosterspruit was assessed to determine the health and deterioration of the stream.

Methodology: For water quality assessment, water samples were collected from 11 sampling sites in Bloemspruit stream and its tributaries during four sampling rounds. These water samples were assessed for four physical, seven chemical and two microbiological properties as well as eight emerging contaminants. The impact of pollution on the macroinvertebrate population and the macroinvertebrate habitat was conducted. The calculations of the Water Quality Index (WQI), the South African Scoring System score (SASS), the Average Score per Taxon (ASPT) and the Index of Habitat Integrity (IHI) score were used to determine the health of the Bloemspruit and its tributaries.

Results and discussion: The results revealed that of the twenty-one properties of water measured, only five properties, i.e. temperature, pH, sulphate, total hardness and estradiol, were compliant in all the sampling rounds. The measurements of dissolved oxygen were relatively low. However, turbidity, nitrates, phosphates, *E. coli*, total coliforms, atrazine, metolachlor, terbuthylazine, carbamazepine, estrone, 17a-ethinylestradiol and bisphenol A all demonstrated relatively high

measurements when compared with the proposed water quality limits for the protection of aquatic ecosystems. The WQI revealed that none of the sampling sites were of good quality. However, 45% demonstrated a fair condition, 45% were marginal and 10% poor. The SASS and ASPT scores revealed that 44% of the sampling sites were severely impaired with only a few tolerant macroinvertebrate taxa present, while the remaining 56% of the sampling sites were critically impaired. The IHI score demonstrated that 89% of the macroinvertebrates sampling sites had a moderately modified habitat, however, the remaining sampling site, S9, revealed a largely modified macroinvertebrate habitat.

Conclusion: This study revealed that the water quality of the Bloemspruit and its tributaries is poor. Several anthropogenic activities along the stream may have attributed to the poor status of the Bloemspruit water quality. These include the waste water treatment plant (WWTP), agricultural farming of animals and pastures as well as formal and informal settlements. Therefore, humans that use the water are at risk of being exposed to water-borne pathogens caused by high levels of *E. coli* and coliforms in the water. These pathogens can cause gastrointestinal diseases, such as diarrhoea, typhoid fever, cholera and other conditions including ear and eye infections. Additionally, the animals are also in danger of contracting diseases transmitted by this polluted water.

Chapter 1

Introduction

1.1 Introduction

Water is an indispensable natural resource. It plays a vital role in the survival of all living organisms (Brack*et al*., 2017). All living organisms and ecological systems need a safe and adequate supply of water for their physiological and ecological functions (Gunawardhana, 2016). Water is also used in a wide range of activities. These activities include agriculture, industry, transportation and the generation of power, as well as for recreation and mining (Matthews & Bernard, 2015). In households, water is used for drinking, food preparation and washing as well as for cleaning (Department of Water Affairs & Forestry [DWAF], 1996a).

Most living organisms use surface water and groundwater as the main supply source. Surface water is referred to as water located on the surface of the earth. It originates mainly from rainfall and surface runoff (Balasubramanian, 2017). Rivers, streams, lakes, dams and ponds are examples of surface water sources (WWF South Africa [WWF-SA], 2016). In contrast, groundwater includes all underground water that is either stored in soil, aquifers or in rock pores (WWF-SA, 2016). In most countries, such as South Africa, water is commonly sourced from the surface of the earth with rivers being the most common source of water supply (Department of Water Affairs [DWA], 2010a; Osunmakinde *et al*., 2013; WWF-SA, 2016). Therefore rivers and streams in South Africa are used for various purposes, which are either domestic or industrial in nature. In recent years, surface water volumes in South Africa have become relatively low, therefore, most rivers and streams can be regarded as perennial. As a result of the relatively low surface water volumes, South Africa is generally viewed as an arid country and has been rated as the 30th driest country in the world

(Department of Forestry, Fisheries and the Environment [DFFE], 2011; Kohler, 2016; Kidd *et al*., 2017).

Surface water resources may be affected by a wide range of natural processes and anthropogenic activities in their immediate environment. The natural processes, such as climate change, occur mostly through rainfall and weathering of rocks that may affect the quality and quantity of surface water (DWA, 2012; Khatri & Tyagi, 2015; Mathebula, 2015; Li & Qian, 2018). Moreover, some anthropogenic activities including the use of fertilisers and pesticides in agriculture, industries like mining, poor settlements and urbanisation, degrade the quality of surface water in many ways (Harizanova-Bartos *et al*., 2019; Rutting *et al*., 2018). The fertilisers and pesticides used in agricultural activities contain nutrients, such as nitrogen and phosphorus, which support the growth of crops (Food and Agriculture Organisation [FAO], 2017). However, the unutilised nitrogen and phosphorus may then be washed off by rain into rivers and streams and affect the concentration of available nutrients in the water (U.S. Environmental Protection Agency [US EPA], 2017b). This results in a “dead zone” for aquatic organisms and is referred to as eutrophication (US EPA, 2017b). The increased levels of nitrates also cause acidification of streams which kills the aquatic life (Belle *et al*., 2018). Consequently, this results in a declined biological integrity of the stream.

The stream water pollution by industrial agencies occurs through dumping and discharging of improperly treated effluent into water bodies. The effluent discharged may contain chemicals, such as mercury, arsenic, cadmium and ammonia, which are the by-products introduced through indiscriminate solid waste and wastewater dumping (Ratikane, 2013; Ezbakhe, 2018). If water containing these chemicals is consumed, it may cause adverse health problems to humans and animals. These chemicals may also adversely affect the aquatic life. Recently in Pietermaritzburg, South Africa, there was a spillage of crude oil from Willowton Oil into the Duzi River. The two

collapsed tanks had a capacity of 1600 tons (Department of Water and Sanitation [DWS], 2019b), which put the aquatic life under severe danger.

Due to rapid urbanisation, human activities have also become one of the leading causes of pollution in stream water. As the human population increases, water shortages are aggravated due to the high demand for fresh water as well as an increase in pollution and catchment destruction (Esterhuizen, 2014; Rand Water, 2017). Pollution in water escalates as more people move into cities and towns and consequently physically disturb the land during construction of houses and roads (Liyanage & Yamada, 2017). These physical disturbances of soil and vegetation affect the concentration of salts and minerals in water. Also, as more fertilisers containing high amounts of nitrates and phosphates are applied to grow more crops, the greater the possibility for surface water bodies being polluted during run-off. Furthermore, the increased population growth puts pressure on the waste water treatment plants (WWTP) (Seanego & Moyo, 2013; Liyanage & Yamada, 2017). Greater volumes of waste are generated with some being potentially hazardous and complex. The WWTPs are designed for a specific capacity, making it difficult to treat excessive volumes of waste and as a result, water is poorly treated and then discharged into water bodies. This makes the water bodies' repository for waste (Seanego & Moyo, 2013).

The presence of emerging contaminants (ECs) in surface water is of great concern for the aquatic ecosystem and human health. These ECs include classes of personal care products (PCPs), pharmaceuticals (PhACs), plasticisers and herbicides (Gogoi *et al*., 2018; Godfray *et al*., 2019). The ECs are derived from non-point sources and point sources from industry and agriculture as well as households. Emerging contaminants can be found in surface water, ground water, drinking water and discharges from waste water treatment plants (Fairbairm *et al*., 2018; Swartz *et al.,* 2018). The wastewater from treatment plants displays high numbers of emerging contaminants (Swartz *et al*., 2018). The conventional treatment system of WWTPs is designed in a way that cannot eradicate the

presence of emerging contaminates in the effluent discharged into water bodies, thus affecting surface water quality and posing a threat to the aquatic ecosystem, plants and humans that use the water (Swartz *et al*., 2018).

Surface water pollution has become a major challenge in the 21st century. Polluted water does not only affect the aquatic life, but also humans that use the water (Ezbakhe, 2018). In instances where people accidentally ingest polluted water containing high levels of faecal coliforms, they may suffer from gastrointestinal diseases, such as dysentery, cholera and typhoid fever. Other health issues linked to polluted water are eye irritations, ear infections and damage to the mucous membrane (DWAF, 1996a; Keraita *et al*., 2003; Rehman *et al*., 2018). The polluted water may also destroy certain tissues in animals and cause reproductive system failures (Rechenmacher *et al*., 2010). Therefore, water pollution has resulted in threats to water security and to the achievement of Sustainable Development Goals (SDGs) (Ezbakhe, 2018).

A critical aspect required to meet the basic environmental and human needs is surface water quality. Good water quality is referred to as the suitability of water to sustain various uses or processes and for the protection of the health of aquatic ecosystems (DWS, 2015; New South Wales [NSW] Government, 2017). It also supports and protects human health and aquatic life (Edokpayi *et al*., 2017; Tanjung *et al*., 2018). The water quality of streams is described based on the physical, chemical and microbiological properties of the water (Tanjung *et al*., 2018). In South Africa, the South African Water Quality Guidelines (SAWQG) stipulates the acceptable levels of the water quality properties for the different uses (DWAF, 1996). The physical properties of water generally have no direct impact but can affect the aesthetic characteristics of water. The chemical properties are defined by dissolved substances, while the microbiological properties refer to the presence of pathogenic organisms, like *E.coli* and total coliforms, in water (DWAF, 1996; Ratikane, 2013).

South Africa, as one of the members of the United Nations (UN), has adopted seventeen (17) Sustainable Development Goals (SDGs). These goals aim to provide peace and prosperity to people and on the planet (United Nations [UN], 2019). The 17 goals are built on the achievements of the Millennium Development Goals (MDGs) which expired in 2015. The 2030 agenda for Sustainable Development Goals has brought the issue of water quality to the forefront of international actions with the setting of SDG 6 that aims at "ensuring availability and sustainable management of water and sanitation for all" (DWS, 2019a; Ezbakhe, 2018). It is the UN's target that by 2030, SDG 6 promotes improved water quality by identifying and reducing pollution, increases water-use efficiency across all sectors, manages water resources in an integrated manner and protects water-related ecosystems (Ezbakhe, 2018).

The Bloemspruit stream is situated on the eastern part of the Bloemfontein urban area within the Mangaung Metropolitan Municipality (MMM) in the Free State province. Bloemspruit, together with its tributaries, namely; the Fonteinspruit and Renosterspruit, form part of the Modder River catchment. Taking place along the stream are anthropogenic activities comprised of industrial activities, such as food processing plants and agricultural activities such as cattle rearing and small scale crop farming as well as recreational activities, petrol stations and the waste water treatment plant (WWTP). These activities may affect the quality and quantity of water in the Bloemspruit stream. Therefore, humans and animals that use water from the Bloemspruit for any designated purpose are at risk of being exposed to the dangers that polluted water harbours.

1.2 Aim and objectives

In recent times there has been a rapid growth of human settlements, increased agricultural and industrial activities and a newly built waste water treatment plant along the Bloemspruit stream, which has brought an upsurge in pollution and further deterioration of water quality in the Bloemspruit stream (Belle, 2015). Water pollution degrades the aquatic ecosystem which later

affects humans, animals and plants that use the water. Therefore, the aim of this study was to evaluate the quality of the water in the Bloemspruit together with its tributaries; the Fonteinspruit and Renosterspruit. This was conducted to promote goal 6 of the SDGs and assist in improving water quality. Furthermore, the study was aimed at distinguishing the effects of anthropogenic activities and emerging pollutants on the surface water.

To meet this aim, the following objectives were devised:

- to identify appropriate sampling sites along the Bloemspruit, Fonteinspruit and Renosterspruit streams for the study;
- to measure water quality in terms of physical, chemical and microbiological properties;
- to measure the concentration values for existing emerging contaminants in the water;
- to obtain quantitative information concerning the physical, chemical and biological characteristics of water through statistical sampling;
- to measure water quality in terms of ecological parameters and analysis of emerging contaminants;
- to calculate a number of water quality indices (WAI) and ecological quality indices (EQI); and
- to make recommendations, if necessary.

1.3 Layout of the dissertation

This dissertation has been sectioned into seven chapters. Briefly, these chapters cover the following topics:

Chapter 1: Introduction

In this chapter, a brief synopsis of the study, including the problem statement, background, motivation for the study, aim and the objectives is provided.

Chapter 2: Literature review

In Chapter 2, a detailed introduction on the description of water as well as the relevant literature on water resources, uses and several factors attributed to pollution in water sources. Also included is an outline on surface water quality parameters and factors that exacerbate water quality.

Chapter 3: Materials and methods

In this chapter, materials and methods executed in analysing the physical, chemical and microbiological parameters are presented. Also included is an analysis of the emerging contaminants in the Bloemspruit stream and its tributaries. A detailed ecological survey using biota, particularly macroinvertebrates, at sampling sites was also included in the study.

Chapter 4: Results and analysis on the properties of water

In Chapter 4, the results of the water quality analysis of the physical, chemical, microbiological parameters in the Bloemspruit stream and its tributaries are presented. Results of physical parameters were recorded on-site while the rest we performed in the laboratories. A comparison

analysis of the results recorded from the Bloemspruit, Fonteinspruit and Renosterspruit streams was conducted. The analysis if variance (ANOVA) test was widely utilised to compare any seasonal variations in the results. WQI was also calculated to give a single description of the quality of water.

Chapter 5: Results and analysis on emerging contaminants in water

The results of the measurement and data analysis of emerging contaminants in the water are presented in this chapter.

Chapter 6: Results from the ecological study

In Chapter 6, results of the ecological assessment of the macroinvertebrate population and the macroinvertebrate habitat are provided.

Chapter 7: Discussion and conclusions

In this concluding Chapter, discussion and conclusion on the key findings of the study are presented and these findings are integrated into the existing knowledge. A discussion on the challenges and further prospects for surface water quality is also presented.

References: The references for this thesis were generated by the reference manager Mendeley.

Chapter 2

Literature review

2.1 Introduction

Water is a fundamental natural resource. It is a vital nutrient to sustain life of all living organisms, namely; humans, animals and plants (Abhineet & Dohare, 2014; Ratikane, 2013; Noreen *et al*., 2019). Water plays a crucial role in natural processes and in many physical and chemical reactions in nature as well as in domestic households and industries. In nature, water provides a healthy home for numerous aquatic animals and plants. In domestic households and industries, water is used for many different purposes. For domestic use, water is used in food preparation and for sanitation purposes. Industrial water is used in agriculture, mining and many manufacturing industries (Ahmad *et al*., 2016; DWA, 2010; City of Phoenix, 2017).

Most of the earth's surface is covered by water with approximately three-quarters of the earth's surface covered (Patil *et al*., 2012). It is estimated that 96.5% of the water occurs in oceans and seas, while 1.7% is sub-surface groundwater and 1.7% is frozen in the ice caps of the Antarctica (Khatri& Tyagi, 2015). Besides the sub-surface groundwater, less than 1% of the freshwater is contained in surface water resources.

South Africa has been classified as the 30th driest country in the world (DWAF, 2002a; Reddick & Kruger, 2019). Water resources in South Africa are under pressure, mostly because of the ever-growing human population and increasing anthropogenic activities. It has been estimated that by 2030, the demand for fresh water in certain developing countries is likely to exceed the supply by over 50% (Khatri & Tyagi, 2015). In South Africa, it has been estimated that by 2025 the demand for fresh water will greatly exceed the supply (Welch *et al.,* 2009). This upsurge in freshwater demand is driven by a combination of several human activities which include domestic activities, agricultural

activities, industrial activities, power generation, manufacturing and urbanisation (Donnenfeld *et al*., 2018).

2.2 Sources of water

2.2.1 Introduction

Most living organisms in nature depend on either surface water or groundwater for survival. Although surface water and groundwater are usually viewed as separate water masses, they are connected by the groundwater-surface water transition zone in the hydrological continuum (Winter et al., 1998; EPA, 2001). Therefore, any change in surface water bodies may eventually cause a change in groundwater.

2.2.2 Groundwater sources

Groundwater is the largest source of freshwater worldwide. It originates mostly from the infiltration of rain falling on the surface of the earth (Global water Partnership [GWP], 2014). In the hydrological cycle, groundwater is an important source as it is used in a number of different activities. These include domestic households, industries and in many agricultural activities (Strydom, 2010).In South Africa, groundwater plays a crucial role in supplying water for the rural and farming communities (Seaman *et al*., 2010; Mpenyana-Monyatsi *et al*., 2012). For domestic use, groundwater is mainly used as a source of freshwater for drinking and other sanitary purposes, such as washing and cleaning (Braune & Xu, 2008). At least 67% of the population in South Africa rely on groundwater for their daily water needs (Makoae *et al*., 2015).

The quality of groundwater may be affected by several natural processes and anthropogenic activities. Generally the groundwater quality depends on the quality of the surface water as groundwater is recharged water and precipitation moving down from the surface (Vasanthavigar *et al*., 2010). Groundwater quality also depends on the geology and the nutrient composition of the

water (Thibeault & Seth *et al*., 2014; Khatri & Tyagi, 2015). Some effluent produced by anthropogenic activities, such as municipal solid waste, agricultural activities, chemical spills from industries and domestic waste, may also contaminate groundwater through its connected system with the surface water (Fawell & Nieuwenhuijsen, 2003; Chen *et al*., 2016). Thus, to meet water demands, groundwater and surface water are integrated together in the planning of water resources and uses.

2.2.3 Surface water sources

Surface water originates mostly from rainfall. It is a mixture of surface run-off and groundwater flowing on the surface of the earth (World Health Organisation [WHO], 2017). Rivers, lakes, dams, oceans and streams are natural surface water resources that supply water to all living organisms. In South Africa, a water scarce country, nearly 80% of the population relies on rivers and streams as the main sources for domestic water and other purposes (Zamxaka *et al*., 2004). For quality and quantity, surface water greatly depends on several factors, such as the intensity of rainfall, climate, geology and vegetation as well as being governed by the diversity and health of living organisms in the water (WWF-SA, 2013; Khatri & Tyagi, 2015). However, more than 60% of the rivers in South Africa have been impacted due to pollution and only one third of the rivers are in a good condition (Donnenfeld *et al.,* 2018).

South Africa has approximately ten major rivers. The Orange River is regarded as the largest river in South Africa. It flows from Lesotho through the Free State province and the Northern Cape Province (Rand Water, 2016). The second largest river is the Vaal River that rises in the Mpumalanga province and forms the border between the Gauteng province and Mpumalanga (Rand Water, 2016). These rivers, together with their tributaries, provide water to the economic heartland of South Africa and are used for agriculture as well as for industrial purposes (WWF-SA, 2013; Rand Water,

2016). However, these rivers and streams are susceptible to pollution caused by a wide range of activities taking place in their vicinity (DWA, 2012).

2.3 Surface water use

South Africa is one of many countries that depend mainly on surface water resources to meet basic needs. Surface water is extensively utilised for domestic, industrial, energy production, navigational, recreational, manufacturing and agricultural activities (UN, 2011; Edokpayi *et al*., 2017; US EPA, 2017b). However, all these activities put pressure on water resources. In line with global trends, agricultural activities, mainly irrigation, are the largest water consumers in South Africa (Donnenfeld *et al.,* 2018, Reddick & Kruger, 2019). Agricultural activities use approximately 62% of the total water withdrawal in South Africa (Figure 2.1). The other large consumers of water at 27% are the municipalities, who supply commercial, industrial and residential users.

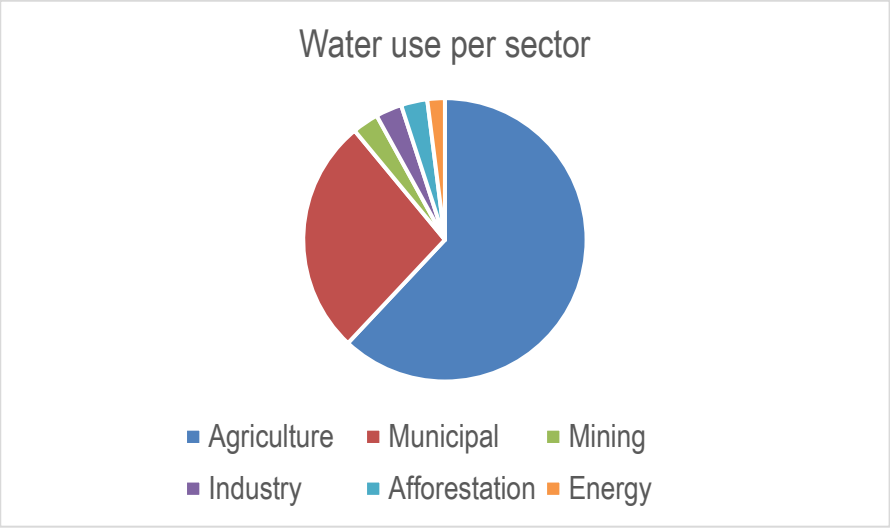


Figure 2.1 Water use per economic sector (Reddick & Kruger, 2019).

2.4 Surface water pollution

2.4.1 Introduction

Water pollution is a global challenge that has affected both developed and developing countries. Water pollution occurs when unwanted substances enter the water bodies and cause a change in the physical, chemical and microbiological properties of water from a beneficial state to a dangerous state (Baumgartner, 1996; Galadima *et al*., 2011; Owa, 2014; Haseena *et al*., 2017). Water pollution can be caused by a number of natural processes and anthropogenic activities as shown in the flow diagram below (Figure 2.2). Climate change and geological and hydrological factors are the most important contributors of the natural impacts (Gleik & Palaniappan, 2010; WHO, 2011; Kibena *et al*., 2014; Mathebula, 2015; Edokpayi *et al*., 2017). Anthropogenic activities, such as agricultural activities and industrial businesses that include waste water treatment plants (WWTP) as well as some domestic activities may discharge untreated effluent into the nearby rivers and streams (Kibena *et al*., 2014). The effluent from these enterprises may contain trace amounts of emerging contaminants (EC), which are highly toxic (Gogoi *et al*., 2018). Furthermore, a rapid human population growth in formal and informal settlements due to urbanisation may also play a major role in surface water pollution (Ratikane, 2013). Industrial effluents and domestic discharge may release phosphates, nitrates, metals, toxic chemicals and micro-pollutants into streams and this poses a significant impact on aquatic organisms, animal and people who use the water.

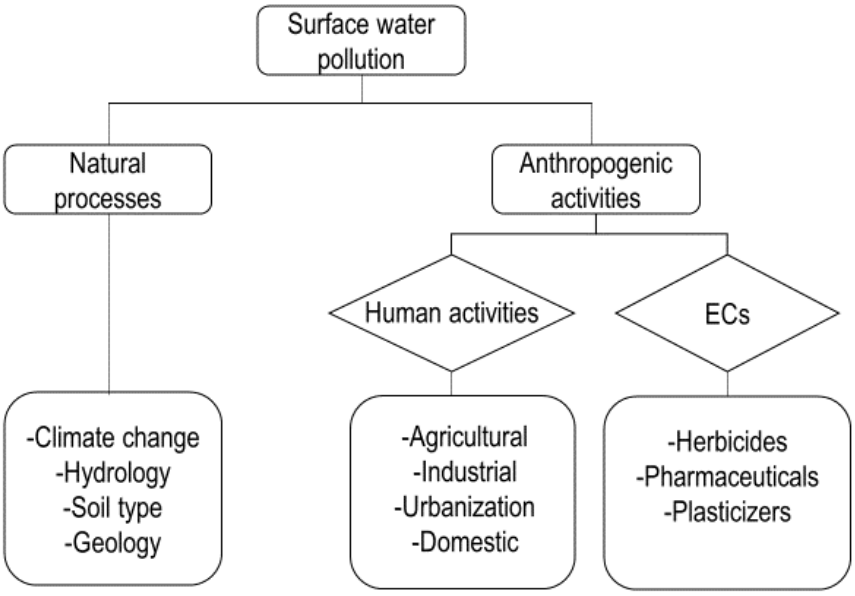


Figure 2.21 Natural and anthropogenic factors of surface water pollution

2.4.2 Water pollution by natural factors

Climate change and hydrology

Climate change is one of many factors that play a key role in surface water pollution. Any change in the statistical weather patterns that extend over a certain period of time is labelled as climate change (USEPA, 2017a). Climate change affects the hydrology of rivers and streams as the availability and variability of the flow of water is altered (Watts *et al*., 2015). During heavy rain falls and high precipitation events, the water quality of streams is severely affected (USEPA, 2017a). Increased volumes of water lead to flooding, which may cause problems with the sewage systems and water treatment plants (Mujere & Isaac, 2017). Heavy rain falls are also attributed to increasing the amount of suspended sediments and solids, such as nutrients, animal waste, domestic waste and emerging contaminants as well as some microbial fluxes such as *Escherichia coli*(*E. coli*) into rivers, streams and lakes (USEPA, 2017a). In Asheville, North Carolina, the city water system was damaged by heavy rainfall (US Global Research Program [USGCRP], 2009).

Climate change causes a change in the temperature and any change in the air temperature may affect the water temperature because water temperature is directly regulated by the ambient air temperature (Haghshenasi *et al*., 2017). This temperature variation may also change the concentration of contaminants in stream water. During the summer season, the water temperature is usually high and the flow of water is generally slow, which may affect the quality of water. When the water temperature is high, the phytoplankton and macrophytes grow extensively and reduce the dissolved oxygen concentrations in water (Haghshenasi *et al*., 2017). This causes the organisms that live in water to suffocate and eventually die. The reduced flow in summer also increases the phosphorus and nitrate concentrations and biological oxygen demand which may ultimately reduces the ammonium concentrations in water (Komatsu *et al*., 2007).

Drought is a natural climate change condition which usually affects some parts of the world every year. Drought is characterised by the reduced available water resources in both surface and groundwater levels that results in a lower to no flow in streams and rivers (Nosrati & Kazami, 2011). Drought reduces the supply of fresh water from rivers, resulting into more saline water resources (USEPA, 2017a).

Geology and soil type

The geology of a catchment has a high impact on the quality of water. The geology is characterised by the soil and rocks in the catchment. These rocks are composed of different chemical components which may affect the quality of water (Mathebula, 2016). As water flows through the rocks, the rocks break down and different components such as metals, ions and nutrients are released into the water (Alloway, 2013). Metals may affect the quality of water and kill the aquatic organisms. Properly managed soil may act as an effluent receiver during rainfall, however, when improperly managed, it leads to soil erosion as the soil particles are conveyed with the runoff into water bodies. Soil erosion

has been proven to be one of the main causes of degradation in water quality. Soil with high iron content may affect the iron concentration in the water that flows through it.

2.4.3 Water pollution caused by anthropogenic activities

Agricultural activities

Agricultural activities may contribute to surface water pollution through a number of activities. Agriculture is a non-point source of pollution into surface water bodies particularly by agrochemicals, organic matter, pesticides, herbicides and fertilisers (Chidya *et al*., 2011; Kibena *et al*., 2014; FAO, 2017). Agricultural pollutants enter the streams through surface runoff and leaching of nutrients (FAO, 2017; Belle *et al*., 2018) usually from gardens and fields adjacent to the streams and rivers. The fertilisers and pesticides used in agriculture release high amounts of nitrates, phosphorus, nitrogen and faecal coliforms (Hooda *et al*., 2000; Monaghan *et al*., 2007; Dafter, 2019), which are added to the natural nutrient concentration in the stream. Phosphates and nitrates are essential nutrients used for the growth of aquatic flora. However, excessive amounts of these nutrients may result in water eutrophication (Kiedrzynska *et al.*, 2014; Rand Water, 2017). Agriculture is the main source of pollution in the rivers and streams in the United States of America (USEPA, 2017b). A study conducted in the European Union (EU) demonstrated that 38% of the water bodies are highly polluted due to agricultural activities (World Water Assessment Programme [WWAP], 2015).

Animal husbandry and dairy farms also contribute to stream pollution in the vicinity of the operations. Cattle, sheep and other animals produce large amounts of urine and faeces that are rich sources of nitrates, ammonia and other pathogens such as *E. coli, Salmonella, Campylobacter, Clostridiums and Leptospira* which are washed down into streams during rainy days (Saxena & Sewak, 2016). These nutrients may affect the quality of water, thus killing animals and aquatic organisms (Esterhuizen *et al*., 2012).

Industrial activities

Industrial effluent contamination in surface water has emerged as a major contributor to diverse water pollution worldwide. Industrial agencies pollute stream water by dumping and discharging improperly treated effluent that contains solid substances, oil, chemicals and other substances into the streams (DWAF, 1996). Chemicals, such as ammonia, lead and arsenic, from industrial effluents are detrimental to aquatic ecosystems and pose a health risk to humans and animals (DWAF, 1996e; Hussain & Rao, 2013). Municipal waste contains a complex mixture of human waste, suspended solids, debris and some chemicals from industries, residential and commercial areas. Approximately 80% of the untreated municipal waste is discharged into water bodies globally (FAO, 2017). Industries, such as waste water treatment plants (WWTP), are responsible for dumping tonnes of heavy metal, nutrients, microorganisms, pharmaceuticals and personal care products into water bodies annually (WWAP, 2017). This effluent may affect the quality of water, aquatic organisms and humans (Edokpayi *et al*., 2017).

Urbanisation

Urbanisation, human settlements and an increase in the human population are the leading causes of water pollution in recent times. Due to limited water supply chains, as the human population increases water shortages are aggravated due to a higher demand for a freshwater supply (Esterhuizen *et al*., 2012). This results in an increase in pollution and catchment destruction (Esterhuizen *et al*., 2012; Ratikane, 2013; Rand Water, 2017). As urban development expands, pollution increases from point sources and non- point sources such as waste water treatment plants, industrial effluents, storm water discharge and domestic pollutants (Fourie, 2005). In urban areas, water and sanitation infrastructures are continually and significantly challenged because of the escalating production of waste from the growing population. Population growth also puts pressure on

the wastewater treatment plants, which are required to purify high volumes of effluent (Ratikane, 2013). Consequently, waste is discharged into surface water bodies without proper treatment. The studies conducted by OchandaOgola *et al*. (2009) and Edokpayi *et al*, (2017) demonstrated that the existing waste water treatment plants in South Africa fail to treat their waste water to safe and acceptable levels.

The increasing human population plays a negative role in surface water pollution. As the human population increases, more solid waste is generated and discharged into the nearby rivers and streams (Jabeen *et al*., 2011; Haseena *et al*., 2017). Solid waste may contain human excreta, diapers, chemicals and pharmaceuticals, which are harmful to aquatic life (Haseena *et al*., 2017). Moreover, as population growth increases, houses, schools, hospitals and roads are constructed. The construction of this infrastructure ultimately disturbs the land. These physical disturbances of soil and vegetation as well as deforestation affect the amount of salts and minerals in the water (Seanego & Moyo, 2013). When it comes to the production of food, high volumes of fertiliser are utilised to grow more food to feed the people. The increased human population also puts pressure on the waste water treatment plants (Seanego & Moyo 2013).

Emerging contaminants

The introduction of emerging contaminants (EC) in surface water is of great concern to aquatic ecosystems and human health. Emerging contaminants are referred to as natural or synthetic micro organic contaminants that cause undesirable effects on both humans and aquatic fauna and flora (Stuart *et al*., 2012; Meffe & Bustamante, 2014; Gogoi *et al*., 2018). ECs were known as micro-pollutants when they were first discovered in the 1900s. ECs include chemicals and microbial constituents that have been in the environment for a long time, but they have recently being elucidated due to the advancement in technology (US EPA, 2008). Residues of ECs, including

personal care products (PCPs), pharmaceutical (PhACs), illicit drugs and pesticides are widely present in faeces, medical waste, sewage treatment plants, drinking water, surface water and ground water (Jiang *et al*., 2014; Meffe & Bustamante, 2014).

The rate at which ECs are used on a daily basis worldwide is increasing annually. Chemicals are widely used, produced in high quantities and released into the environment in high volumes (Daughton & Ruhoy, 2009; Ncube, 2009). ECs are bioactive in the environment, significantly persistent, can accumulate in living organisms and have the ability to disrupt endocrines (UN & WHO, 2012). Their presence in the environment is of utmost concern because they do not appear individually, but rather as complex mixtures (Petrie *et al*., 2015). A study conducted in the US and Europe reported the presence of these chemicals in marine water (Jiang *et al*., 2014; Manzetti & Ghisi, 2014).

Emerging contaminants can enter surface, ground and drinking water in different ways. Waste water treatment plants, industrial effluent, agricultural runoff and farming operations play a significant role in releasing ECs into water bodies (Liu & Wong, 2013; Gogoi *et al*., 2018). A portion of all pharmaceuticals and personal care products is excreted by humans and animals through urine and faeces that end up in the soil then washed down into surface water bodies and eventually in the groundwater, the source of drinking water (Gaso-Sokac *et al*., 2017). The ECs are also transported as waste from showers and baths into wastewater systems and ultimately discharged into receiving water bodies (Caliman & Gavrilescu, 2009). Furthermore, ECs enter water resources through direct discharge from septic tanks, leaking landfills and agricultural runoff that contains high volumes of pesticides and fertilisers (Ncube, 2009). Even though ECs exist in very low concentrations in the environment, their prolonged exposure presents a high risk to human health and that of aquatic fauna and flora. Veterinary medicines are used to treat diseases in animals such as livestock and poultry. These veterinary drugs can affect the soil and consequently enter the food chain (Ebele *et*

al., 2017).Manure is used ad fertiliser to help with the nutrition of plants in agriculture. However, animal waste (excretion) used as manure may contain large amounts of PPCPs.

2.5 Effects of water pollution

2.5.1 Introduction

Water pollution has become a major challenge worldwide. Water pollution affects the quality of water and in turn the less tolerant organisms. It also affects other plants, humans and animals that use the water for any purpose. Polluted water is a medium for the transmission of water-related diseases, which in humans may lead to death.

2.5.2 Effects of water pollution on aquatic ecosystem

Eutrophication

Eutrophication of surface water bodies is one of the causes of water quality impairment worldwide. Eutrophication is a result of the enrichment of streams by nutrients, specifically nitrates and phosphorus (Kong *et al*., 2018). Eutrophication is a natural process, however, when accelerated by human activates it becomes detrimental to the aquatic ecosystem (Rathore *et al*., 2016). High levels of nitrates and phosphorus are attributed to excessive growth of microscopic blue-green plants and algae which produce cyanobacteria (Department of Environmental Affairs and Tourism [DEAT], 2006; European Union [EU], 2013). The blue-green plants and algae hinder the penetration of sunlight which results in the reduction of dissolved oxygen needed by aquatic organisms (EU, 2013). Therefore, the sensitive organisms that live in water may die, which leads to a declined ecosystem. Eutrophication also produces a scum on the surface of water, which is visually unappealing and can emit a nasty odour. Eutrophication has been identified in the Vaal River as the algae blooms in the river are due to high levels of nitrates, phosphates, sulphates and ammonia (DWAF, 2009).

Salinization and acidification

Salinization and acidification are two of the most challenging environmental problems worldwide. Salinization results from the accumulation of salts in the water bodies. Although some rivers are naturally saline due to the geological conditions, some dissolved salts are the result of human activities, such as industrial runoff, irrigation and dry land farming, which cause the leaching of salts into streams and thus elevating the concentration of salts in the water (Schulz, 2011; Velasco-Munoz *et al*., 2018). High levels of salt in water have an adverse impact on crop production (Clarke *et al*., 2015). When the irrigation water salinity exceeds 5ppt, the crop yield decreases (Ayers *et al*., 2017). High salinity also prevents the growth of aquatic plants and kills the less tolerant organisms as they are that are sensitive to high levels of salt (Schulz, 2011).

Acidification may also affect the aquatic organisms in water. Acidification of water is caused by high levels of nitrates emitted by agricultural activities (Petrin *et al*., 2008). Acidification of a stream is a measured on a pH scale. The lower pH measurements indicate acidic water, which may threaten the survival of acidic sensitive organisms (Harrould-Kolieb *et al*., 2010). Acid rain is caused by sulphur dioxide and nitrogen oxides lowering the pH value in any form of participation as well as the emission of carbon dioxide, which lead to water acidification (Owa, 2014).

2.5.3 Effects of water pollution on human and animal health

Water pollution is a serious health hazard worldwide. In most rural communities, stream water is often used for irrigation and other domestic purposes like washing and cleaning. Accidental ingestion of contaminated stream water may cause water-related diseases in humans and animals (Perveen & Zaidi, 2018). Polluted water is a medium for pathogenic bacteria, viruses and parasites (Esterhuizen, 2014). These are introduced to stream water through runoff from WWTP and other sewage lines (WHO, 2011). However, the disease depends on the infectious dose, invasiveness and virulence of

the pathogen. Water-borne diseases are associated with the ingestion of water contaminated by pathogens, which may cause acute and chronic human health illnesses such as diarrhoea, cholera, dysentery and typhoid fever (DWAF, 1996a; DWAF, 2002a, WHO, 2016). In developing countries, diarrhoea remains the leading cause of ill health and deaths amongst children under the age of five years (WHO; 2005; UN Children's Fund [UNICEF] & WHO, 2009). On the African continent, diarrhoea accounts for 46% of children under the age of five dying and 19% in South Africa (Msemburi *et al*., 2014). Polluted water containing high levels of nitrates from fertilisers may cause methaemoglobinaemia in infants if ingested (Ward *et al*., 2018). The increased amount of nitrates in water not only affects infants but may cause cancer and may affect the reproductive system in all humans. Exposure to high levels of lead may cause severe brain and kidney damage, while in pregnant women, it may lead to miscarriages (Inyinbor *et al*., 2018).ECs contain endocrine disruptive properties that can affect the reproductive system of animals and humans. Prolonged exposure to ECs may result in abnormalities in the human reproductive process such as a decline in the sperm quality and a low sperm count. Aquatic animals, like fish, may experience enlargement of the liver due to the presence of chronic estrogenic pollutants in the water (Gunnarsson *et al*., 2009; Yang *et al*., 2017). Human who come in contact with ECs, either by eating raw vegetables or drinking polluted water, may experience deformities of the kidneys and intestines. ECs have been proven to increase breast and ovary cancer in women when exposed to them for a long term (WHO, 2002). Emerging contaminates have been proven to cause gene alterations and may also cause a persistent antibiotic resistance (Swartz *et al*., 2018).

Pharmaceuticals and personal care products (PPCs) create an antibiotic resistant strain of bacteria in the body (Ebele *et al*., 2017). When they are used extensively on a daily basis, they become pseudo-persistent in the body (Kostich *et al*., 2014) and as a result, they tend to build resistant antibiotic bacteria. Therefore, the treatment and prevention of various infectious diseases becomes more difficult. A study conducted by the US Environmental Protection Agency (USEPA) identified

some drugs of concern such as antibiotics, antimicrobials, estrogenic steroids and antiepileptic drugs, in water sources these pose a threat to people and animals that use the water containing them (Environmental Working Group [EWG], 2009; USEPA, 2009).

2.6 Surface water quality

2.6.1 Introduction

Water quality is a significant component assessed on the basis of fitness for use. Water quality is aimed at meeting basic environmental and human needs as well as to support and maintain a healthy ecosystem (Fourie, 2005; Gleick *et al*., 2014). The quality of water is illustrated by its suitability to sustain certain uses and processes and can be defined by a range of parameters that have certain influences on water quality (Mathebula, 2015). A relatively good water quality aids in protecting the health and integrity of the aquatic ecosystem (DWAF, 1996e). However, when the stream water quality deteriorates, it may have a detrimental effect on the aquatic ecosystem making the water unsuitable for human and animal consumption and other agricultural and irrigation activities. Therefore, it is highly imperative to determine the quality of surface water by measuring and analysing the water properties, namely; the physical, chemical and microbiological determinants and comparing the measurements to the standards and guidelines to establish compliance (Khatri & Tyagi, 2015).

2.6.2 Water quality assessment

Water quality is assessed by measuring the physical, chemical and microbiological properties as well as measuring some other chemicals found in water. The physical properties are referred to as the aesthetic characteristics of water, such as, taste, dour and colour. These properties include temperature, pH, turbidity and electrical conductivity (Jordaan & Bezuidenhout, 2016). The physical properties of water generally have no direct impact on public health, but can affect the aesthetic

characteristics of water (WHO, 2008). However, if these properties exceed the limits for set standard, they may cause a change to the chemical and microbiological determinates.

Water temperature is considered as the most fundamental and basic parameter as it influences the quality of water in many ways, such as the rate of the biological and chemical reactions. It has a direct effect on chemical reactions, rates of reaction and aquatic life (Mathebula, 2015). Any sudden alterations to the ambient water temperature indicate a source of unnatural warming of the water or thermal pollution. Temperature affects the solubility of gases, mostly oxygen, in water. More gases can be dissolved in water that has a cold temperature than in warm temperatures.

Turbidity is the measurement of light transmitting properties of water. It indicates the colloidal and residual suspended matter in the surface water (Mathebula, 2015). Turbidity is caused by suspended particles, such as sediment, clay and silt, organic and inorganic matter, algae and other microscopic organisms. It is an indication of problems with infrastructure and operational challenges. High levels of turbidity pose a harmful impact on aquatic life for it hinders the amount of sunlight needed for photosynthesis. Turbidity is measured on-site using a Hach 2100Q turbidity meter and the results are reported as nephelometric turbidity units (NTU) (Metcalf & Eddy Inc., 2004). pH is the other essential parameter in water quality testing. It is a measure of the amount of free hydrogen ions in the water (Barnes *et al*., 1998; Holmbeck-Pelham & Rasmussen, 1997) and as an intensity factor of acidity in the water bodies (Mathebula, 2015). The pH scale usually ranges from 0-14 (Gorde & Jadhav, 2013). Electrical conductivity expresses the capacity of an aqueous solution to carry an electrical current (Gorde & Jadhav, 2013). This is attained through the presence of ions, mobility, relative concentrations and the temperature of the liquid. It is generally an indication of the taste and freshness of the water as it's a measure of the salinity of the water (Ratikane, 2013).

Chemical water quality factors involve dissolved substances, such as organic and inorganic substances. They include dissolved oxygen (DO), chemical oxygen demand (COD), nitrates, phosphates, ammonia, sulphates and total hardness. Dissolved oxygen is an essential form of oxygen used by fish to respire and by other aquatic fauna and flora (Gorde & Jadhav, 2013). The two main sources of dissolved oxygen are diffusion of oxygen from the atmosphere that enters the water bodies as well as a by-product of the photosynthesis process in aquatic plants and algae. Therefore, to survive, fish and other aquatic organisms need at least 3-5mg/l of dissolved oxygen. COD is a measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide in water (Holmbeck-Pelham & Rasmuseen, 1997; Barnes *et al*., 1998). This is an important water quality parameter used to indirectly measure the amount of organic compounds in water (Graham & Taylor, 2013). Nitrates and phosphates are natural nutrients needed by aquatic organisms for survival. However, excessive nutrients from the weathering of rocks and some anthropogenic activities, such as agriculture and industries, lead to water eutrophication (Sulaiman *et al*. 2014).

The microbiological indicators are widely used to detect the water quality in surface water bodies. The microbes in the water are measured by testing the amount of indicator bacteria or viruses present (Jordaan & Bezuidenhout, 2016). The most commonly used indicators of microbes are *Escherichia Coli* (*E. coli)*, faecal coliforms and coliform bacteria belonging to the family of Enterobacteriaceae. However, some of the faecal bacteria such as *E. coli* and coliforms are non-pathogenic (Mathebula, 2015). These indicator bacteria are exploited because they are easily identified, less expensive to test and are not dangerous to detect. *E. coli* is a thermo-tolerant coliform widely used to detect water quality in water bodies used for recreational purposes, environmental freshwater sources and drinking water. The coliform bacteria, the faecal coliforms (FC) and *E. coli* are always present in human and animal faeces, which end up in surface water bodies (Mathebula,

2015). Therefore, they are used to indicate the risk of infection through consuming, as stated in the World Health Organization Guidelines for Drinking Water (WHO, 2008).

Water quality of surface water can also be assessed through measuring some chemicals, such as emerging contaminants (ECs) in the water. Emerging contaminants include classes of pharmaceuticals, herbicides, personal care products and plasticisers (Gogoi *et al*., 2018). Pharmaceuticals include any medicine or drug, prescribed or over the counter. Pharmaceuticals are therapeutic drugs mainly used to treat human and animal diseases (Ebele *et al*., 2017). Such medicines include antibiotics, anti-diabetic drugs, anti-anxiety drugs and analgesics, such as paracetamol and lamivudine as well as blood lipid regulators, cytostatic drugs and hormones. (Osunmakinde *et al*., 2013). Pharmaceuticals are persistent drugs with high pharmacological and psychoactive properties. Most ECs are referred to as endocrine disrupting compounds (EDC) as they have the ability to disrupt the hormonal functions in the body (Osunmakinde *et al*., 2013). In South Africa, several studies that have been conducted found substantial amounts of endocrine disrupting compounds in fish tissues and other amphibians (Barnhoorn *et al*., 2004; Marchand *et al*., 2008).

Personal care products (PCPs) are ingredients used daily to improve the quality of life. The PCPs includes cosmetics, shampoos, soap, toothpaste, fragrances, sunscreen, hand lotions and preservatives (US EPA, 2014a; Osunmakinde *et al*., 2013; Ebele *et al*., 2017). Drugs used for vector control, such as bactericides or disinfectants and insect repellents, are also regarded as personal care products (Kosma *et al*., 2010; Liu & Wong, 2013). All these products have the ability to alter the quality of water and thus kill the aquatic life.

2.6.3 Ecological assessment

An ecological assessment is often conducted to evaluate the quality of water and the effects of water pollution on the aquatic macroinvertebrate population and their habitat. This involves the enumeration of aquatic organisms and the assessment of their habitat biotopes, such as stones and vegetation as well as gravel, sand and mud.

Macroinvertebrates population

The assessment of macroinvertebrates is often used to ascertain the overall quality of water in streams and rivers. The overall quality of water can be determined by assessing the abundance and diversity of the aquatic organisms, such as macroinvertebrates, algae and fish in the stream. However, tin several studies, the macroinvertebrates were mostly used as biological indicators of stream and river health (Monda *et al*., 1995; DWAF, 1996e; Dickens & Graham, 2002; Odume *et al*., 2012; Seanego, 2014; Wagenhoff *et al*., 2016; Belle, 2018). Macroinvertebrates are organisms without a backbone and can be easily identified with the naked eye. They are ubiquitous and immobile in stream water, with a life span ranging from several weeks to several years (Seanego & Moyo, 2013). They are highly sensitive to any physical or chemical stressors in their environment (Dickens & Graham, 2002; Selvanayagam & Abril, 2017; Belle *et al*., 2018). Moreover, macroinvertebrates play a key role in the function of the food web as they are primary consumers. Therefore, macroinvertebrates have been effectively used to monitor water quality worldwide (Selvanayagam & Abril, 2017)

To assess the ecological health of a stream based on the presence and diversity of macroinvertebrates, biological indices are used. These include indices such as the Ethiopian Biological Scoring Index (ETHbios), Biological Monitoring Working Party (BWMP), Zambian Invertebrate Scoring System (ZISS), South African Scoring System (SASS) and the Tanzanian River

Scoring System (TARISS) (Oigara & Masese, 2017). However, in South Africa, only the South African Scoring System version 4 (SASS4) has been validated for use (Oigara & Masese, 2017). The SASS version 4 was updated to version 5 due to some limitations that occurred in SASS4. These include the omission of some macroinvertebrates taxa and their sensitivity values. The SASS5 was then created by Chutter (1994 &1998), but more recently, it has been revised and updated (Dickens & Graham, 2002).

SASS is a fast and easy index used to determine the degree of stream water pollution by assessing the presence of macroinvertebrates in the water. Some macroinvertebrates are more sensitive to any change in their environment, therefore, where there is pollution, only pollution-tolerant macroinvertebrates will survive (Dickens & Graham, 2002; Ollis, 2005). SASS involves enumerating macroinvertebrates in the stream to determine their abundance and diversity at a particular sampling site for a certain period of time. Macroinvertebrates are collected from different biotopes, namely the stone biotopes, vegetation biotope and the gravel, sand and mud biotope (Dickens& Graham, 2002; RHP, 2017). The SASS5 method involves the calculation of the SASS score, number of taxa and the ASPT score.

Macroinvertebrate habitat

Even though the SASS data, through the assessment of macroinvertebrates, provides a true indication of stream health, it is important to include some factors that may have an influence on the macroinvertebrate data. The physical habitat structure is a key factor affecting the presence and diversity of macroinvertebrates in surface water bodies (Ollis *et al*., 2006; Khudhair *et al*., 2019). Therefore, it is important to include some form of habitat assessment in order to get an accurate interpretation during the ecological assessment (Chutter, 1994 & 1998; McMillian, 1998; Dickens& Graham, 2002; Ollis *et al*., 2006). Examples of habitat monitoring programmes include the

Qualitative Habitat Evaluation Index (QHIE) and River Habitat Survey (RHS). However, in South Africa, the Habitat Quality Index (HQI), Habitat Assessment Matrix (HAM) and Habitat Score Version 1 (HABS1) were used interchangeably, but they produced unreliable results (McMillian, 1998). Therefore, the Integrated Habitat Assessment System (IHAS) was developed. The Index of Habitat Integrity (IHI) and the IHAS are the tools mostly used to assess the habitat of macroinvertebrates in South Africa (Ollis et al., 2006).

Index of Habitat Integrity (IHI)

The IHI is an important tool that is used to assess any disturbance factors in the macroinvertebrate habitat. This index was created by Kleynhans (1996) to assess the anthropogenic factor along the streams and rivers and their impact on the habitat integrity (Dallas, 2005). Disturbance factors include water abstraction, flow regulation, bed and channel modification as well as the presence of alien plants in both the in-stream zone and the riparian zone (Dallas, 2005; Kleynhans *et al*., 2008). The in-stream zone represents the macroinvertebrate habitat in the current of the stream, while the riparian zone represents the macroinvertebrate habitat on the embankments (Kleynhans et al., 2008).

Integrated Habitat Assessment System (IHAS)

The Integrated Habitat Assessment System (IHAS) is also predominantly used to assess the condition of the macroinvertebrate habitat. IHAS was developed by McMillian (1998) to summarise the quality and diversity of the macroinvertebrate habitat at each sampling site (McMillian, 1998; Dallas, 2000; Olli *et al*, 2006). The IHAS is conducted on-site and the scoring sheet is comprised of a total of 100 points.

2.6.4 Water quality indexes

Several approaches have been used to best describe the quality of water in streams and rivers. The Water Quality Index (WQI) has gained popularity and is widely used because it expresses water quality as a single value. The WQI was initially developed by Horton in 1965 (Ghosh *et al*., 2019). It is a rating scale that reflects the composite impact of various water quality parameters (Ishaku, 2011).WQI is among the most effective tools used to communicate information with regard to water quality and water quality trends (Esterhuizen, 2014) to policy makers and concerned citizens (Puri *et al*., 2015). Water quality indexes provide a comprehensive picture of the quality of surface/ground water by reporting the overall multi-parameter water analysis as a single number (Boah *et al*., 2015). The intention of the WQI tool is to turn complex water quality data into information that can be easily understood. WQI is also helpful in selecting the appropriate water treatment technique to meet concerning issues. WQI is a mathematical means that involves the calculation of a single value to represent the level of water quality in any water body, such as streams and lakes (Etim *et al*., 2013).

The WQI was originally proposed by Horton in 1965. It was further developed by several other researchers who included Brown *et al*. (1970) and Ishaku (2011).In recent years, several researchers have developed different types of WQI, but the most commonly used are the Canadian Council of Ministers of the Environment Water Quality Index (CCE WQI), National Sanitation Foundation Water Quality Index (NSFWQI), Oregon Water Quality Index (OWQI), British Columbia water Quality Index (BCWQI), Recreational Water Quality Index (RWQI) and the Florida Stream Water Quality Index (FSWQI).

2.7 Prevention of surface water pollution

2.7.1 Introduction

Surface water pollution has gained recognition in several years due to the detrimental impacts it harbours. To safe guard water quality, several legislations, guidelines and standards have been instituted in an endeavour to prevent water pollution.

2.7.2 Surface water legislation and guidelines to prevent pollution

Surface water pollution is a serious challenge affecting developed and developing countries worldwide. To ensure good quality of water, legislation has been implemented to reduce the pollution in stream and river water. In South Africa, the South African National Standards (SANS) were developed to specify the physical, chemical and microbiological water quality properties for drinking water in 2006 (SANS241, 2006) but modified in 2011 (SANS 241, 2011). However, in 1996, the Department of Water Affairs (DWA) developed the South African Water Quality Guidelines (SAWQG) for surface water purposes. These guidelines were introduced for the protection of aquatic ecosystems (DWAF, 1996e), recreational use (DWAF, 1996b), agricultural use (1996d), industrial use (1996c) and for domestic activities (1996a). The SAWQG specify the Target Water Quality Ranges (TWQR) for each water quality property to ensure that an acceptable quality of water for any designated use is maintained. More recently, the Department of Water Affairs and Forestry has been renamed the Department of Water and Sanitation (DWS). DWS is a government organisation that leads and regulates water and sanitation sectors and is responsible for developing strategies and policies (DWS, 2016). In order to accomplish effective water use and management, DWS is governed by two acts, namely the National Water Act (1998) and the Water Services Act (1997) all within the national strategic objectives and the governance and regulatory framework.

Not all guidelines for other physical and chemical properties were available in the SAWQG, therefore research was undertaken to source guidelines from different countries. Other guidelines were sourced from the United States Environmental Protection Agency (US EPA, 2002) and the World Health Organization (WHO, 2013). For the analysis of emerging contaminants (ECs), the guidelines used are from the Canadian Council of Ministers of the Environment (CCME, 1998 & 2018) and the World Health Organization (WHO, 2013).

2.7.3 Implementation of the Sustainable Development Goals (SDGs)

South Africa as one of the members of the United Nations, has adopted seventeen (17) Sustainable Development Goals (SDGs). These goals are a universal call to end poverty, protect the planet and to ensure peace and prosperity for all people by the year 2030 (UN, 2019). The 17 goals are built on the achievements of the Millennium Development Goals (MDGs) that expired in 2015. The 2030 Agenda for Sustainable Development Goals has brought the issue of water quality to the forefront of international actions by establishing SDG 6 that aims at "ensuring availability and sustainable management of water and sanitation for all" (Ezbakhe, 2018; DWS, 2019a). It is the UN's target that by 2030, Goal 6 that aims to improve water quality by identifying and reducing pollution, increase water-use efficiency across all sectors, manage water resources in an integrated manner, and protect water-related ecosystems, will be achieved (Ezbakhe, 2018). In South Africa, it is the responsibility of the DWS to implement Goal 6 of the SDGs and thus ensure accessibility and availability of good water quality as well as ensure that surface water bodies, such as rivers and streams, are of good a quality (Statistics South Africa [Stats SA], 2013; Stats SA, 2017; Stats SA, 2019).

2.7.4 Blue Drop and Green Drop certification

To improve the quality and management of water in South Africa, the Department of Water Affairs (DWA) developed the Blue Drop and Green Drop certification programme in 2009. The Blue Drop

programme is aimed at improving the quality of drinking water while the Green Drop programme strives to improve waste water management (DWA, 2009; Molewa, 2011 cited in Esterhuizen, 2014). The Green Drop programme focuses on the management and the treatment of municipal waste water by monitoring the effluent discharged into the environment (DWS, 2017). The Green Drop certification programme measures and compares results of different water service providers. Based on their performance and evidence of excellence or failure, providers are rewarded or penalised. The organisations that score 90% or higher are awarded with the prestigious Green Drop status. However, the wastewater systems that score less than 30% receive a "Purple Drop" and are given 30 days to correct the issue.

2.8 Discussion

The assessment tools to measure the quality of water and the impact water quality has on the ecology provides an idea of the health status of the water. Therefore, these tools help to devise interventions, thus ensuring that the ecosystems of the water bodies remain healthy.

Chapter 3

Materials and methods

3.1 Introduction

This study was undertaken to assess the quality of water and the impact of pollution on the ecology in the Bloemspruit stream and its two tributaries, namely Fonteinspruit stream and the Renosterspruit stream. The water quality of the Bloemspruit stream was assessed by measuring the physical, chemical and microbiological properties as well as emerging contaminates in the water. However, the potential impacts of the polluted water on the ecology were evaluated using the macroinvertebrate population and macroinvertebrate habitat at the selected sampling sites in this study.

3.2 Study design

The study design was partitioned into three distinct stages. Stage 1 involved scouting the Bloemspruit stream and its tributaries (Fonteinspruit and Renosterspruit) to identify and select suitable sampling sites for this study (Figure 3.1). The second stage involved the collection of water samples at the selected sampling sites and recording the measurements for the water quality and ecological assessment. Measurements of the physical properties were recorded on-site, however, some measurements of the chemical, microbiological and emerging contaminants were recorded in the laboratory. Lastly, the third stage involved analysing the water quality and ecological assessment data.

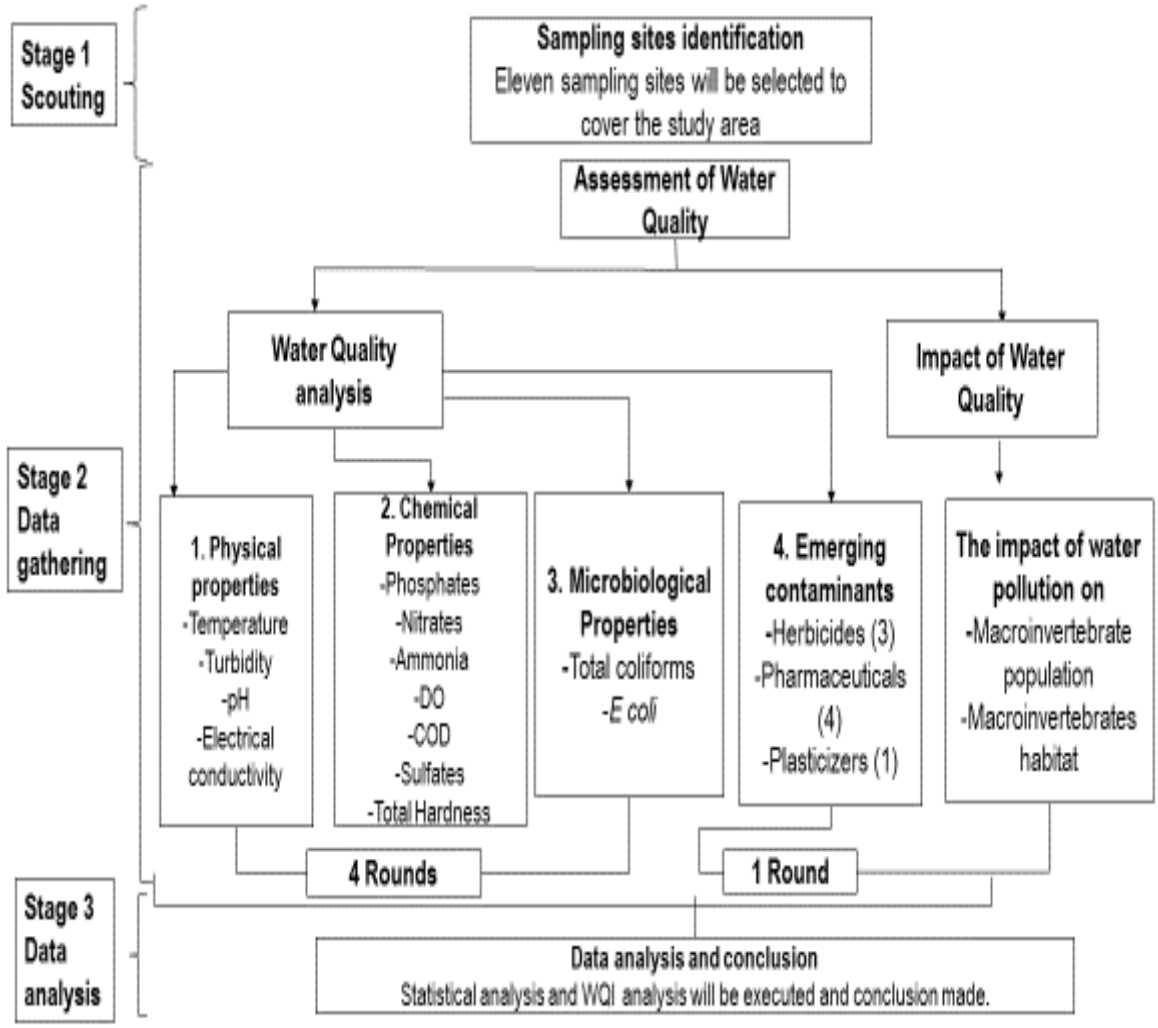


Figure 3.1 Experimental study design of the water quality and ecological assessment

3.2.1 Stage 1: Scouting for sampling sites

The scouting stage involved a visit to the Bloemspruit stream and its tributaries (Fonteinspruit and Renosterspruit) to identify appropriate sampling sites within the study area. Scouting was conducted on the 5th February 2018 and eleven sampling sites were selected for analysis in this study. These sampling sites were located downstream of the potential pollution agents, such as farming, industries and informal settlements along the streams. The sampling sites were identified from recommendations made by the supervisor and guidance gained from previous studies conducted on other streams included in this study.

3.2.2 Stage 2: Data collection

At each sampling site, water and macroinvertebrates were collected for water quality assessment and ecological assessment. For water quality assessment, the water samples were collected from the Bloemspruit and its tributaries during four separate sampling rounds. The first water collection round was conducted on the 3rd July 2018, the second on the 6th August 2018, the third on the 10th September 2018 and the last on the 18th of February 2019. For the ecological assessment, the aquatic macroinvertebrates were collected only during the last round of sampling.

For the water quality assessment, measurements of water quality properties in the Bloemspruit stream and its tributaries were taken. The properties of water that were measured in this study included four physical, seven chemical and two microbiological as well as eight emerging contaminants. The selected physical properties were temperature, pH, turbidity and electrical conductivity (EC). The physical properties affect different biological processes and chemical reactions in the stream. The chemical properties, namely nitrates (NO3), phosphates (PO4), dissolved oxygen (DO), ammonia, COD, sulphate and total hardness, were measured because they contribute to the pollution in streams from anthropogenic sources. The presence of two microbiological properties, total coliforms and *E. coli,* were measured because they indicate the presence of faecal pollution in water. The ECs that were measured in this study included three herbicides, four pharmaceuticals and one plasticiser. The herbicides measured were atrazine, metolachlor and terbuthylazine, while the pharmaceuticals were carbamazepine, estrone, estradiol and 17a-ethinylestradiol. Lastly, one important plasticiser, bisphenol A that is used in the production of plastics, was measured.

The ecological assessment of the Bloemspruit and its tributaries was performed on the aquatic macroinvertebrate population and macroinvertebrate habitat. The aquatic macroinvertebrates were collected because they are good indicators of the health of the water source that has either very high

or low pollutants. The South African Scoring System (SASS) created by Chutter (1998) was used to collect and enumerate the aquatic macroinvertebrates from the different biotopes along the stream. Furthermore, the macroinvertebrate habitats were assessed by visually observing and quantifying the conditions of the macroinvertebrate habitat. The macroinvertebrate habitats were measured using the Index of Habitat Integrity (IHI) to determine the impact of the human disturbance factors on the macroinvertebrate habitat.

3.2.3 Stage 3: Data analysis

In this study, data from the measured water and ecology samples from the Bloemspruit and its tributaries were analysed for water quality and ecological health indicators. The water quality assessment data obtained from the 11 sampling sites were entered into an Excel spread sheet for analysis. The measurements of the water quality properties were first compared with the standard limits for compliance. The measurements of temperature, turbidity, DO, *E. coli* and total coliforms were compared with the South African Water Quality Guidelines (DWAF, 1996e). However, the standard limits for pH, electrical conductivity, nitrates, phosphates, ammonia, sulphates, COD, and total hardness are not prescribed for South African. Therefore, the standard limits set by the United States Environmental Protection Agency (2002) were used.

The concentration values for emerging contaminants in the water samples were compared with the standard limits set by the World Health Organisation (2013), the Canadian Council of Ministers of the Environment (2018) and the European Union (2018) for compliance. A statistical analysis was performed on the physical, chemical and microbiological properties and emerging contaminants to determine the descriptive statistics, such as the mean, median and standard deviation of each water quality property. The analysis of variance (ANOVA) tests, at a significant level of 0.05, were performed on the data to ascertain if there were any significant differences between the different sampling rounds. Tuckey pos- hoc tests were also performed on the data where the ANOVA tests

were significant. Lastly, the Water Quality Index (WQI) assessment was calculated for each sampling site to describe the overall quality of water at each site.

An assessment of the impact of pollution on the macroinvertebrate population was performed by classifying the results of the SASS5 scores and the ASPT scores. At each sampling site, the SASS5scores and the ASPT scores were interpreted using the reference guidelines for the Highveld Eco-region. However, the macroinvertebrates habitat at each sampling site was analysed by classifying the IHI scores for each site.

3.2.4 Conclusion

Water quality of the streams may be measured, analysed and described using several methods and indexes. This may help to identify the pathway of pollution and the possible impacts of the pollution on the ecosystem, thus determining the health of the stream.

3.3 Study area

The Bloemspruit steam and its tributaries form part of the Modder River catchment. Bloemspruit stream is located on the eastern part of Bloemfontein in the Free State province. It is a medium flow stream that receives the majority of its surface runoff from the suburban part of Bloemfontein, such as Loch Logan (Belle *et al*., 2018). Bloemspruit is also fed by a small tributary, the Fonteinspruit that is downstream towards the Tau Pele Waste Water Treatment Plant (WWTP) (Figure 3.2). Fonteinspruit is a small stream that channels surface runoff from the informal residential zones of the Batho and Heidedal areas into the Bloemspruit stream. Within a few kilometres downstream, the Bloemspruit joins the Renosterspruit stream forming a confluence in the vicinity of the industrial area. The Renosterspruit is a medium flow stream that transports pollutants from Bloemfontein city, such as treated effluent from waste water treatment plants, and channel them into the Modder River (Figure 3.2).

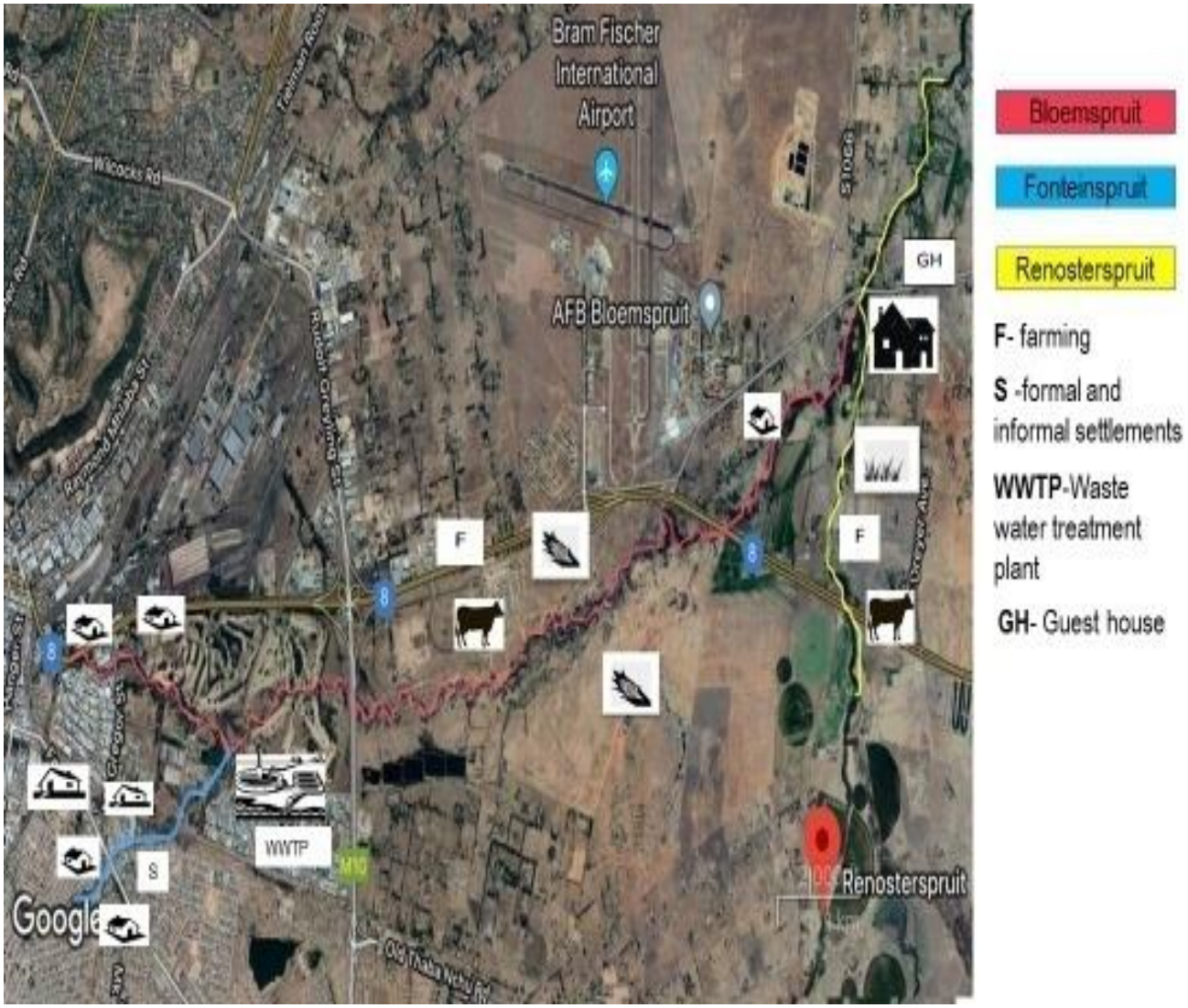


Figure 3.2 Study area and the activities taking place in the vicinity of the streams

3.4 Methods and materials for water quality assessment

3.4.1 Introduction

At each sampling site, the measurements of all the physical water quality properties were taken directly on-site in the stream. While the measurements of all but one chemical and all microbiological properties as well as the emerging contaminates required the collection of water samples that were then measured in the laboratory.

3.4.2 On-site measurements of the water quality properties

The stream's temperature, electrical conductivity, pH and dissolved oxygen were measured on-site using a battery operated Hach HQd hand held meter, while the stream's turbidity was measured on-site using a battery operated Hach 2100Q turbidity meter.

Measurement of turbidity

For measurement of turbidity, water samples were collected directly from the streams by using clean appropriate sample cells of approximately 10mL for readings. Prior to water collection, the sampling cell was first rinsed with distilled water and then rubbed with a clean cotton cloth (Figure 3.3). The turbidity meter was turned on and the cell containing the water sample was inserted into the instrument with its orientation mark aligned with the orientation mark in front of the cell compartment. The 'read' key was then pressed. Turbidity results were displayed in nephelometric turbidity units (NTU) which were recorded on the appropriate field record sheet for every sampling site.

Figure 3.3 Turbidity meter and the sample cells

Measurements of pH, dissolved oxygen, temperature and electrical conductivity

The pH, temperature, electrical conductivity and dissolved oxygen of the water in the streams were measured on-site using a battery operated Hach HQd handheld meter. The Hach HQd meter uses

digital probes that are designated for each property measured (Figure 3.4). Each sampling probe was submerged into the water, with the head attached to the handheld meter for recordings. To avoid any contamination, the sampling probes were first rinsed with distilled water before and after sampling at each sample point.

(a) (b)

Figure 3.4 (a) **Hach HQD handheld meter** (b) **Measuring probes**

3.4.3 Measurement of the chemical properties

The chemical properties, namely nitrates (NO_3), phosphates (PO_4), sulphates, ammonia (NH_3) and water hardness were measured using the DR 3900 Spectrophotometer in the water laboratory at the Central University of Technology. A spectrophotometer is an instrument that measures the intensity of each colour of light at a given wavelength while passing through a chemical (CRAIC, 2018) (Figure 3.5). This instrument is arranged so that the liquid, in a cuvette, can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer, which delivers a voltage signal to a galvanometer. The signal changes as the amount of light absorbed by the liquid changes, thus providing the means to quantify the chemical content. This instrument provides the simplest way of operation with fast execution and comprehensive documentation (Hach, 2011).

Figure 3.5 DR3900 Spectrophotometer

Sulphates, nitrates, ammonia and phosphates were measured in the following manner:

1. The spectrophotometer was first switched on so that the stored programs could be displayed and the relevant barcode program for either sulphate, nitrates, ammonia or phosphate could be selected for the measuring.
2. The sample cells were prepared by filling 10mL of the sample water and adding the appropriate powder pillows for each chemical into a sample cell. Cells were shaken for a few minutes according to the required procedure and then put down for the prescribed time. This was to allow the reagents to dissolve in the sample water. While waiting, the blank sample was prepared.
3. The instrument was then zeroed by placing a blank sample cell into the instrument's cell holder. Then the appropriate sample cell was cleaned and inserted into the sample cell holder and the readings were displayed.
4. The readings for phosphates, sulphates, nitrates and ammonia were displayed in mg/L.

Total hardness was measured in the following manner:

1. The spectrophotometer was switched on to start the program and the 225 Hardness, Mg barcode was selected.

2. 100mL of the water sample were poured into a 100-mL graduated mixing cylinder. A 1.0mL dropper was used to add 1.0 mL of calcium and magnesium indicator solution to the sample water.

3. A stopper was put on the mixing cylinder so that the mixing cylinder could be inverted several times in order to properly mix the solution. Another 1.0 mL dropper was used to add 1.0 mL of alkali solution for calcium and magnesium test into the solution and then inverted again to allow the solution to mix.

4.10 mL of the solution was then poured into each of the three sample cells. A blank sample was prepared by adding one drop of 1M EDTA solution to the first sample cell and then swirled to mix. For the magnesium sample, one drop of EGTA solution was added to the second sample cell, then swirled to mix.

5. The blank sample cell was cleaned and inserted into the cell holder before pushing the 'read' key. The display showed 0.00mg/L $CaCO_3$.

6. For the magnesium readings, the prepared magnesium sample was inserted into the cell holder. The results were displayed in mg/L magnesium as calcium carbonate. So, this value was the amount of magnesium in the sample expressed as $CaCO_3$.

7. Lastly, the magnesium program was exited and the new program 220 Hardness, Ca was selected. The instrument was zeroed to display 0.00mg/L $CaCO_3$. The third sample cell was then inserted into the cell holder for readings. The results were shown in mg/L calcium as calcium carbonate. Thus this value was the amount of calcium in the sample expressed as $CaCO_3$.

3.4.4 Measurement of the microbiological properties

The microbiological analysis of *E. coli* and total coliforms was executed using the IDEXX (Colilert 18) Quanti-Tray™ method at the accredited Test-it laboratory. The IDEXX (Colilert 18) Quanti-Tray™ method is a biotechnological detection approach that uses a multi-well most probable number (MPN)

process. It integrates a defined substrate medium which contains *O*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG). Subsequent to incubating samples at 37^{0}C for 18-22 hours, when illuminated under ultraviolet (UV) light, bacteria produce a yellow colour due to the production of β-galactosidase, while *E coli* produces a blue fluorescence that is the result of the action of β-glucuronidase (Health Protection Agency [HPA], 2004). The number of positive wells helps to calculate the MPN.

Measurements of *E. coli* and total coliforms using the IDEXX (Colilert 18) Quanti-Tray™ method were conducted in the following manner:

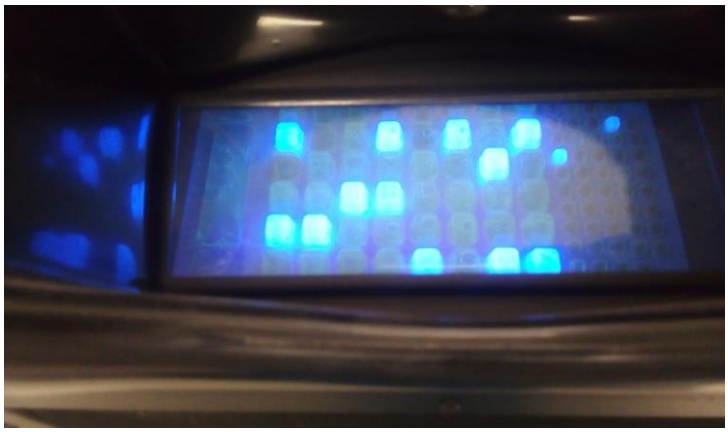
1. Water samples collected from each sampling site were filled up to the 100mL mark on the bottle used for microbiological tests.
2. The contents of the Colilert 18 medium pack were added to the 100mLof the sample water. The sample bottle was gently shaken and then left to stand for a few minutes to allow the Colilert 18 to dissolve.
3. The appropriate Colilert Quanti-Tray™, i.e. the 97-well Colilert 18 Quanti-Tray™ 2000 for untreated water was identified.
4. After selecting the appropriate Colilert Quanti-Tray™, it was then labelled with the sample number and date.
5. The 100mL water sample solution was poured into the Colilert 18Quanti-Tray™ 2000 and sealed in the pre-warmer sealer.
6. Thereafter, the Colilert 18 Quanti-Tray™ was incubated for 18 to 22 hours at 37^{0}C.
7. The Colilert 18 Quanti-Tray™ was removed from the incubator after 22 hours in order to quantify *E coli* and total coliforms in each sample.

Interpretation of the microbiological results

The enumeration of *E. coli* and total coliforms was conducted by counting the different colours in the 97-well Colilert 18 Quanti-Tray™ 2000 (Figure 3.6). The number of yellow coloured wells was counted to quantify the total coliforms while the number of fluorescent blue coloured wells with the help of the UV illumination was used to quantify *E. coli*.

a) The 97-well Colilert 18 Tray™ 2000 showing yellow wells that represent the number of coliforms

b) The 97-well Colilert 18Quanti-Tray™ 2000 under UV light is showing the fluorescent blue colour that represents *E. coli.*



After recording the number of wells that indicate the presence of both coliforms and *E. coli* in a 100mL water sample, the colony forming unit (CFU) was calculated by referring to the IDEXX 97-well Quanti-Tray™ MPN table in the following manner:

1. For coliforms, the number of large yellow wells on the MPN table was matched against the small yellow wells. For example, after incubation, if 14 large yellow wells and 10 small wells were counted, they were matched together to provide the MPN of 28.1 (Figure 3.7)

   The same procedure was undertaken for *E. coli* but, the 97-well Quanti- Tray™ was placed under UV light in order to count the fluorescent blue wells and the number of large fluorescent blue wells was matched against the number of small fluorescent blue wells. For instance, if 17 large fluorescent wells and 15 small fluorescent wells were counted, the MPN for *E. coli* was 39.3.

2.

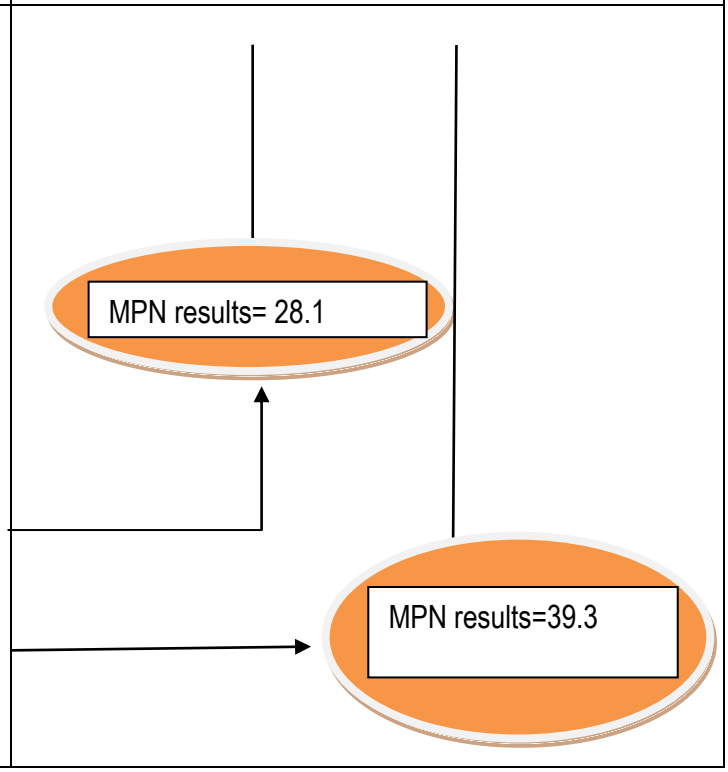
Large wells positive	Small wells positive 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15……49
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 . 49	

Figure 3.6 The most probable number (MPN) of *E. coli* and total coliforms

3.4.5 Measurement of emerging contaminants

In this study, water samples were collected from 10 of the 11 sampling sites in the Bloemspruit and its tributaries. Water samples were collected from the streams using a clean 1L plastic bottle and transported to the University of Free State's (UFS) chemistry laboratory for analysis. The selected contaminants, namely herbicides, plasticisers and pharmaceuticals were used for different purposes.

The extraction and analysis of ECs were conducted in the following manner:

1. The internal standards (positive mode: Atrazine-D5 and negative mode) were added to 500mL of the sample.
2. Samples were then filtered through a glass fibre filter and concentrated into methanol conditioned C18 6mL solid phase extraction cartridges (Strata, Phenomenex) at the flow rate of 5mL/min.
3. The bound analytes were slowly eluted from the dried cartridges using 2 mL methanol followed by 2mL Ethyl acetate.
4. The extracts were vacuum dried (Thermo Scientific Savant Speed vac) until almost dry and reconstituted in I mL water containing 10mM ammonium acetate.

The analysis of emerging contaminants in the water was performed using an ABSCIEX 4000 QTRAP hybrid quadrupole ion trap mass spectrometer (Figure 3.8), with a Shimadzu HPLC stack as the front end. All data acquisition and processing was manipulated using Analyst 1.5 (AB SCIEX) software. The analysis is clearly described below.

1. Firstly, twenty microliters (µL) of each extracted sample were separated on a C18 (150mm x 4.6mm, Gemini NX, Phenomenex) column at a flow rate of 300 µL/min using a five minute

gradient, in a positive ionisation mode, 5% solvent A (H_2O/0.1% formic acid) to 95% solvent B (MeOH/0.1 formic acid).

2. Then in a negative ionisation mode, 5% solvent A (H_2O/10 mM NH_4OH) to 95% solvent B (MeOH/10 mM NH_4OH) with a total run time of 15 minutes to allow for column re-equilibrium was performed.
3. Eluting analytes were ionised by an electro spray with 500^oC heater temperature to evaporate the excess solvent, 30 psi nebuliser gas, 30 psi heater gas and 20 psi curtain gas in the TurboV ion source.
4. In the positive ionised mode, the ion spray voltage (V) was set at 5500 V and —4500 V in the negative ionised mode.
5. Targeted analysis of pesticides using 2 MRM (multiple reaction monitoring) transitions per analyte was performed.

Figure 3.7 ABSCIEX 4000 QTRAP hybrid quadrupole ion trap mass spectrometer

3.5 Methods and materials for the ecological assessment

3.5.1 Introduction

An ecological assessment was conducted to measure the impact of water pollution on the benthic fauna, particularly macroinvertebrates. The macroinvertebrates spend their life time in water, thus

making them appropriate indicators of water quality for a long period of time. As a result, when the macroinvertebrates die because of pollution, the biological integrity is degraded. Therefore, in this study, the ecological assessment was conducted to assess the degree and impact of pollution on macroinvertebrates.

3.5.2 Enumeration of macroinvertebrates

The macroinvertebrate samples were collected from 11 identified sampling sites along the Bloemspruit and its two tributaries in accordance with the SASS5 (South African Scoring System, Version 5) sampling protocol (Dickens &Graham, 2002). A standardised SASS collection net of 2mm mesh size (Figure 3.9) was used with a kick and sweep technique in all biotopes (stones-in-current, stones-out-of-current, marginal vegetation, aquatic vegetation, gravel, sand and mud). The SASS5 tool is the most utilised instrument to assess the ecological state of aquatic ecosystems, to assess the spatial and temporal trends and to assess emerging problems in water in many different studies (Roux, 1997). The SASS tool was initially developed by Chutter (1998), but was modified by Dickens and Graham (2002).

As per protocol, SASS involves collecting water samples from different habitat biotopes that include stones and vegetation as well as sand, mud and gravel biotopes. Stones biotopes are comprised of stones-in-current (SIC) and stones-out-of-current (SOOC) (Dickens &Graham, 2002). For the stones-in-current, the macroinvertebrates were collected from the bedrocks or any solid object situated in the water for approximately two minutes. In contrast, for SOOC, macroinvertebrates were collected from moveable stones situated out of the perceptible current for one minute. Samples collected from SIC and SOOC were combined into a single biotope (Dickens &Graham, 2002). The vegetation biotope is characterised by marginal vegetation and aquatic vegetation. Marginal vegetation hangs or grows along the edge of the stream and can be both in current (MVegIC) and out of current (MVegOOC) (Dickens &Graham, 2002). Sampling of macroinvertebrates in the gravel,

sand and mud (GSM) biotopes required shuffling one's feet for about one minute. Visual observation of macroinvertebrates was also conducted for one to two minutes.

Figure 3.8 SASS net

The macroinvertebrates were collected according to the SASS5 protocol from different biotopes in the follow manner:

Sampling from stones biotopes

1. Macroinvertebrates were collected from stones using a net, which was placed downstream facing in the opposite direction to the water flow in order to catch the dislodged biota. Moveable and loose stone with an average size 2–25 cm, both in current and out current were collected.
2. The stones were kicked, turned and scraped with hands or feet to allow the disturbed macroinvertebrates in the area to be swept inside the net. Kicking, turning or scraping of stones-in-current (SIC) was conducted for approximately two minutes, while stones-out-of-current (SOOC) for one minute.
3. Macroinvertebrates collected from SIC and SOOC were combined into a single stones biotope sample.

Sampling from vegetation biotope

1. For the vegetation biotope, macroinvertebrates were collected from the marginal and aquatic vegetation. Sampling of the marginal vegetation along the embankment was done along a total length of two metres of vegetation, while the aquatic vegetation sampling was executed over an area of approximately one square metre.
2. The net was pushed vigorously against and through the vegetation in order to dislodge and collect the macroinvertebrates.
3. Macroinvertebrates sampled from marginal and aquatic vegetation were combined together into a single vegetation biotope.

Sampling from the gravel, sand and mud (GSM) biotopes

1. Macroinvertebrates were collected from the gravel, sand and mud by shuffling or scraping one's feet using wader boots for one minute and then sweeping the net over the disturbed area to catch the dislodged biota.
2. Gravel is made up of small stones that range from < 2 cm in size, while sand is any grains that are <2 mm in diameter and mud is any particles <0.06mm in diameter.
3. Macroinvertebrates sampled from the gravel, sand and mud were added together into a single gravel, sand and mud (GSM) biotope.

In this study macroinvertebrate samples were collected on the 19th February 2019 from five representative sampling sites in the Bloemspruit, Renosterspruit and Fonteinspruit streams using a 600 –micron mash net as depicted in (Figure 3.10).

Figure 3.9 Collection of macroinvertebrates in the stones and vegetation biotopes

3.5.3 Preservation and transportation of macroinvertebrates

Although it is advisable to examine macroinvertebrate samples in the field, it was a challenge for this study as many sites had to be sampled in one day. Therefore, preservation was the best alternative choice. Preservation can be conducted in two different ways, i.e. cold preservation and chemical preservation (Dickens &Graham, 2002). However, the chemical preservation method is not allowed in SASS, so the cold preservation mode was utilised. For the cold preservation, the sample was drained of all the excess water, placed in a 2 litre clean container and sealed with a lid. The sample container was then a put in cold box containing cold ice packs in accordance with SASS5 requirements and transported to the laboratory. The samples were refrigerated above freezing point for up to 72 hours.

3.5.4 Identification of macroinvertebrates

The identification of the macroinvertebrates was executed in the laboratory the day following the sampling, which was within the 72 hours as per the SASS5 procedure (Dickens & Graham, 2002). To identify and record the number of different taxa, the SASS5 score sheet was used for each sampling site in the following manner:

1. Macroinvertebrate samples were first removed from the refrigerator 30 minutes before enumeration and placed in a white tray containing clean water. The waiting period of three minutes that is advised to allow organism to come back to life, was observed.
2. The identification of macroinvertebrates was conducted with the aid of a hand lens to check on characteristic features for a maximum 15 minutes. The Aquatic Invertebrates of South African Rivers Illustration Guide (Gerber & Gabriel, 2002a) and Aquatic Invertebrates of South African Rivers Field Guide (Gerber& Gabriel, 2002b) were used in the identification.
3. The macroinvertebrates were identified according to each family level and recorded on the SASS score sheet. An estimated abundance of present taxa was entered, making use of the letters A, B, C and D. For example, a single individual macroinvertebrate was recorded as "1", but 2 to10 was allocated a "A", from 10 to 100 a "B", from 100 to 1000 a "C" and >1000 a "D".
4. Each family was assigned a quality score depending on their sensitivity or tolerance towards pollution (Table 3.1). The lowest scores were allocated to taxa resistant to pollution while the highest scores were assigned to taxa susceptible to pollution.
5. The SASS score, number of taxa and the ASPT score were calculated for all the biotope groups combined.

Table 3.1 The macroinvertebrate taxa and the corresponding sensitivity scores

Families	Sensitivity score	Families	Sensitivity score
ANNELIDA		**DIPTERA**	
Oligochaeta	1	Ceratopogonidae	5
Leeches	3	Chironomidae	2
EPHEMEROTERA		Culicidae	1
Baetidae	4	Muscidae	1
ODONATA		Psychodidae	1
Coenagrionida	4	Simulidae	5
Aeshnidae	8	Syrphidae	1
Gomphidae	6	**GASTROPODA**	
Libelludae	4	Ancylidae	6

HEMIPTERA		Lymnaeidae	3
Belostomatidae	3	Physidae	3
Notonectidae	3	Planorbonae	3
COLEOPTERA			
Elmidae	8		
Dytiscidae	5		
Hydeaenidae	8		
Hydrophilidae	5		

Included on the SASS scoring sheet is additional information, which is of utmost importance in the later stage during the interpretation of results. The information included is the site code, the description of the site and the signs of disturbance in the vicinity. The SASS5 method involved the calculation of three indices for each sampling site. These indices are the SASS score, number of taxa and average score per taxon (ASPT) (Dickens &Graham, 2002). The SASS score was calculated by adding all sensitivity scores for the identified macroinvertebrate families at each sampling site (Dickens & Graham, 2002). Macroinvertebrates were collected are identified to family level therefore, the number of taxa represented the number of families identified at each sampling site. The taxa were allocated sensitivity scores ranging from 1 to 15, with respect to water pollution. Each species is to some degree unique, so possess different tolerances to change in their environment. The sensitivity of 1 refers to extremely pollution-tolerant organisms, while a sensitivity score of 15 refers to extremely pollution-sensitive organisms (Ollis, 2005). Dickens and Graham (2002) explained the ASPT as the overall sensitivity of macro invertebrates at a certain sampling site. For that reason, ASPT was calculated by dividing the SASS score by the number of taxa.

Chapter 4

Water quality assessment of the Bloemspruit stream

4.1 Introduction

In this study the water quality assessment of the Bloemspruit stream and its tributaries was determined by analysing several physical, chemical and microbiological properties. The physical properties assessed were, pH, temperature, turbidity and electrical conductivity. The concentration of dissolved substances in the water was determined by measuring the chemical properties, such as dissolved oxygen, nitrates, phosphates, ammonia, chemical oxygen demand and total hardness. The analysis of the microbiological properties was conducted by measuring the total coliforms and *E. coli* in the water. Water was sampled over four rounds at 11 identified sampling sites in the Bloemspruit and its tributaries, the Fonteinspruit and Renosterspruit.

To determine compliance of the water quality properties, the results of all the physical and microbiological properties were compared with the South African Water Quality Guidelines for agricultural irrigation activities and the protection of aquatic ecosystem developed by the Department of Water Affairs and Forestry (1996). However, standards for all chemical properties except dissolved oxygen and total hardness, are not prescribed in South Africa, therefore the standards set by the Environmental Protection Agency of USA (US EPA, 2002) were used.

4.2 Sampling sites in the Bloemspruit stream and its tributaries

After the first stage of scouting, 11 sampling sites were identified. These sampling sites were located upstream and downstream of pollution sources, such as cattle farming and a waste water treatment plant (WWTP) as well as some formal and informal settlements (Table 4.1).

Table 0.1 Coordinates and the description of the study sampling sites as well as the reasons for sampling

Sampling sites	Coordinates	Area	Description of sampling site vicinity	Reason for choosing sampling site
S1	S29.12651 E026.25139	Industrial area	Bloemspruit –situated on Marula Street, under a bridge	This sampling site was included because it is located upstream of a suburban area of Bloemfontein
S2	S29.06079 E026.20598	Residential area	Fonteinspruit– situated downstream from Batho and Heidedal location	Sampling site S2 was included because it is located upstream before the Fonteinspruit joins the Bloemspruit
S3	S29.12719 E026.25139	WWTP area	Confluence of Bloemspruit and Fonteinspruit – downstream from Tau Pele WWTP	This sampling site was included because it is located upstream from the WWTP
S4	S29.11641 E026.32869	WWTP area	WWTP effluent before it reaches the oxidation plant	This sampling site was included to determine the quality of the effluent before being discharged into the Bloemspruit stream
S5	S29.12727 E026.26340	WWTP area	Bloemspruit –under the bridge, located on M10	This sampling site was included because it is located downstream from the WWTP which may be responsible for pollution
S6	S29.09959 E026.32963	Cattle farming area	Renosterspruit – on the N8 road, passing through a cattle farm	This sampling site was included because it was located downstream the farming area which could affect the quality of water
S7	S29.09694 E026.33155	Kopano-nokeng area	Renosterspruit – situated next to Kopano-nokeng guest house.	This sampling site was included to determine water quality of the Renosterspruit before it joins the Bloemspruit
S8	S29.11639 E026.32870	Kopano-nokeng area	Bloemspruit –situated next to Kopano-nokeng guest house	This sampling site was included to determine water quality of the Bloemspruit before it joins the Renosterspruit

S9	S29.09851 E026.33103	Kopano-nokeng area	Confluence of Bloemspruit and Renosterspruit	This sampling site was included to assess the water quality at the confluence of the Bloemspruit and Renosterspruit
S10	S29.12192 E026.26790	Kopano-nokeng area	Renosterspruit	This sampling site was included because it is located downstream from the confluence of the Bloemspruit and the Renosterspruit stream and to determine the amount of pollutants still present after the confluence
S11	S29.09698 E026.33153	Indaba area	Renosterspruit – situated next to Indaba Lodge	This sampling site was included because it is located downstream from the Indaba Lodge which may be responsible for pollution in the Renosterspruit stream

4.3 Determination of compliance limits for water quality properties

Data obtained from the measurements of water quality were captured for the statistical analysis in an Excel spread sheet. All the water quality properties, i.e. physical, chemical and microbiological, were first compared with the standard guideline limits for compliance.

The physical, chemical and microbiological properties of water were first compared with the South African Water Quality Guidelines (DWAF, 1996e) and the United States Environmental Protection Agency (US EPA, 2002) standards for compliance (Table 4. 2). The descriptive statistics including mean, median, standard deviation and compliance percentages were calculated to summarise the water quality properties. The ANOVA and the Tukey post hoc tests were also conducted on the physical, chemical and microbiological water properties to ascertain if there were any differences in the sampling rounds.

Table 0.2 Water quality property limits for stream water

Water quality property	Proposed limit	Purpose of limit	Reference
Physical properties			
Temperature	≤ 25^{0}C	Aquatic ecosystem	DWAF (1996e)
Turbidity	≤ 5.6NTU	Aquatic ecosystem	DWAF (1996e)
pH	4.2 9	Aquatic ecosystem	US. EPA (2002)
Electrical conductivity	≤ 1000 µs/cm	Aquatic ecosystem	US. EPA (2002)
Chemical properties			
Nitrates	≤ 2 mg/L	Aquatic ecosystem	US. EPA (2002)
Phosphates	≤0.7 mg/L	Aquatic ecosystem	US. EPA (2002)
Sulphates	<200 mg/L	Aquatic ecosystem	US. EPA (2002)
Ammonia	<1.5 mg/L	Aquatic ecosystem	US. EPA (2002)
DO	6.5 – 9.5 mg/L	Aquatic ecosystem	DWAF (1996e)

COD	≤40 mg/L	Aquatic ecosystem	US. EPA (2002)
Total hardness	≤150 mg/L	Aquatic ecosystem	US. EPA (2002)
Microbiological properties			
E. coli	≤200cfu/ 100mL	Irrigation	DWAF (1996d)
Total coliforms	≤200cfu/ 100mL	Irrigation	DWAF (1996d)

DWAF= Department of Water Affairs and Forestry, US. EPA= United States Environmental Protection Agency, DO= dissolve oxygen, COD= chemical oxygen demand, *E. coli*= Escherichia coli

4.4 Calculation of a Water Quality Index

The Water Quality Index (WQI) is the most frequently used tool to describe water quality. WQI is defined as a mathematical rating scale that communicates information on the quality of water in the most effective way (Esterhuizen, 2014; Belle, 2015). WQI is a criterion used in several studies to classify the surface water quality (Sener *et al*., 2017). When describing the overall water quality, large data obtained from the water quality measurements can be expressed as a single number (Sarkar & Abbasi, 2006, Sener *et al*., 2017). Therefore, a review of the literature was undertaken to identify the most suitable WQI for this study. Among the three indexes identified, namely the arithmetic (Brown *et al*., 1972), weighted arithmetic (Cude, 2001) and Canadian Council of Ministers of the Environment Water Quality Index (CCME, 2001), the CCME WQI was the most appropriate for this study.

The CCME Water Quality Index is calculated using the three main factors, namely, the scope (F_1), the frequency (F_2) and the amplitude (F_3) (CCME, 2001).

CCME is calculated using the following formula:

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

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

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

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

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

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

- When the measurement must not exceed the guideline

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

- Where the measurement must not fall below the guideline

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

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

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

c) Calculation of F3:

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

After the three factors were obtained, the CCMEWQI was calculated in the following manner

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

After calculations, water quality conditions are classified into five categories (CCME, 2017). These categories provide a description of the condition of water quality at each sampling site. The classification starts from an excellent WQI down to poor WQI of less than 44% (Table4.3).

Table 0.3 CCMEWQI ranking and description (CCME, 2017)

Condition	CCMEWQI	Description
Excellent	95 – 100	Water quality is protected with an absence of threat. Condition is very close to natural levels.
Good	80 – 94	Water quality is protected with a minor degree of threat. Condition rarely departs from natural levels.
Fair	65 – 79	Water quality is protected by occasional threats. Condition sometimes departs from natural levels.
Marginal	45 – 64	Water quality is frequently threatened. Conditions often depart from natural levels.
Poor	0 – 44	Water quality is always threatened. Condition usually departs from natural levels.

4.5 Water quality assessment results

4.5.1 Physical properties

In this study, the four physical properties of water were measured over a series of four rounds. For both temperature and pH, 100% compliance was recorded in all rounds of sampling when compared with the limits of the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996e)

(Table4.4). For EC, three of the four sampling rounds demonstrated 100% compliance when compared with the limits of the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996e), showing all but one measurement (S10) within the prescribed limits. In contrast, compliance for turbidity was low, ranging from zero in sampling Round 4 to 36%in sampling Round 2. The mean values for turbidity and pH in all sampling rounds were outside the standard limits.

Table 0.4 Measurements and summary statistics of the physical properties of the Bloemspruit and its tributaries.

Properties	Temperature (°C)				Turbidity (NTU)				pH				Electrical conductivity (µs/cm)			
Standard limit	(≤25)[1]				(≤5.6)[1]				(5.5-9.0)[1]				(≤1000)[1]			
Site no.	R 1	R 2	R3	R 4	R 1	R 2	R 3	R 4	pH 1	pH 2	pH 3	pH 4	EC 1	EC 2	EC 3	EC 4
S1	7,3	8,5	9,2	22,8	16,3	2,4	3,5	86,6	6,9	6,8	6,9	7,7	595	436	560	846
S2	6,3	8,3	9,3	12,2	4,5	4,2	9,7	29,3	6,5	6,9	7,2	6,5	723	724	698	376
S3	6,0	7,8	9,0	20,3	5,6	4,8	6,3	34,9	7,2	6,7	6,8	6,7	753	732	718	401
S4	5,8	13,4	13,8	24,7	4,9	62,9	30,3	31,3	7,1	6,9	7,2	6,8	656	749	679	777
S5	6,1	8,5	9,4	20,8	8,3	2,4	5,6	19,2	6,8	6,7	7,4	6,5	697	607	694	541
S6	5,7	7,9	9,2	21,8	8,9	11,4	16,0	36,4	6,7	7,2	7,6	7,2	688	677	711	612
S7	7,7	9,7	11,5	-	9,3	8,6	13,8	-	6,9	7,0	7,7	-	707	712	710	-
S8	8,8	8,8	10,5	20,3	9,6	10,3	17,0	61,2	7,2	6,9	7,4	6,8	716	683	688	520
S9	8,6	10,6	11,6	22,8	8,0	12,0	14,0	87,1	7,0	7,0	7,3	6,8	690	728	718	437
S10	7,5	10,5	12,1	22,7	9,4	7,7	13,3	170,0	7,1	6,9	6,9	7,0	675	710	698	1975
S11	14,0	9,8	11,6	24,7	14,9	8,7	14,1	37,8	7,1	6,9	6,9	6,5	680	676	700	586
Mean	7,6	9,4	10,7	21,3	9,1	12,3	13,1	59,4	7,0	6,9	7,2	6,9	689	676	689	707
Median	7,3	8,8	10,5	22,3	8,9	8,6	13,8	37,1	7,0	6,9	7,2	6,8	690	710	698	564

Minimum	5,7	7,8	9,0	12,2	4,5	2,4	3,5	19,2	6,5	6,7	6,8	6,5	595	436	560	376
Maximum	14	13,4	13,8	24,7	16,3	62,9	30,3	170	7,2	7,2	7,7	7,7	753	749	718	1975
SD	2,38	1,64	1,58	3,57	3,73	17,12	7,27	45,52	0,22	0,14	0,305	0,38	40,71	88,51	44,33	470,74
% COMPLIANCE	100	100	100	100	27	36	18	0	100	100	100	100	100	100	100	91

[1] = Standard limit according to South African Water Quality Guidelines (1996e), (–) = no sample obtained, red colour= measurement exceeded limit

To obtain a visual perspective of the turbidity measurements at the sampling sites in this study, a histogram was constructed. For the most, turbidity measurements in sampling Round 4 were relatively higher when compared with the turbidity measurements for the other three sampling rounds, except for sampling site S4 (Figure 4.1). Observation of individual sampling sites revealed that at sampling site S3 all turbidity measurements, except in sampling Round 4 were within the limit. Furthermore, at sampling sites S1, S2, S5, S6, S7, S8, S9, S10 and S11, turbidity measurements in sampling Round 1, 2 and 3 were slightly above the standard limit.

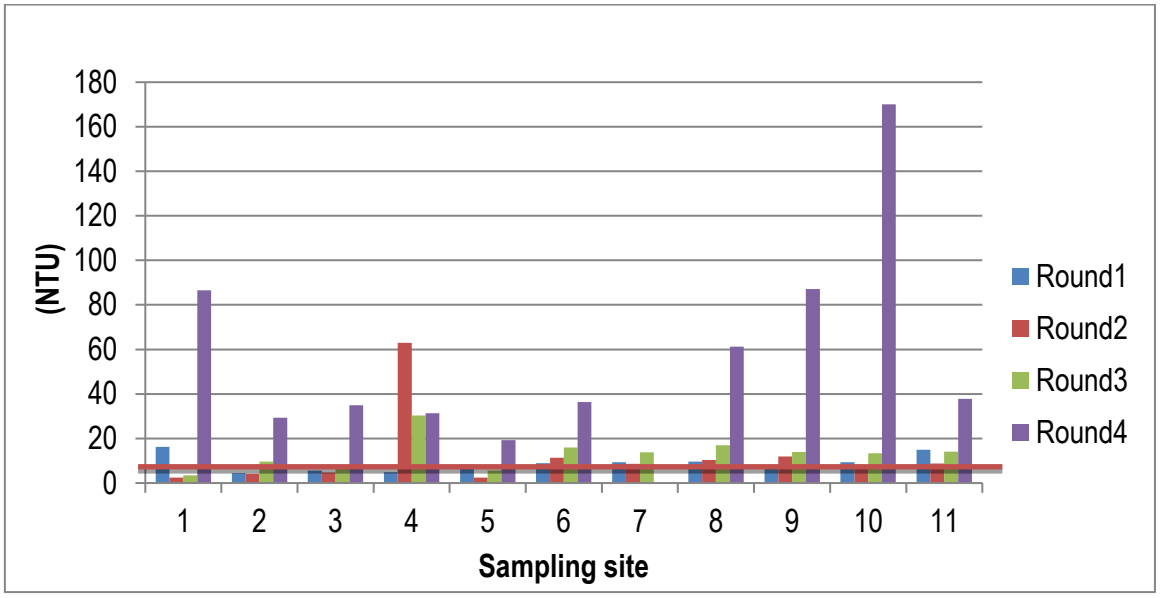


Figure 0.1 Histogram of the turbidity measurements for the four sampling rounds (Red horizontal line indicates the limit for turbidity).

The comparative analysis of the physical properties was conducted using the ANOVA single factor test to ascertain if any significant difference existed over the four rounds of sampling. Apart from temperature, turbidity and electrical conductivity (EC), the pH measurements demonstrated significant differences between the four rounds of sampling with a p-value of 0.02 (Table 4.5).

Table 0.5 ANOVA test results of the physical properties

Physical properties	*df*	*SS*	*MS*	*F*	*F crit*	*P-value*
Temperature	3	1170,09	390,03	68,05	2,85	1,48
Turbidity	3	17710,44	5903,48	10,35	2,85	3,82
pH	3	0,82	0,27	3,72	2,85	0,02
EC	3	5166,01	1722,00	0,03	2,85	0,99

df= degree of freedom, SS=sum of squares, MS= mean of sum of squares, f= variance of group means, p= probability, significant = ($p<0.05$).

The Tukey post hoc tests were conducted on pH measurements to ascertain which sampling rounds differed significantly from other rounds. The test revealed that a significant difference for the pH measurements was established between sampling Rounds 3 and 4 at $\alpha= 0.05$ (Table 4.6).

Table 0.6 Results of Tukey post hoc test conducted on pH measurements

pH	R1	R2	R3	R4
R1		NS	NS	NS
R2			NS	NS
R3				S
R4				

R=round, NS= not significant, S= significant

4.5.2 Chemical properties

The seven chemical properties of water quality were measured for the four sampling rounds. Only two of the seven chemical water quality properties, sulphate and total hardness, demonstrated 100% compliant measurements for all four sampling rounds when compared with the limits as prescribed by the United States Environmental Protection Agency (US EPA, 2002) (Table 4.7). However, ammonia revealed 100% compliance in all the sampling rounds with an exception of sampling Round 3, where compliance was the 91%. Furthermore, compliance with regard to COD was greater than 80% in all sampling rounds. In contrast, DO, nitrate and phosphorus were ≤80% compliant when compared with the Environmental Protection Agency (US EPA, 2002) limits. Phosphates revealed the lowest compliance of less than 10% for all four sampling rounds with only two (5%) of the measurements within the limit. The mean values for nitrates were slightly higher than the standard limit, whereas for phosphates, all the mean values were significantly higher than the limit (Table 4.7).

Table 0.7 Measurements and summary statistics of the chemical properties of the Bloemspruit and its tributaries

Properties	Dissolved oxygen				Nitrates				Phosphates				Sulphates				Ammonia				Total hardness				Chemical oxygen demand		
Standard limit	6.6-9.5				≤2				≤0.7				<200				<1.5				≤150				≤40		
Site no	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3
S1	7,0	8,4	6,0	1,0	0,8	0,1	2,1	0,6	0,7	0,6	1,1	4,8	22	23	39	42	0,001	0,00	0,01	0,02	3,1	10,8	23,3	0,3	2,0	8,0	156,0
S2	7,6	6,1	5,7	3,2	1,6	2,7	2,4	1,7	1,7	3,1	1,4	1,1	63	68	51	29	0,00	0,04	0,05	0,01	11,2	0,8	21,3	2,0	3,0	20,0	38,0
S3	5,6	3,8	4,7	3,1	2,7	2,0	2,1	1,4	1,6	3,1	1,0	2,5	50	48	51	37	0,00	0,07	1,70	0,01	12,4	11,5	23,2	2,7	1,0	21,0	10,0
S4	8,6	2,7	4,7	3,4	1,4	3,5	10,8	7,5	1,8	4,7	4,6	4,5	43	55	52	57	0,00	0,04	1,00	0,00	14,0	12,6	31,6	3,4	126,0	78,0	40,0
S5	7,6	8,3	8,4	4,9	2,8	0,5	2,8	1,1	5,2	1,4	1,8	1,9	46	38	56	45	0,02	0,02	0,30	0,03	15,4	12,0	27,0	1,4	22,0	18,0	22,0
S6	8,4	7,2	8,1	6,7	2,6	5,0	4,7	1,3	4,4	1,5	4,8	2,9	45	45	52	56	0,00	0,05	0,40	0,00	15,5	11,5	28,9	0,4	2,0	31,0	22,0
S7	8,6	8,0	7,4	-	1,2	1,7	1,2	-	5,5	5,3	5,6	-	38	39	35	-	0,01	0,00	0,40	-	12,9	12,5	27,3	-	11,0	34,0	-
S8	7,4	7,2	7,6	6,5	1,5	3,5	4,3	1,6	5,8	5,4	5,1	2,4	44	45	51	43	0,01	0,00	0,20	0,01	11,5	9,9	25,2	2,6	11,0	30,0	16,0
S9	6,9	6,5	3,8	7,3	1,4	2,1	2,7	2,2	5,4	5,7	5,4	2,3	40	48	48	34	0,01	0,00	1,30	0,04	12,1	11,2	26,8	1,7	12,0	37,0	14,0
S10	10,5	7,3	6,1	6,0	4,0	0,1	0,8	3,0	5,1	5,2	5,5	1,9	43	40	33	13	0,03	0,00	0,20	0,01	25,1	26,4	27,4	3,2	42,0	38,0	18,0
S11	3,1	8,7	9,7	7,6	6,4	3,3	4,4	1,6	5,3	5,4	4,7	2,7	45	50	54	47	0,03	0,00	0,40	0,05	26,1	27,2	27,9	2,0	2,0	39,0	25,0
Mean	7,4	6,8	6,6	4,9	2,4	2,2	3,4	2,2	3,9	3,8	3,7	2,7	43,5	45,4	47,5	40,3	0,00	0,02	0,55	0,02	14,5	13,3	26,4	2,0	21,3	32,0	36,1
Median	7,6	7,2	6,1	5,5	1,6	2,1	2,7	1,6	5,1	4,7	4,7	2,5	44	45	51	42,5	0,01	0,02	0,40	0,01	12,9	11,5	27	2,0	11,0	31,0	22,0

Minimum	3,1	2,7	3,8	0,2	0,8	0,1	0,8	0,6	0,7	0,6	1,0	1,1	22	23	33	13	0,00	0,00	0,01	0,00	3,1	0,8	21,3	0,3	1,0	8,0	10,0
Maximum	10,5	8,7	9,7	7,6	6,4	5,0	10,8	7,5	5,8	5,7	5,6	4,8	63	68	56	57	0,00	0,07	1,70	0,05	26,1	27,2	31,6	3,4	126	78,0	156,0
SD	1,9	1,9	1,8	2,4	1,6	1,6	2,8	2,0	2,0	1,9	1,9	1,2	9,7	11,2	8	13	0,01	0,02	0,55	0,02	6,4	7,5	2,9	1,2	36,8	18,0	43,2
% Compliance	**73**	**73**	**36**	**40**	**55**	**45**	**27**	**80**	**9**	**9**	**0**	**0**	**100**	**100**	**100**	**100**	**100**	**100**	**91**	**100**	**100**	**100**	**100**	**100**	**82**	**91**	**90**

(–)= no sample could be obtained, red colour= measurement exceeded limit

A visual perspective in the form of a histogram, demonstrates the pattern and extent to which the phosphates measurements exceeded the standard limit. All but two measurements greatly exceeded the limit (Figure 4.2). The pattern from the sampling sites showed that five of the sampling sites (S7, S8, S9, S10 and S11) demonstrated relatively high phosphate measurements for three sampling rounds. However, for most sampling rounds, the phosphate measurements at sampling sites S1, S2, S3 and S5 were slightly higher than the standard limit of <2mg/L.

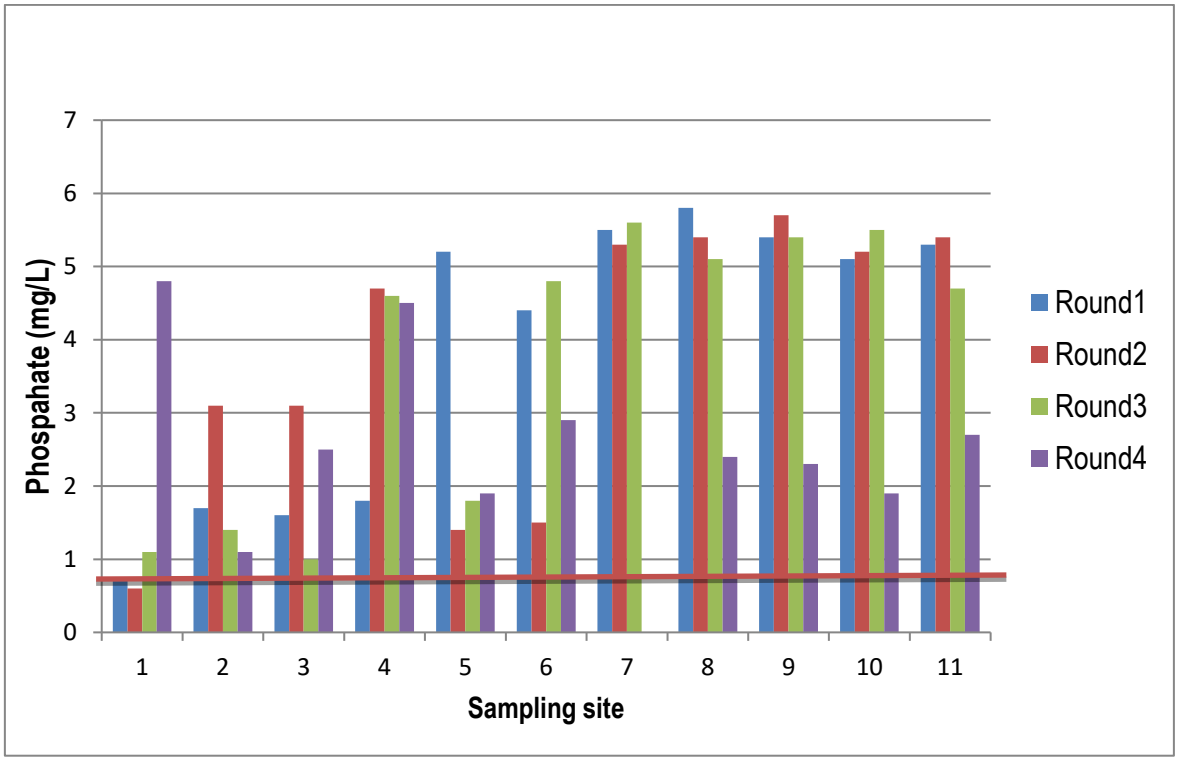


Figure 0.2 Histogram of the phosphate measurements for the four sampling rounds. (Red horizontal line indicates the standard phosphate limit).

The ANOVA single factor test was carried out to determine if there was a significant difference in any of the measurements of the chemical properties over the four rounds of sampling. Only one of the seven properties assessed, namely; DO, showed a significant difference with a p-value of 0.05 (Table 4. 8).

Table 0.8 ANOVA test results of the chemical properties

Chemical properties	*df*	*SS*	*MS*	*F*	*F crit*	*P-value*
DO	3	32,74	10,91	2,85	2,85	0,05
Nitrates	3	12,11	4,04	0,98	2,85	0,41
Phosphates	3	9,14	3,05	0,96	2,85	0,42
Sulphates	3	288,09	96,03	0,86	2,85	0,47
Ammonia	3	2,26	0,75	9,76	2,85	6,22
Total hardness	3	3129,88	1043,29	38,41	2,85	1,01
COD	2	1258,5	629,25	0,54	3,33	0,58

DF= degree of freedom, SS=sum of squares, MS= mean of sum of squares, f= variance of group means, p= probability, significant = ($p<0.05$).

The Tukey post hoc test was conducted on the dissolved oxygen data to ascertain if there was any significant difference between the sampling rounds. Significant differences were revealed between sampling Rounds 1 and 4 at $\alpha= 0.05$ (Table 4.9).

Table 0.9 Results of Tukey post hoc test for DO measurements

DO	R1	R2	R3	R4
R1		NS	NS	S
R2			NS	NS
R3				NS
R4				

R=round, NS= not significant, S= significant

4.5.3 Microbiological properties

The two microbiological properties measured, namely total coliforms and *E. coli,* displayed exceptionally high measurements, which were above the proposed guidelines set out in the South African Water Quality Guidelines for Irrigation (DWAF, 1996d) most of the sampling sites (Table

4.10). However, *E. coli* revealed compliance of greater than 50% in sampling Rounds 1 and 2 but less in sampling Round3.Furthermore, compliance for total coliforms was <30% in all sampling rounds, with 0% compliance in sampling Round 2 when compared with the South African Water Quality Guidelines for Irrigation (DWAF, 1996d).

Table 0.10 Summary statistics of the microbiological water quality properties of the Bloemspruit and its tributaries

Properties	E coli/100mL			Coliforms/100mL		
Irrigational limit	≤200cfu/ 100mL			≤200cfu/100mL		
Site no.	R 1	R 2	R 3	R1	R 2	R 3
S1	>2419,6	325,6	390,0	340,0	>2419,6	1000,0
S2	207,1	648,8	188,0	820,0	>2419,6	410,0
S3	149,5	196,8	391,0	644,0	>2419,6	40,0
S4	251,0	1046,2	500,0	508,0	>2419,6	63,0
S5	58,8	24,3	210,0	286,0	>2419,6	1000,0
S6	80,5	16,0	64,0	285,0	>2419,6	680,0
S7	12,7	1,0	–	97,0	>2419,6	
S8	14,5	25,9	64,0	174,0	>2419,6	512,0
S9	61,2	547,5	928,0	440,0	1986,3	914,0
S10	2,0	5,2	10,0	201,0	>2419,6	456,0
S11	62,6	10,9	290,0	1000,0	>2419,6	190,0
Minimum	2,0	1,0	10,0	97,0	1986,3	40,0
Maximum	>2419,6	1046,2	928	1000,0	>2419,3	1000,0
% COMPLIANCE	73	64	40	18	0	27

Cfu=colony forming unit, mL=millilitres, (–) = no sample could be obtained, red colour= measurements exceeded limit

To obtain a better perspective of the data, a histogram was constructed using the measurements of the total coliforms. The histogram shows that in sampling Round 2, measurements of total coliforms were exceptionally higher than the standard limit (Figure 4.3). For the most, measurements in the sampling round exceeded 2000cfu except for a measurement at sampling site S9. During sampling Round 1, measurements at sampling sites S7 and S8 were within the limit, whereas during sampling Round 3, measurements at sampling site S3, S4 and S9 were within the standard limit recommended by the DWAF(SAWQG Irrigation) (DWAF, 1996d).

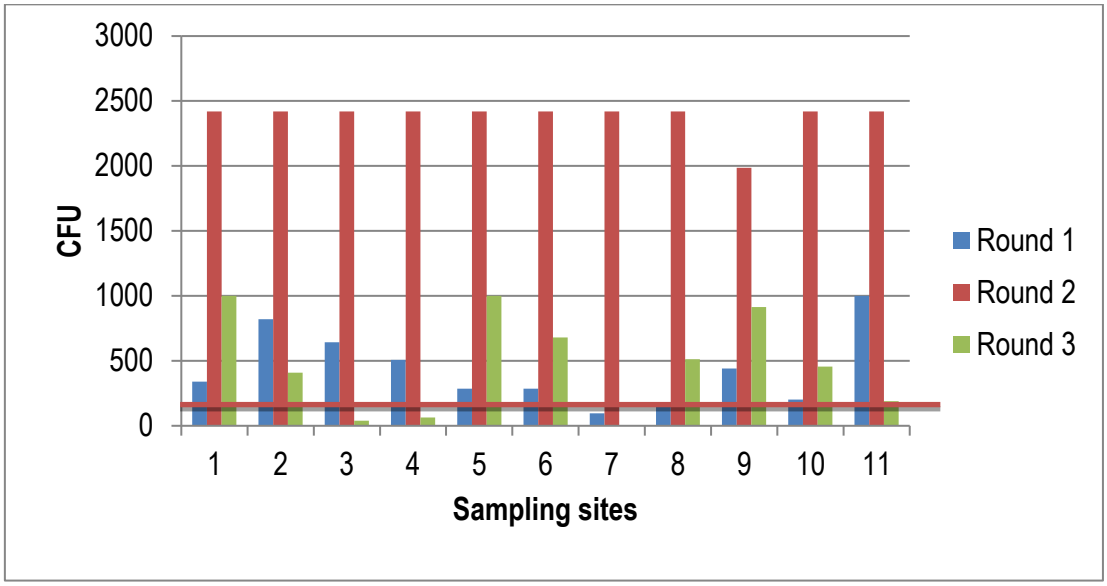


Figure 0.3 Histogram of the total coliforms measurements for the three sampling rounds. (Red horizontal line indicates the standard phosphate limit).

ANOVA tests were performed on measurements of the microbiological properties to ascertain if any significant difference existed for *E. coli* and total coliforms over the three sampling rounds. Both of the properties revealed no significant differences in four rounds of sampling (Table 4.11).

Table 0.11 ANOVA test results of the microbiological properties

Microbiological properties	*SS*	*df*	*MS*	*F*	*F crit*	*P-value*
E. coli	13781,00	2	6890,499	0,03	3,33	0,97
Total coliforms	26134545,41	2	13067273	173,54	3,33	7,30

DF= degree of freedom, SS=sum of squares, MS= mean of sum of squares, f= variance of group means, p= probability, significant = (p<0.05).

4.5.4 Water Quality Index

To evaluate the overall quality in the stream, WQI was calculated for all the sampling sites. Out of all the 11 sampling sites, none of them demonstrated good water quality, however, five sampling sites revealed a fair condition (Table 4.12). For the remaining sampling sites, five demonstrated a marginal condition and only one sampling site demonstrated a poor WQI of 35%.Therefore, the quality of water of the Bloemspruit may affect the aquatic ecosystem, vegetation and humans that use the water.

Table 0.12 Water Quality Indexes for each of the sampling sites

Sampling sites	WQI	Condition	Description
S1	65		The fair Water Quality Index can be associated with less anthropogenic activities in the vicinity of the sampling sites S1 and S2. However, at sampling site S5, the increased stream flow may have diluted the pollution discharged from the waste water treatment plant. The fair WQI at sampling sites S6 and S7, might be attributed to the impact of cattle farming alongside the stream.
S2	68		
S5	69		
S6	67		
S7	70	**Fair**	
S3	61		The marginal Water Quality Index is influenced by the effluent discharged from the waste water treatment plant at sampling sites S3 and S4. At sampling
S4	58		
S8	64	**Marginal**	

S10	51		site S10 and S11, marginal WQI might be influenced by the confluence of the Bloemspruit and Renosterspruit.
S11	59		
S9	35	**Poor**	The poor Water Quality Index is influenced by the confluence of the Bloemspruit and the Renosterspruit.

4.6 Discussion

The analysis of the water quality properties provided a clear indication of the water quality in the Bloemspruit and its tributaries. Of the thirteen water quality properties analysed, only four (31%) demonstrated 100% compliance in all rounds of sampling. For most of the physical properties, 100% compliance was recorded, except for turbidity measurements that had a compliance of less than 40%. For chemical properties, only sulphate and total hardness demonstrated 100% compliance. Compliance for the other chemical properties was less than 80%. On the other hand, both of the microbial properties showed compliance percentages of less than 40%. When considering the Water Quality Index (WQI) of the respective sampling sites, none of the WQI values indicated that the water quality could be classified as good or excellent (>80). However, five of the sampling sites were classified fair. Overall, this study reveals that the water quality of the Bloemspruit is poor.

Chapter 5

Emerging contaminants in the Bloemspruit stream

5.1 Introduction

Besides studying the regular physical, chemical and microbiological properties, water was also collected and analysed for the presence of a few emerging contaminants (EC). The ECs that were analysed in this study included three herbicides, four pharmaceuticals and one plasticiser. The selected herbicides were atrazine, metolachlor and terbuthylazine. These herbicides are commonly used as weed killers in agriculture. The pharmaceuticals, namely carbamazepine, estrone, estradiol and 17a-ethinylestradiol, were analysed, because they often end up in streams and rivers through sewage water and effluent discharged from waste water treatment plants. Lastly, the presence of one important plasticiser, bisphenol A, which is used in the production of plastics, was also analysed in this study. These ECs were assessed at 10 of the 11 sampling sites because access to sampling site S7 was impeded due to trees being uprooted during a storm a few days prior to sampling..

In South Africa, there are no standards for ECs found in surface water bodies. Therefore to establish compliance, international standards were used to compare the EC concentration values against. The concentration values for the herbicides, namely atrazine and metolachlor, were compared with the standard limits set by the Canadian Council of Ministers of the Environment (CCME, 2018), while for terbuthylazine, the concentration values were compared with the standard limits set by the World Health Organisation (WHO, 2013). The concentration values of three of the four pharmaceuticals, namely estrone, estradiol and 17a-ethinylestradiol, were compared with the standard limits specified by the European Union (EU, 2018). For one pharmaceutical, carbamazepine and the plasticiser, bisphenol A, the standard limits stipulated by the Canadian Council of Ministers of the Environment (CCME, 2018) were used to compare the concentration values in order to determine compliance.

5.2 Determination of compliance limits for emerging contaminants

Data obtained from the emerging contaminants measurements were captured in an Excel spread sheet for the statistical analysis to be executed. For compliance, all the measurements of the ECs found in the water samples, except for one EC, were compared with the guidelines for the protection of aquatic ecosystems of the Canadian Council of Ministers of the Environment (CCME, 2018) and the European Union (EU, 2018) (Table 5.1). However, standards for the analyte terbuhylazine were not stipulated, therefore, the standard for drinking water specified by of the World Health Organisation (2013) was used. The descriptive statistics, including mean, median, standard deviation and compliance percentages, were calculated to summarise the water quality properties.

Table 0.1 Emerging contaminant guidelines for stream water

Emerging contaminant analyte	Proposed limit	Purpose of limit	Reference
Herbicides			
Atrazine	≤ 1.8µg/L	Aquatic ecosystem	CCME (2018)
Metolachlor	≤ 7.8µg/L	Aquatic ecosystem	CCME (2018)
Terbuthylazine	≤ 7µg/L	Drinking water	WHO (2013)
Pharmaceuticals			
Carbamazepine	≤ 10µg/L	Aquatic ecosystem	CCME (2018)
Estrone	≤ 0.4ng/L	Aquatic ecosystem	EU (2018)
Estradiol	≤ 0.4ng/L	Aquatic ecosystem	EU (2018)
17a-ethinylestradiol	≤ 0.035ng/L	Aquatic ecosystem	EU (2018)
Plasticiser			
Bisphenol A	≤ 1.4µg/L	Aquatic ecosystem	CCME (2018)

CCME= Canadian Council of Ministers of the Environment, WHO= World Health Organization, EU=European Union

5.3 Water quality assessment results

5.3.1 Herbicides

The concentration values for the three herbicides, namely atrazine, metolachlor and terbuthylazine measured at the 10 sampling sites were relatively similar. To determine if the concentration values were compliant with the standard limits, the concentration values for the terbuthylazine analyte were compared with the standard limit stipulated by WHO (WHO, 2013) and the concentration values for the atrazine and metolachlor analytes were compared with the limits specified by the CCME (CCME, 2018). This comparison revealed that all the concentration values were non-compliant (Table 5.2). Moreover, the concentration values for all three herbicides were lowest at sampling site S1, when compared with the concentration values at the other nine sampling sites.

Table 0.2 Herbicide concentration values (limit) and summary statistics for the sampling sites

Sampling site no.	Herbicide		
	Atrazine (≤1.8µg/L)[1]	Metolachlor (≤7.8µg/L)[1]	Terbuthylazine (≤7 µg/L)[2]
S1	28	16	32
S2	46	53	179
S3	40	39	87
S4	57	55	52
S5	40	44	116
S6	55	44	103
S8	97	80	181
S9	36	50	115
S10	68	68	153
S11	80	64	131
Mean	55	51	115

Median	51	52	116
Minimum	28	16	32
Maximum	97	80	181
SD	21,56	17,59	49,37
% Compliance	**0**	**0**	**0**

[1]= Standard limit according to CCME (2018); [2]= Standard limit according to WHO (2013), red colour= exceeded the limit

To obtain a better understanding of the pattern of the concentration values for the three herbicides at the 10 sampling sites, a histogram was constructed. The upstream sampling sites (i.e. S1, S2, S3, S4, S5 and S6) demonstrated similar low concentration values for all three herbicides (Figure 5.1). In contrast, high herbicide concentration values were recorded at the four downstream sampling sites, namely S8, S9, S10 and S11.

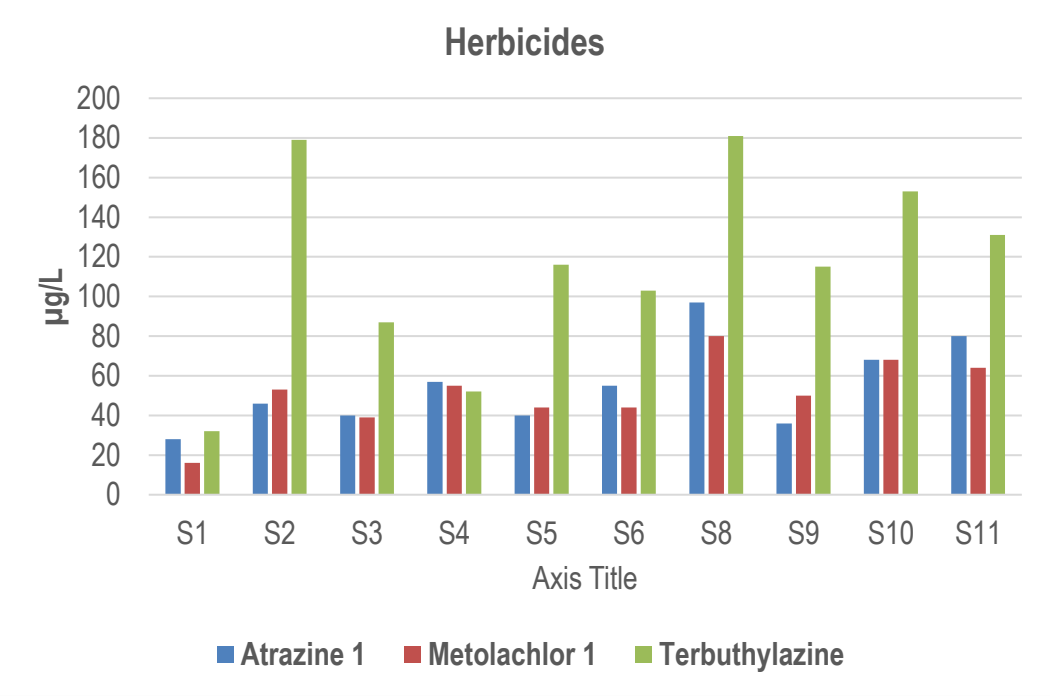


Figure 0.1 Histogram showing the concentration values for the three herbicides

5.3.2 Pharmaceuticals

The concentration values for three pharmaceuticals, namely carbamazepine, estrone and 17a-ethinylestradiol analytes, that were measured in this study were relatively high. Out of the four pharmaceuticals analysed, only one pharmaceutical, estradiol, demonstrated 100 % compliance when compared with the limit specified by the European Union (EU, 2018). Furthermore, when looking at the concentration values for all four pharmaceuticals, sampling site S10 demonstrated the lowest values overall (Table 5.3).

Table 0.3 Pharmaceutical concentration values and summary statistics for the sampling sites

Sampling site no.	Pharmaceuticals			
	Carbamazepine (≤10ng/L)[1]	Estrone (≤0.4 ng/L)[2]	Estradiol (≤0.4 ng/L)[2]	17a-ethinylestradiol (≤0.035ng/L)[2]
S1	244	40	0	338
S2	42	180	0	211
S3	101	10	0	158
S4	335	80	0	221
S5	147	70	0	171
S6	157	30	0	309
S8	221	240	0	277
S9	52	20	0	83,1
S10	54	0	0	66,9
S11	197	120	0	342
Mean	155	79	0	217,7
Median	152	55	0	216
Minimum	42	0	0	66,9
Maximum	335	240	0	342
SD	96,03	79,09	0	99,13

% Compliance	0	10	100%	0

[1] = Standard limit according to CCME (2018),[2] = European Union (2018), red colour= exceeded limit, carbamazepine=10^3, estrone=10^2, 17a- ethinylestradiol=10^4

A histogram was constructed to obtain a better understanding of the pattern of the concentration values for the four pharmaceuticals at the 10 sampling sites. In contrast to the herbicides, no pattern was demonstrated, however, the concentration values at sampling sites, S9 and S10, were the lowest when compare with the concentration values at the other sampling sites (Figure 5.2).

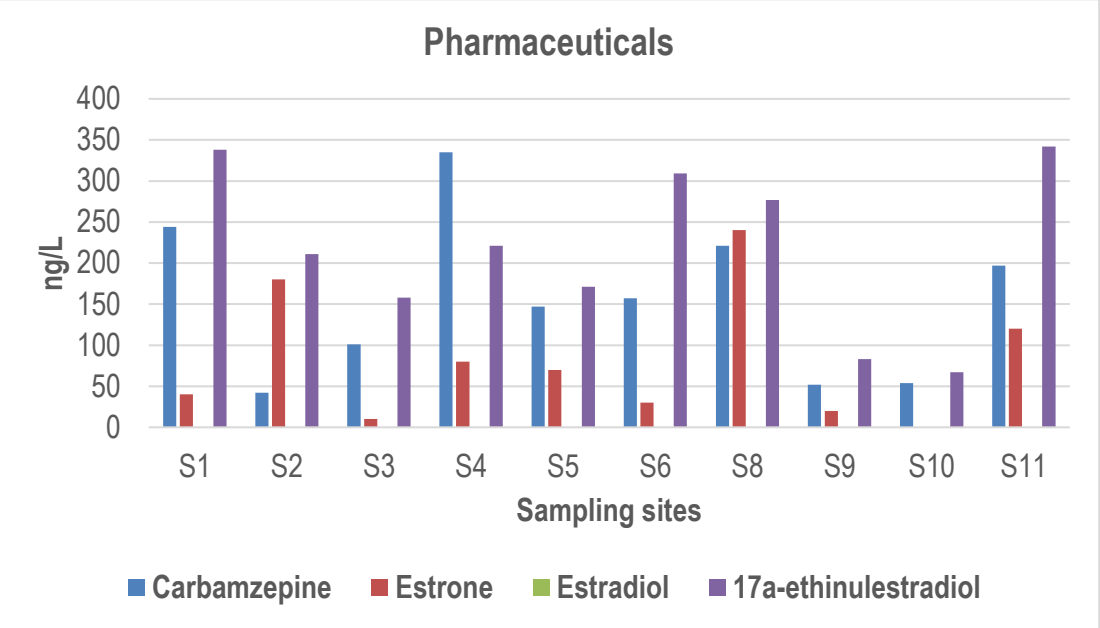


Figure 0.2 Histigram showing the concentration values for the pharmaceuticals at different sampling sites

5.3.3 Plasticiser

The concentration values for the bisphenol A analyte measured in this study were relatively high. All concentration values for bisphenol A were not complaint when compared with the limit specified by the CCME, (CCME, 2018) (Table 5.4). At four of the ten sampling sites, relatively high concentration values which exceeded 100µg/L were detected.

Table 0.4 Plasticiser concentration values and summary statistics for the sampling sites

Sampling site no.	Plasticiser
	Bisphenol A (≤1.4µg/L)[1]
S1	39
S2	113
S3	82
S4	51
S5	169
S6	86
S8	172
S9	54
S10	43
S11	199
Mean	100,8
Median	84
Minimum	39
Maximum	199
SD	59,50
% Compliance	**0**

[1]= Standard limit according to CCME (2018

A histogram of the concentration values for bisphenol A did not reveal any particular pattern. High concentration values for bisphenol A were detected at some upstream as well as downstream sampling sites (Figure 5.3). In particular, sampling sites S5, S8 and S11 stood out with substantially higher concentration values when compared with the concentration values at the other sampling sites (Figure 5.3)

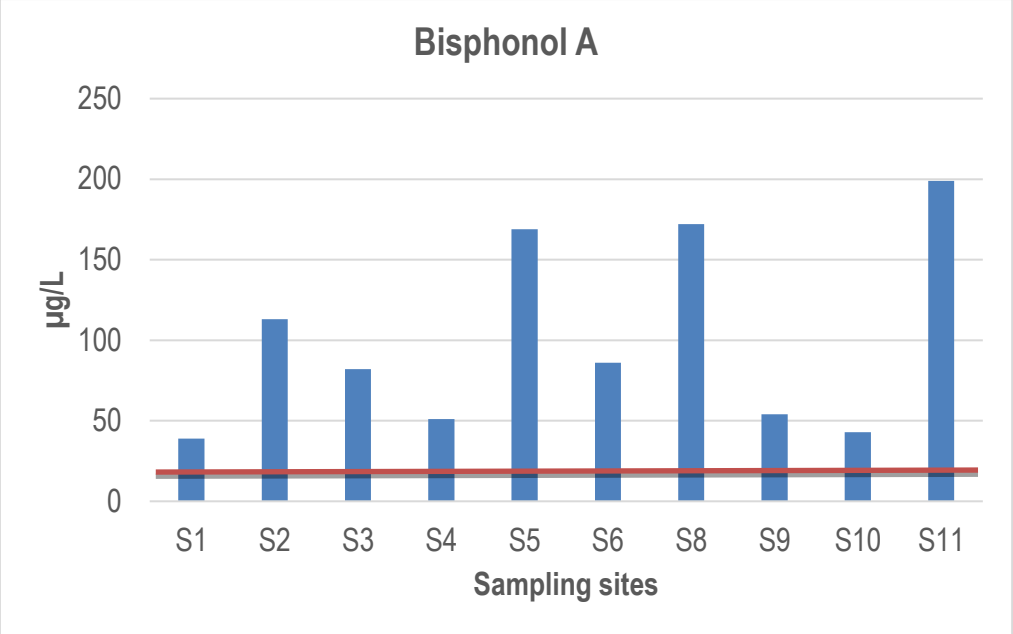


Figure 0.3 The pattern of the bisphenol A concentration values at the 10 sampling sites

5.4 Discussion

This study revealed that the ECs occurred at relatively high concentrations in the Bloemspruit and its tributaries, Fonteinspruit and Renosterspruit. Of the eight ECs studied, only estradiol demonstrated 100% compliance. The other ECs were 100% non-complaint, except for estrone that demonstrated only 10% compliance. Of interest is sampling site S8 that overall had the highest EC concentration values. This sampling site is located where the Bloemspruit stream flows through the property of the Kopano Nokeng Guest House before it flows into the Renosterspruit stream. The land surrounding sampling site S8 incorporates a number of plots where cabbage and other crops are cultivated as well as the guest house and its activities. These activities probably contributed to the relatively high concentration values for the herbicides, pharmaceuticals and plasticisers analysed in this study. Although only a few ECs were assessed in this study, the results strongly indicated that the Bloemspruit may very well be highly contaminated with many other ECs as well.

Chapter 6

Ecological quality of the Bloemspruit stream

6.1 Introduction

In this study, the impact of pollution on the aquatic ecosystem was determined by assessing the macroinvertebrate population and the macroinvertebrate habitat. Macroinvertebrates were enumerated and analysed in accordance with the SASS5 protocol to produce the SASS score, number of taxa and the ASPT scores for each of the nine sampling sites in the Bloemspruit and its tributaries. For the assessment of the macroinvertebrate habitat, the Index of Habitat Integrity (IHI) was used as the tool to quantify the impacts of disturbance factors that may adversely affect the water quality. These factors include water abstraction, flow regulation, bed and channel modification on the in-stream and riparian zones.

The SASS and ASPT scores were computed to determine the level of pollution and the diversity of macroinvertebrates according to the classification scoring system for the Highveld Eco-region (Dallas, 2007). However, for habitat integrity, the percentage scores calculated from the IHI were categorised using the description of the macroinvertebrate habitat rating system developed by Kleynhans *et al*. (2008).

6.2 Measurements and classification of the macroinvertebrate population

The SASS scoring system data was interpreted using guidelines to classify an overall analysis. The guideline uses a classification system that incorporates the SASS score together with the ASPT scores in order to calculate the condition of pollution and the diversity of the different macroinvertebrates and thereby categorise the ecological integrity of the stream (Ollis, 2005; Dallas,

2007). The interpretation of data also involved the use of biological bands generated from the reference for the Highveld Eco-region. This eco-region includes the classification and scoring system of biological bands from A – F (Table 6.1). The scoring systems were used to delineate the pollution condition and the diversity of macroinvertebrates at each site.

Table 0.1 Scoring system used to classify SASS and ASPT scores

SASS score	ASPT	BAND	Condition	Description
>124	>5.6	A	Unimpaired	High diversity of taxa with high sensitivity
82 – 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

6.3 Measurements of the macroinvertebrate habitat

When assessing the health status of the stream, analysis of the habitat integrity is of great concern in ecological studies (Ollis, 2005). Habitat integrity has emerged as one of the key issues to be considered when examining the health of an ecosystem. The habitat integrity refers to the maintenance of a balanced composition of physico-chemicals and habitat characteristics of a region (Kleynhans, 1996; Kleynhans *et al*., 2007a). It evaluates a range of major physical disturbances in the macroinvertebrate habitat, such as water abstraction and flow regulation as well as bed and channel modification, on both the in-stream zone and the riparian zone (Kleynhans *et al*., 2005). The in-stream zone represents the macro-invertebrate habitat in the current of the stream, while the riparian zone represents the macroinvertebrate habitat on the embankments (Kleynhans *et al*., 2008).

Determination of Index of Habitat Integrity

To measure the impacts of the disturbance factors at each sampling site, the IHI scoring sheet was used. The IHI scoring sheet includes the assessment of a number of criteria in the in-stream and riparian zones (Dallas, 2005). This system score is based on six descriptive class ratings which ranges from 0 – 25 (where 0 delineates zero to little impact on the habitat), 1 – 5 (few or small impact), 6 – 10 (moderate impact) 11 – 15 (large impact), 16 – 20 (serious impact) and lastly, the score of 20 – 25 demonstrates an adversely affected habitat. However, the original IHI scoring sheet listed some criteria that were not suitable for this study, such as the extent of inundation, presence of exotic aquatic fauna and presence of exotic aquatic macrophytes. Therefore, the modified Index of Habitat Integrity (mIHI) scoring sheet created by Belle (2015) was applied (Table 6.2). A modified mIHI is comprised of five criteria in the in-stream zone and fine criteria in the riparian zone (Table 6.2). Therefore, the percentage weight was adjusted to exclude the non-existing criteria in this study.

In this study, the proposed weight for each criterion was calculated in the following manner (Belle, 2015):

- Proposed weight of one criterion = $\frac{Original\ weight\ of\ one\ criterion}{Sum\ of\ the\ original\ weights\ of\ all\ criteria} \times 100$
- For example, for water quality, the proposed weight = $\frac{14}{113} \times 100 = 12$

Table 0.2 Example of a modified IHI scoring system to determine the disturbance factors on macro-invertebrates

Criterion	Score	Original weight (%)	Proposed weight (%)	Estimation of impact of criterion
In-stream zone				
Water abstraction e.g. pumps, irrigation, cultivated lands	4	14	12	1.92
Water quality: clarity, odour, presence of macrophytes, etc. due to untreated sewage, urban, and agricultural runoff	12	14	12	5.76
Flow modifications: relating to effects of abstraction or regulation by impoundments	5	7	7	1.4
Bed modification: indirect indications of sedimentation are stream bank and catchment erosion	6	13	12	2.88
Solid waste disposal	10	6	5	2
Riparian zone				
Water abstraction : presence of pumps, irrigation, etc.	2	13	12	0.96
Water quality: clarity, odour, presence of macrophytes etc.	13	13	12	6.24
Flow modifications: This shows the consequence of abstraction or regulation by impoundments	6	7	6	1.44
Channel modification :This results in change in flow which alters the in-stream and riparian habitat	3	12	11	1.32
Bank erosion	2	14	12	0.96

TOTAL		**113**	**100**	**24.88**

The calculation of the IHI score is based on three steps. This includes calculating the moderation of impact score for each criterion and calculating the estimation of impact of criterion before calculating the IHI score for each sampling site.

The IHI scores were calculated using the following formulas:

1. Calculate the moderation of impact score: Each criterion was moderated by multiplying the score with the proposed weight
   - Moderated score = assigned weight × proposed weight
   - An example for water abstraction criterion

     Moderated weight = 4 × 12
2. Calculate the estimated impact of criterion: Estimated impacted was calculated by dividing the moderated score of criterion by the maximum possible value of a score (25).
   - $\frac{Moderated\ score\ of\ criterion}{Maximum\ possible\ value\ of\ a\ score}$
   - An example for water abstraction $\frac{4 \times 12}{25}$ = 1.92
3. Calculating the IHI score: The sum of all estimated impact scores of all criteria expressed as a percentage
   - $\frac{Sum\ of\ all\ estimated\ impact\ scores}{Number\ of\ criteria} \times 100$
   - An example presented in Table 3.8, the total impact score was 24.88. The impact score is as a percentage, so the IHI is simply 100 minus the impact score
   - IHI score = 100 – 24.88= 75.12

Classification of the macroinvertebrate habitat

The interpretation of the mIHI scores was conducted using the rating system developed by Kleynhans *et al*. (2008) (Table 6.3). This system aims to describe the disturbance factors on the macroinvertebrate habitat ranging from small modifications to sampling sites that are critically or severely modified.

Table 0.3 Description of the macroinvertebrate habitat

Habitat integrity category	Condition	Description	Rating (% of the total)
A	Unmodified	Habitat is largely natural	90 – 100
B	Largely natural	The flow regime is only slightly modified and pollution is limited to sediment	80 – 89
C	Moderately modified	Loss and change of natural habitat and biota have occurred, but the basic ecosystem functions are unchanged.	60 – 79
D	Largely modified	Large loss of natural habitat, biota and basic ecosystem functions have occurred	40 – 59
E	Seriously modified	The loss of natural habitat, biota and basic ecosystem functions is extensive	20 –39
F	Critically/ Extremely modified	Modifications have reached a critical level and the system has been modified completely with an almost complete loss of natural habitat and biota.	0 – 19

6.4 Results of the impact of pollution on the macroinvertebrates

6.4.1 Analysis of the macroinvertebrate population data

The pollution sensitivity scores in this study were awarded to different macroinvertebrates per the SASS5protocol. Most of the macroinvertebrate families identified had relatively low sensitivity scores ranging from 1 – 5 (Table 6.4) which indicated that they are tolerant towards different stream conditions and to high levels of pollution. However, five of the macroinvertebrate taxa, namelyGomphidae, Ancylidae, Aeshnidae, Elmidae and Hydeaenidae, had sensitivity scores of over 6 (Table 6.4).

Table 0.4 Macroinvertebrates identified at nine sampling sites in this study.

Families	Sensitivity score	Families	Sensitivity score
ANNELIDA		**DIPTERA**	
Oligochaeta	1	Ceratopogonidae	5
Leeches	3	Chironomidae	2
EPHEMEROTERA		Culicidae	1
Baetidae	4	Muscidae	1
ODONATA		Psychodidae	1
Coenagrionida	4	Syrphidae	1
Aeshnidae	8		
Gomphidae	6	**GASTROPODA**	
Libelludae	4	Ancylidae	6
HEMIPTERA		Lymnaeidae	3
Belostomatidae	3	Physidae	3
Notonectidae	3	Planorbonae	3
COLEOPTERA			
Elmidae	8		
Dytiscidae	5		

Hydeaenidae	8		
Hydrophilidae	5		

A total of 23 macroinvertebrate families were identified at the nine macroinvertebrate sampling sites. The sampling sites S1 and S11 accounted for the highest number of macroinvertebrate families, while the sampling site S9 had the lowest number of families (Table 6.5). Moreover, the order Diptera was the most ubiquitous macroinvertebrate taxa when compared with the number of other families identified in this study (Table 6.5).

Table 0.5 Macroinvertebrate families identified at each macroinvertebrate sampling site

Site no.	S1	S2	S3	S5	S6	S8	S9	S10	S11
ANNELIDA									
Oligochaeta	1	2	4	3	3	1		8	5
Leeches	2		2	2	1				2
EPHEMEROPTERA									
Baetidae	3	2		5					
ODONATA									
Coenagrionida			2		1			3	
Aeshnidae	1	1		4					
Gomphidae	6								
Libelludae					5				1
HEMIPTERA									
Belostomatidae	4			1			2		
Notonectidae		2	1						
COLEPTERA									
Dytiscidae						4			
Elmidae		10			2				
Hydeaenidae	1								
Hydrophilidae		2		1					
DIPTERA									
Ceratopogonidae	3			6	6				
Chironomidae	6	8	14		8	3	24	16	12
Culicidae		2	3	5	6	1	16	4	5
Muscidae			3			1	3	3	2
Psychodidae	4		2			1	8	6	2
Syrphidae	2		3			2	2	4	1

GASTROPODA									
Ancylidae									4
Lymnaeidae					2	3			1
Physidae		1						4	4
Planorbonae					1			2	
Mean	3	3,3	3,8	3,4	3,5	2	9,2	5,6	3,5
Median	3	2	3	3,5	2,5	1	5,5	4	2
Range	5	9	13	5	7	3	22	14	11
Minimum	1	1	1	1	1	1	2	2	1
Maximum	6	10	14	6	8	4	24	16	12
SD	1,8	3,3	3,9	1,9	2,5	1,2	9	4,3	3,2
Count	11	9	9	8	10	8	6	9	11

6.4.2 Comparative analysis of the sampling biotopes

A comparative analysis was conducted on the macroinvertebrate biotopes to ascertain which biotope had the highest number of macroinvertebrates collected. The vegetation biotope had a relatively high number of 35 macroinvertebrates when compared with the number of macroinvertebrates collected from the other sampling biotopes (Table 6.6). However, an individual assessment demonstrated the highest number of macroinvertebrates sampled occurred in the stone biotope at sampling site S11 and I the vegetation biotope at sampling site S5 (Table 6.6).

Table 0.6 The number of macroinvertebrates collected from the different sampling biotopes

Sampling site no.	Biotopes		
	Stone	Vegetation	GSM
S1	3	6	2
S2	2	7	0
S3	2	3	4
S5	0	8	0
S6	5	3	2
S8	1	0	7
S9	1	5	0
S10	5	1	3
S11	8	2	1
Mean	3	4	2
Median	2	3	2
SD	2,5	2,8	2,3

SUM	27	35	19

To obtain a better understanding of the pattern of the macroinvertebrates sampled in the vegetation biotope at the nine sampling sites, a histogram was constructed. The pattern of the macroinvertebrates collected from the sampling sites along the Bloemspruit stream and its tributaries showed that five of the upstream sampling sites (S1, S2, S3, S5 and S6) demonstrated a relatively high number of macroinvertebrates collected from the vegetation biotope (Figure 6.1). However, at sampling site S8, no macroinvertebrates were present in the vegetation biotope (Figure 6.1)

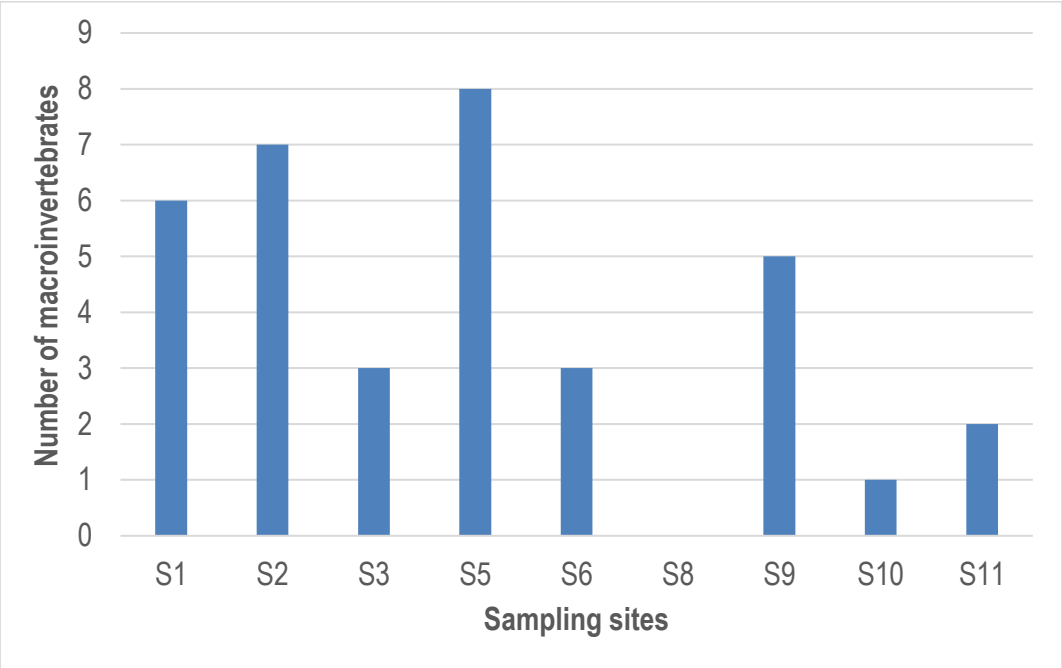


Figure 0.1 Pattern of the macroinvertebrates sampled in the vegetation biotope

6.4.3 Calculations of the SASS scores and ASPT scores

In this study, the highest SASS scores were recorded at sampling sites S1, S2, S5, S6 and S11 with the scores greater than the mean value of 24,9 (Table 6.7). For the number of taxa, relatively high numbers were recorded at sampling sites S1 and S11. However at sampling sites S2, S3 and S10,

the number of taxa was equivalent to the mean value of 9.The ASPT scores demonstrated that56% of the sampling sites obtained a score less than the mean value of 2, 7.

Table 0.7 SASS scores and ASPT scores for each of the sampling sites

Site no	SASS score	Number of taxa	ASPT
S1	42	11	3,8
S2	34	9	3,8
S3	17	9	1,9
S5	30	8	3,8
S6	34	10	3,4
S8	15	8	1,9
S9	9	6	1,5
S10	17	9	1,9
S11	26	11	2,6
Mean	24,9	9,0	2,7
Median	26,0	9,0	2,6
Minimum	9,0	6,0	1,5
Maximum	42,0	11,0	3,8
SD	11,0	1,6	1,0

SASS= South African Scoring System. ASPT= average score per taxa, SD, standard deviation

6.4.4 Classification of the SASS scores and ASPT scores

Data obtained from the calculation of the SASS scores and the ASPT scores were interpreted using the scoring system for the Highveld Eco-region created by Dallas and Day (2007). None of the sampling sites demonstrated an unimpaired condition. However, 44% of the sampling sites were

severely impaired (E) while the remaining 56% of the sampling sites were critically impaired (F) (Table 6.8).

Table 0.8 Classification of each sampling site using the SASS score and ASPT score

Site no	SASS score	ASPT	Condition	Class
S1	42	3,8	Severely impaired	E
S2	34	3,8	Severely impaired	E
S3	17	1,9	Critically impaired	F
S5	30	3,8	Severely impaired	E
S6	34	3,4	Severely impaired	E
S8	15	1,9	Critically impaired	F
S9	9	1,5	Critically impaired	F
S10	17	1,9	Critically impaired	F
S11	26	2,4	Critically impaired	F

SASS= South African Scoring System. ASPT= average score per taxa, red colour=critically impaired, orange colour=severely impaired

6.5 Results of the impact of pollution on the habitat

6.5.1 Analysis of the macroinvertebrate habitat

In this study, the macroinvertebrate habitats were analysed using a modified Index of Habitat Integrity (mIHI) created by Belle (2015). The mIHI scores demonstrated that 89% of the macroinvertebrate sampling sites had a moderately modified habitat while the remaining sampling site S9 demonstrated a largely modified macroinvertebrate habitat (Table 6.9).

Table 0.9 Index of Habitat Integrity at each macroinvertebrate sampling site

Site no	IHI score	Classification
S1	75	C
S2	78	C
S3	64	C
S5	77	C
S6	74	C
S8	61	C
S9	55	D
S10	65	C
S11	73	C

C= moderately modified macroinvertebrate habitat, D= largely modified macroinvertebrate habitat

6.6 Discussion

This study revealed that the ecological quality of the Bloemspruit and its tributaries, the Fonteinspruit and Renosterspruit is impaired. This was illustrated by the presence of mostly pollution-tolerant macroinvertebrates at most sampling sites. The low SASS scores and low ASPT scores also demonstrated a degradation of the water quality of the Bloemspruit and its tributaries. Furthermore, the majority of the sampling sites demonstrated a moderately modified macroinvertebrate habitat, where only the pollution-tolerant macroinvertebrates survive. A degraded ecological quality may lead to a declined ecosystem. Therefore, the aquatic organisms, animals and people that use the water are exposed to dangers if no precautions are taken will eventually die or be compromised.

Chapter 7

Discussion and conclusion

7.1 Introduction

This study was undertaken to investigate the quality of the Bloemspruit stream and its tributaries, the Fonteinspruit and Renosterspruit. The Bloemspruit stream was selected because it drains a large part of suburban Bloemfontein, such as Loch Logan. This area includes several industrial businesses, such as the waste water treatment plant (WWTP), formal settlements and other small business, such as cattle and sheep farming that dump effluent into the stream. The Bloemspruit tributary, the Fonteinspruit, was included because it receives runoff from the Batho and Heidedal locations. These areas are susceptible to the dangers of polluted water, particularly because the community relies solely on surface water for different domestic activities, such as for sanitary purposes. Pollution from these sources affects the quality of stream water, leading to a temporal and spatial degradation of the water quality. The macroinvertebrate population and macroinvertebrate habitat was also assessed to indicate the overall health and ecological quality of the stream (Ollis *et al*., 2006).

A Water Quality Index (WQI) is often used to describe the overall quality of water. WQI is a mathematical calculation computed to transform the measurements of the physical, chemical and microbiological properties of water into a single descriptive value (Ramakrishnaiah *et al.,* 2009; Kilgour *et al*., 2013). In this study, the Canadian Council of Ministers of the Environment Water Quality Index (CCMEWQI) (CCME, 2001) was used. The CCMEWQI percentages displayed categories of fair (highest 70%) and marginal (highest 64%) as well as a poor category of 35% which was recorded at sampling site S9, the confluence of the Bloemspruit and the Renosterspruit in the Kopano Nokeng area. The impact on pollution of the macroinvertebrate population and the

macroinvertebrate habitat also supports this outcome. The SASS scores and ASPT scores were relatively low ranging from 9 – 42 and 1.9 – 3.8 respectively. The Index of Habitat Integrity (IHI) scores confirmed to the deterioration of the water quality by revealing that most of the sampling sites were moderately modified ranging from 61 – 77, except for one sampling site which was largely modified with a score of 55%. These results pointed to a major loss of natural habitat and the functioning of an impaired ecosystem. Therefore, it may be concluded that the overall water quality of the Bloemspruit was relatively poor in all sampling rounds.

7.2 Impact of the water quality properties of the Bloemspruit stream

The physical properties mostly revealed measurements of compliance. Compliance for pH and temperature were 100% for all the sampling rounds, whereas EC was 91% compliant during sampling Round 4. However, turbidity demonstrated a relatively low compliance with values of 27% in Round 1, 36% in Round 2, 18% in Round 3 and 0% in Round 4. The highest turbidity measurements, ranging from 19.2 NTU to 170 NTU in sampling Round 4, were recorded after an episode of heavy rainfall. The rains may have possibly contributed to the elevated turbidity measurements due to runoff from the industrial, domestic and agricultural area as well as the WWTP effluent discharged into the streams in the vicinity of the sampling sites. Turbidity in surface water bodies has a direct impact on the ecology. High levels of turbidity can affect the ecological productivity and the habitat quality (USGS, 2020). Particles formed by turbidity also provide places for other pollutants, such as metals and bacteria to attach. Therefore, turbidity is an indicator of pollution in the stream.

Of the seven chemical water quality properties measured in this study, three properties demonstrated a relatively large number of measurements beyond the limits set by the US Environmental Protection Agency (US EPA, 2002). These properties included, nitrates, phosphates and dissolved oxygen. Additionally, only one measurement of ammonia was slightly above the limit

at sampling site S3 situated below the WWTP, while some measurements of the chemical oxygen demand were above the limit at a few sampling sites. When looking at the compliance values, only sulphate and total hardness were complaint for all sampling rounds. The phosphates measurements demonstrated the lowest compliance values of 9% in sampling Round 1 and 2, but 0% in sampling Round 3 and 4. When the measurements from all the rounds were compared, no significant differences were demonstrated for the chemical properties except for DO ($p \leq 0.05$).

Compared with the phosphate results recorded, the highest nitrate measurements were also recorded at sampling site S8 in the vicinity of the agricultural area. These nutrient measurements were probably as a consequence of runoff containing agricultural fertilisers and manure from the grass farming and animal feeds as well as sewage discharges from domestic households in the area. The presence of nitrates and phosphates in the water can also signify the existence of trace of emerging contaminants which may pose a threat to aquatic life (FAO, 2017; Gogoi *et al*., 2018). The exposure to high levels of nitrates is attributed to excessive growth of microscopic blue-green plants and algae, which produce cyanobacteria (DEAT, 2006; EU, 2013). This, however, has an adverse effect on the macroinvertebrates, such as fish and amphibians. Furthermore, accidental ingestion of polluted water with high levels of nitrates may contribute to a number of health issues such as methaemoglobinaemia and gastrointestinal disturbances in infants which may lead to brain damage (Ward *et al*., 2018).

In this study, most of the sampling rounds demonstrated low measurements of dissolved oxygen. The low levels of dissolved oxygen in this area are mostly attributed to algae growth caused by high levels of nitrates and phosphates from sewage and the effluent from the waste water treatment plant (Huang et al., 2017). Low levels of dissolved oxygen may contribute to a number of severe negative impacts on the aquatic ecosystem. These include disturbances, such as the physiological stress and

mortality of benthic fauna and fish as well as the loss of macroinvertebrate habitats (Zhang *et al*., 2017).

Two of the microbiological properties measured in this study, *E. coli* and total coliforms demonstrated exceptionally high measurements for all the sampling rounds. Compliance for coliforms was the lowest, ranging from 27% to 0%. *E.coli* and coliforms may originate from agricultural runoff containing animal faecal matter, septic and sewage effluents as well as some domestic waste (Shah *et al*., 2013).

In this study, relatively high concentration values for emerging contaminants were demonstrated at all sampling sites. Of the eight ECs studied, that is the herbicides, pharmaceuticals and plasticisers, only estradiol demonstrated 100% compliance at all the sampling sites. The highest concentration values for ECs were recorded at sampling site S8, where the Bloemspruit stream flows past the Kopano Nokeng Guest House before it flows into the Renosterspruit stream. These high levels of ECs may have originated from runoff containing agricultural fertilisers and domestic effluents containing detergents and other medical waste from the terrain surrounding the area (Meffe& Bustamante, 2014). Prolonged exposure to ECs can affect the liver and the reproductive system of benthic fauna, such as fish and the amphibians (Yang *et al*., 2017). Additionally, if humans come in constant contact with water containing ECs, they may be at risk of breast and ovary cancer and other conditions, such as gene alterations.

7.3 Impact of the Bloemspruit stream on the aquatic ecosystem

Several indexes made available to measure and classify the macroinvertebrate population revealed that the quality of the Bloemspruit was poor. This was evident by the absence of sampling sites that can be deemed acceptable to macroinvertebrates. Some of the sampling sites were severely impaired and more were critical impaired resulting in the presence of mostly pollution-tolerant

macroinvertebrates at most of the sampling sites. Moreover, the highest number of macroinvertebrates collected was from the vegetation biotope, making it easier for the macroinvertebrates to survive through the use of atmospheric oxygen with less exposure to organic substances in the water. Additionally, the presence of tolerant macroinvertebrate taxa belonging to the order of Deptera, such as Chironomidae, Culicidae andSyrphidae as well as Oligochaeta from the order of Annelida is indicative that the Bloemspruit stream and its tributaries are in a poor health. Further, the calculations of the relatively low SASS and ASPT scores supported the findings pertaining to the degradation of the water quality of the Bloemspruit and its tributaries.

Similar to the macroinvertebrate population, the macroinvertebrate habitat scores revealed modified macroinvertebrate habitats. A total of 89% of the macroinvertebrate habitats demonstrated a moderately modified macroinvertebrate habitat. However, only one sampling site, S9, revealed a largely modified macroinvertebrate habitat. It was therefore evident that only the pollution-tolerant macroinvertebrates can live in such habitats.

7.4 Conclusion

This study revealed that the overall health of the Bloemspruit stream is degraded. Several anthropogenic activities along the Bloemspruit and its tributaries have contaminated the water and affected the macroinvertebrate population and habitats. Therefore, humans and animals that use the water are at risk of contracting diseases.

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