Water Quality of the Bloemspruit stream in Mangaung, Free State Province, South Africa

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Declaration

Declaration of Independent Work

I, Belle Gladys Nyoh, identity number [redacted] and student number [redacted] do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree Magister Technologiae: Environmental Health, is my own independent work; and 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 in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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2015

I certify that the above statement is correct.

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Professor Annabel Fossey

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Abstract

Introduction: The increasing anthropogenic activities and the number of people living along the Bloemspruit stream have in recent time brought along extensive pollution of the stream. Such polluted water causes death of less tolerant aquatic organisms living in the stream, thus resulting in a decline of biological diversity of the stream. The polluted stream water also becomes a medium of transmission of various water-related diseases affecting humans and animals using the stream for various activities. An assessment of the water quality of the stream provided information about the extent of the deterioration and degradation of the stream.

Methodology: In this study, 12 sampling sites along the course of the stream and its tributaries were assessed for physical, chemical and microbiological properties. An ecological assessment of the stream was also conducted to provide an indication of stream deterioration and degradation. A number of indexes were used to determine the status of the stream's health. These included the calculation of a Water Quality Index (WQI), the South African Scoring System score (SASS), the Average Score per Taxon (ASPT), the modified Invertebrate Habitat Assessment System score (mIHAS), and the Index of Habitat Integrity score (IHI). To ascertain the overall quality of a particular sampling site, a qualitative assessment of all the sampling sites was performed, taking into account the quality of the macro-invertebrate communities, as well as the quality of the macro-invertebrate habitats.

Results and discussion: Findings from the study revealed that the quality of the water in the Bloemspruit stream is poor as evident by high turbidity, nitrate, phosphate; bacterial load as well as a low dissolved oxygen level outside the proposed Aquatic Water Quality Limits for Urban Streams (AWQUS) limits. WQI calculations also support this outcome. 17% of the sampling sites revealed poor water quality conditions while 25% displayed fair water quality and the remaining 58% displayed marginal water conditions. Additionally, the overall qualitative assessment also revealed good quality

conditions for only 25% of the sampling sites. 33% were classified as acceptable while the majority, 42%, were classified as poor. The SASS scores and ASPT values revealed that 19% of the sites sampled were severely impaired, with tolerant macro-invertebrate taxa present. The remainder 81% of the sites demonstrated critically impaired conditions with only few tolerant macro-invertebrate taxa present. The mIHAS scores indicated that only 17% of the sampling sites had good habitat conditions to support macro-invertebrates communities, while 25% displayed poor conditions which were too inadequate to support aquatic macro-invertebrate communities effectively. However, more than half, 58%, of the sites could only adequately support a diverse aquatic macro-invertebrate community. The IHI scores indicated that for all three seasons, 8.3% of the macro-invertebrate habitats had been largely modified by disturbance factors, while the remainder (91.7%) had been moderately modified, but the basic ecosystem functions are still unchanged.

Conclusion: The results indicated that the health of the Bloemspruit stream has been affected by its immediate environment, including informal settlements, extensive industrial activities as well as the waste water treatment plant (WWTP). Therefore, aquatic organisms are threatened, humans and animals that use the water are also at risk of contracting waterborne diseases.

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Abbreviations

AEV	Acute Effect Value
ANOVA	Analyses of variance
ASPT	Average Score per Taxon
AWQUS	Aquatic Water Quality Limits for Urban Streams
BCWQI	British Columbia Water Quality index
BI	Biotic Index
BMWP	British Monitoring Working Party
CCME	Canadian Council of Ministers of the Environment
CCME-WQI	Canadian Council of Ministers of the Environment's Water Quality Index
CEV	Chronic Effect Value
cfu	Colony forming unit
Cl	Chloride
CSIR	Council for Scientific and Industrial Research
DO	Dissolved oxygen
DS	Dissolved solids
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EC	Electrical conductivity
EPA	Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera and Tripchoptera

FSWQI	Florida Stream Water Quality Index
GSM	Gravel, sand and mud
HABS1	Habitat Score Version 1
HAM	Habitat Assessment Matrix
HQI	Habitat Quality Index
IHAS	Integrated Habitat Assessment
IHI	Index of Habitat Integrity
MDG	Millennium Development Goals
mIHAS	modified Invertebrates Habitat Assessment System
MPN	Most Probable Number
MUG	4-methyumbelliferyl-β-D-glucuronide
NGO	Non-Governmental Organisations
NH_3	Ammonia
NO_3	Nitrate
NSF-WQI	US National Sanitation Foundation's Water Quality Index
NTU	Nephelometric turbidity units
^oC	Degree Celcius
ONPG	θ-nitrophenyl-β-D-galactopyranoside
OWQI	Oregon Water Quality Index
PO_4	Phosphate
ppm	Parts per million

QAS	Quality Assessment Score
RHP	River Health Programme
SANS	South African National Standard
SASS	South African Scoring System
SASS4	South African Scoring System version 4
SASS5	South African Scoring System version 5
SAWQG	South African Water Quality Guidelines
SI	saprobic index
SIC	Stones in current
SOOC	Stones out of current
SS	Suspended solids
TBC	Total bacteria count
TDS	Total Dissolved Solids
TWQR	Target Water Quality Range
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultraviolet light
WHO	World Head Organisation
WQI	Water Quality Index
WWTP	Waste Water Treatment Plant
µS	Micro Siemens

Chapter 1

Introduction

1.1 Introduction

Water is a fundamental natural resource to humans, animals, plants and aquatic organisms. In general, water is used for agricultural, industrial and domestic purposes, as well as for recreational activities. Water provides a home and a wide range of support systems for animals, plants and aquatic organisms. In South Africa, the quality of fresh water is deteriorating, mostly because of ever growing human activities (Ashton, 2010; Oberholster et al., 2010). It is a major concern for government that, in the near future, the country will no longer be able to meet the demands for different water uses (Oberholster & Ashton, 2008).

Rivers are one of the most important fresh water resources in a country (Sarkar & Abbasi, 2006). Rivers provide a direct and readily available source of water for different purposes. Most of the rivers in South Africa, particularly in the Free State Province, are small and are referred to as streams. For example, in the Free State Province, many streams flow through urban areas since, historically, cities were built around these streams (Sarkar & Abbasi, 2006). The streams that are bound by urban areas have been useful to the inhabitants for many generations.

Streams provide humans with a ready source of water for drinking, for domestic purposes and serves as a source for food such as fish, clams, crayfish and other edible aquatic organisms. Streams also support agriculture an offer opportunities for recreation. Conversely, streams provide an easy means of disposing of solid waste and waste water from urban activities practiced along their banks (Mueller & Helsel, 2013). When pollution is relatively low, streams are able to dilute pollutants, thereby protecting the biodiversity living within and around a stream (Yan et al., 2012). However, with the growth of human populations living along streams, the range of anthropogenic activities also increases, resulting in an

increase in the amount of waste generated, which is often dumped in nearby streams (Deacon, 2009; Ruminaite, 2011). In instances where pollution is extensive, stream water quality may drop to such an extent that the quality of aquatic life and the health of users of such water are at risk of being affected by pollution (Ashton, 2010).

The Bloemspruit stream originates in the city of Bloemfontein, Free State Province of South Africa, and meanders through the city in concrete channels in an easterly direction towards the outskirts of the city. The main activities along the stream include cattle rearing, small scale crop farming and fishing. Industrial activities include food processing plants, breweries, car washing, train stations, petrol stations, an abattoir, and a waste water treatment plant (WWTP). The increasing anthropogenic activities and number of people living along the Bloemspruit stream has brought along, in recent time, extensive pollution of the stream (Scott & Watson, 2005).

Aquatic life, animals, and people who use the Bloemspruit water for domestic, recreational and agricultural activities are now becoming at risk of being exposed to dangers that polluted water might harbour. Humans that use this water may suffer from waterborne diseases such as cholera, dysentery, typhoid fever including skin and ear infections, irritations of eyes and mucous membranes (Momba et al., 2007). Humans may also contract fungal infections, pneumonia, or even tumours (Camargo & Alonso, 2006). Polluted water can also destroy certain tissues of animals (Rechenmacher et al., 2010). For example, animals that drank polluted water of the Sinos River in Brazil suffered from liver damage (Rechenmacher et al., 2010). On the other hand, when such polluted water is used for irrigation, the chemicals in the water often burn the irrigated vegetation (Camargo & Alonso, 2006). In addition, high levels of faecal coliforms can be found on vegetables irrigated with contaminated water, which can cause gastrointestinal diseases to farmers and consumers (Keraita et al., 2003; Oberholster, 2010).

Aquatic species may be negatively impacted as a result of the addition of contaminants, such as fertilisers, pesticides, nutrients and metals from urban runoff, industrial, agricultural and waste water

discharges (Scott & Watson, 2005). These contaminants degrade a stream's water quality and leads to problems such as eutrophication, acidification and salination (Lehman et al., 2004).

Eutrophication causes an abundant growth of algae in water. The excessive growth of algae depletes the necessary nutrients and oxygen needed for plants and animals, resulting in death of aerobic and other sensitive organisms in the water (Lehman et al., 2004; Mueller & Helsel, 2013). The death of aquatic organisms results in a reduction in the number of taxa of aquatic organisms which leads to a decline in the biological integrity of a stream (Le Roux, 2013). Eutrophication can also produce scum on the water surface, which produces a very unpleasant odour that may affect any recreational activities taking place in and around a stream (Van Ginkel, 2011). The negative impacts of stream pollution result in increased costs of treating water abstracted from a stream (DWA, 2009; Van Ginkel, 2011). In addition, the excessive algal blooms can clog filters and increase the cost of maintenance of equipment (Walmsley, 2000).

Nitrate and ammonia from agricultural activities cause acidification of streams (Camargo et al., 2005). Acidification results in the death of macro-invertebrates, fishes, amphibians and aquatic mammals in fresh water ecosystems (Petrin et al., 2008; Ashon, 2010). Irrigation, industrial discharges and dry land farming cause salts in rocks to be mobilised leading to leaching of salts into streams (Schulz, 2011). Salination may lead to extinction of salt sensitive species in streams and may also cause species which can tolerate high salinities to inhabit these streams (Schulz, 2011).

It appears, the quality of water in the Bloemspruit stream has been degraded, and thus may have adverse effects on aquatic organisms in the stream, human and animals that use the water, as well as irrigated vegetation. An assessment of the quality of water in the stream provides information about the extent of degradation of the stream.

1.2 Aims and objectives

The overall aim of the study was to assess the water quality of the Bloemspruit stream in Mangaung, Free State Province, South Africa. The water quality of the stream was assessed by measuring the physical, chemical and microbiological water quality properties. An ecological assessment of the stream was also conducted because macro-invertebrates living in the stream together with their habitats provide a good indication of stream deterioration, which might not be picked up by a chemical analysis (Masese et al., 2013).

To meet this aim, the following objectives were formulated:

- to scout and identify suitable sampling sites within the study area;
- to analyse the water quality in terms of physical, chemical, microbial properties;
- to analyse the ecological health status of the water;
- to develop an index that can be used to assess macro-invertebrate habitats;
- to calculate a number of indexes describing different aspects of stream health; and
- to identify the industrial and agricultural sources of pollution based on water quality assessment of the stream water.

1.3 Dissertation structure

1.3.1 Chapter 1: Introduction

This chapter provides the problem statement of the study; outlines the aims and objectives for conducting this study and ends with a breakdown of the chapters that make up the dissertation.

1.3.2 Chapter 2: Literature review

This chapter reviews previous studies on water quality and the ecological health status of a stream in

relation to the following aspects; the different sources of pollution in streams, possible effects of such polluted water on the aquatic ecosystems, humans and animals as well as the plants that are irrigated using polluted water. The chapter further explores the different measures that can be used to prevent pollution of water in streams. Different methods used to measure water quality and the ecological health of a stream is also critically reviewed. Tools used in South Africa to describe or evaluate the quality of water in a stream as well as the health status of a stream were also discussed. Lastly, gaps in the existing knowledge were identified and highlighted.

1.3.3 Chapter 3: Study design and sampling sites

This chapter describes the study area with a brief description of the different study sites visited in the study. It further describes the design of the study, which was divided into four phases; identification of sampling sites, data collection, data analysis and conclusions.

1.3.4 Chapter 4: Water quality

This chapter describes the sampling procedures used in collecting the water samples, and also provides a detailed description of the methods used to measure the properties on-site, as well as in the laboratory. The measurements and summary statistics of the water quality data (physical, chemical and microbiology) obtained are presented in this chapter. A description of the method used to calculate the water quality index is also provided. The chapter ends with the results and discussion of the major outcomes obtained from the study.

1.3.5 Chapter 5: Ecological quality

The South African scoring System (SASS version 5) method used to collect macro-invertebrates from their biotopes, as well as enumerating the macro-invertebrates in the laboratory has been described in this chapter. The chapter further includes a description of the procedure used to develop the modified

Invertebrate Habitat Assessment System (mIHAS) from the Integrated Habitat Assessment System (IHAS), which has been used in South Africa to collect macro-invertebrate habitat data in the stream. Additionally, the mIHAS procedure used to quantify the macro-invertebrate habitats within the stream was further discussed. A description of the Index of Habitat Integrity (IHI) method used to quantify the impact of disturbance factors on macro-invertebrate habitats was also presented. Furthermore, the chapter outlines an overall qualitative assessment of the sampling sites, which is a composite score that includes the different scores that describe the macro-invertebrate conditions as well as the habitat conditions. Different indexes used to classify macro-invertebrate data and macro-invertebrate habitat data obtained are also outlined. The chapter concludes with the results and discussion of the major findings from the chapter.

1.3.6 Chapter 6: Discussion and conclusions

This concluding chapter presents the key findings from the study and also integrates these findings into existing knowledge. The chapter further identifies potential polluters of the Bloemspruit stream as well as the effects of their pollutants on aquatic organisms, human health, animals and irrigated vegetation.

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

Chapter 2

Literature Review

2.1 Introduction

South Africa is generally a dry country with limited water resources. Most of its rivers are small with a low flow rate when compared to those of many other countries (DWAF, 2002a). For example, the Orange River, which is one of the largest rivers in South Africa, contains only about 10% of the water that flows in the Zambezi River (DWAF, 2002a). Furthermore, it can be argued that if the water of all the rivers in South Africa could be combined, this amount of water would be less than half that of the Zambezi River (DWA, 2012).

Even though most rivers in South Africa are small and have low flow rates for most of the year; this has not limited developments along many of the rivers. Such developments include urban and rural settlements, industrial activities, agriculture, irrigation and recreation. These anthropogenic activities have resulted in the abstraction of large volumes of water from these rivers and streams for different uses. Additionally, some of the large rivers such as the Orange and Limpopo found in South Africa are shared with other countries, which also use the water extensively (DWAF, 2002a; RHP, 2004). Therefore, many South African rivers are unsustainable for most of the year and are often referred to as streams.

The quality of water in most streams in South Africa has been impacted by various natural and anthropogenic forces (Morgan & Swathe, 2010; Oberholster, 2010). The joint influence of these forces has increased the level of pollution of many streams which may adversely affect the survival of sensitive aquatic organisms, as well as plants, animals and humans that use the water (Ashton, 2010). Such polluted water, which contains pathogenic micro-organisms, may become a medium for transmission of waterborne diseases such as diarrhoea, cholera and dysentery, to humans and animals

using the water. According to the World Health Organisation (WHO), poor water quality is the dominant cause of death in developing countries (DWAF, 1998).

2.2 Quality of stream water

The quality of water in a stream must be able to support and maintain healthy ecosystems. Ecosystems are delicate communities consisting of a variety of different organisms that interrelate with one another in an environment of specific physico-chemical properties (Barbour et al., 2000 cited in Barbour & Paul, 2010; Lui & Lui, 2009). To maintain stable aquatic ecosystems, a balance must exist between the living organisms in the water and their environment. When this balance is disturbed, ecosystems become at risk of destruction (Thirion, 2007). Therefore, streams containing water of a good quality are able to sustain healthy ecosystems, which consist of variety of animals, plants and micro-organisms (Thirion, 2007). But, in a situation where the quality of the water in streams deteriorates, it may result in the death of living organisms within the water, which causes the water system to become sterile (Thirion, 2007). In more advance stages of deterioration of the quality of the water, the water may no longer be suitable for human consumption, agriculture, irrigation and recreation (RHP, 2003a). Therefore, the quality of stream water can be determined by assessing the water quality properties as well as the ecological properties of the stream water.

2.3 Pollution of streams

2.3.1 Introduction

Pollution of surface water sources has become of great concern worldwide (Nikoo et al., 2011). Water becomes polluted through environmental events as well as anthropogenic activities happening in the vicinity of a stream (Kibena et al., 2014). Rainfall, which is one of the major environmental events, may increase the level of pollution in rivers and streams. Runoff generated after heavy rainfall, carry waste such as, plastics, papers, old cloths, faeces, sewage, and channel them into nearby streams. In

addition, runoff flowing through agricultural land also carries faeces and fertilisers into streams. Anthropogenic activities such as industrial businesses and waste water treatment plants (WWTP), may also release their effluents into the streams (Kibena et al., 2014). Many of these waste substances introduce micro-organisms, nutrients, toxic chemicals, toxins, sediments, metals, and pesticides into streams, which may in turn have detrimental impacts on aquatic organisms and ecosystems (Hatt et al., 2004).

2.3.2 Environmental contributions to water pollution

Rain

Rain and thunder storms generate large volumes of water, which flows over land carrying waste into streams. As water flows over the land it gathers large quantities of sediments, sand, rubble, solid waste, fertilisers, and chemicals from human settlements, agricultural areas and industries along the entire course of a stream (Ntengwe, 2006). Such substances introduced into a stream, increase the level of pollution of a stream (Ntengwe, 2006).

A rainfall event that occurred in December 1998 as well as between 2001 and 2002 in the Western Cape Province of South Africa produced runoff that transported large amounts of pesticides and sediments from nearby orchards, into the Lourens River and its tributaries (Schulz, 2001; Dabrowski et al., 2002 ; Thiere & Schulz, 2004). After the rainfall, the turbidity levels of the river was measured, and it was found to be higher than the prescribed standard for water quality in South Africa (DWAF, 1996a) as well as the standard established by the United States Environmental Protection Agency (US EPA) (Schulz, 2001; Dabrowski et al., 2002). In addition, other substances such as azinphos-methyl and prothiofos found in pesticides that can adversely affect macro-invertebrates had increased in one of the tributaries, and remained high for about three months after the storm, although no pesticide had been used at time of the storm (Schulz, 2001; Dabrowski et al., 2002; Thiere & Schulz, 2004). On the other

hand, faecal polluted runoff transported from the city of Bloemfontein in the Free State, to the Renosterspruit which is close to the city, resulted in increased levels of faecal organisms in the stream water (Griesel & Jagals, 2002). The water at that point close to the city was no longer suitable for the irrigation of crops (Griesel & Jagals, 2002). In addition, this water containing pathogens may cause waterborne diseases such as diarrhoea, cholera and dysentery to humans that use the water for domestic and recreational activities (Griesel & Jagals, 2002).

Wind

Wind is another environmental phenomenon that can transport light weight solid materials over very long distances into streams. The water quality of streams can thus be affected by these materials transported by wind. For example, typhoon Kammuri which occurred in the Wenchang and Wenjiao Estuary on the island of Hainan, in 2008 in China, caused and increase in the level of dissolved substances as well as well as suspended materials in the coastal waters (Herbeck et al., 2011). The nutrient levels in the waters also increased and remained high for more than two weeks after the typhoon (Herbeck et al., 2011). The typhoon also caused a reduction in the level of transparency of the coastal waters, as well as siltation and eutrophication, which in turn affected the normal functions of sea grass meadows and coral reefs within the coastal waters (Herbeck et al., 2011). Pollution of streams brought about by wind may therefore influence the survival of the many aquatic ecosystems sustained by streams (RHP, 2005).

Geology

The geology of the surrounding river drainage basin has been considered as one of the main natural factors that affect the quality of water in streams (DWA, 2004). Rocks present in the bed of water can slowly be dissolved by carbonic and sulphuric acids that are absorbed by rain from the atmosphere. The dissolved rocks increase the sediment load as well as alter the acidity of the water in streams (DWA, 2004). For instance, streams in Western Cape Province of South Africa, are particularly affected

by river bed rock composition. The streams flowing over the Table Mountain sandstones within the Fynbos catchment have become acidic, with pH values below four, because the streams drain carbon dioxide from the Fynbos soils which contain bed rocks that is rich in silica (Le Roux, 2013).

2.3.3 Anthropogenic contribution to water pollution

Pollution from agricultural activities

Deterioration of the quality of water of streams from agricultural activities is a global problem (Chidya et al., 2011). The use of toxic chemicals such as pesticides, herbicides and fertilisers in farming practices is increasing and has been found to pollute surrounding streams (Schulz, 2001; Kibena et al., 2014). In South Africa, it has been shown that pesticides are the most important agricultural pollutant affecting aquatic ecosystems (Dabrowski, 2002; Thiere & Schulz, 2004; Mensah et al., 2012). Additionally, fertilisers used in farming practices add nitrate and phosphate to streams, particularly through runoff (Oberholster et al., 2010). Phosphate and nitrate are very important nutrients for plant growth, but when these nutrients are found in excess in streams, it may result in eutrophication (O'Keefe & Day, 2006; Kiedrzy´nska et al., 2014). Runoff from irrigated agricultural areas in Mpumalanga Province South Africa, which flow into the Crocodile River, caused an increase in the level of nitrate and ammonia within the downstream sites of the river. The nitrate and ammonia levels were above the recommended limits of 0.3 mg/L and 0.03 mg/L (DWAF 1993 cited in Deksissa et al., 2004) as stipulated by the Department of Water Affairs in South Africa. The high levels of nitrate and ammonia, caused an increase in the level of eutrophication within the downstream sites that were sampled (Deksissa et al., 2004).

Feedlots and dairy farming also increase the level of pollution of streams in the vicinity, particularly during the rainy season. Cattle confined in feedlots generate large volumes of faeces and urine that can be washed by storm water runoff or leached into nearby streams. Faeces and urine of cattle are rich

sources of nitrate and ammonia, which are then introduced into streams. Also, waste water from dairy farms that is inadequately handled can further contribute to the pollution of the water of streams (Esterhuizen et al., 2012).

Pollution from waste water treatment plant effluents

Disposal of inadequately treated waste water from WWTP into streams increases the level of pollution of streams. Since most of the WWTPs in South Africa are poorly managed, often their effluents are inadequately treated and the effluent overflow from the treatment plants is washed into nearby streams (Oberholster, 2010). This practice may introduce pathogenic micro-organisms into the stream water (Dungeni & Momba, 2010). These streams may thus act as a major source of pathogens that can be transmitted to humans who drink the water or use it for recreation and other domestic activities. The effectiveness of the waste water treatment process at four WWTPs in Gauteng; Zeekoegat, Baviaanspoort, Rayton and Refilwe Water Care Works, revealed that the treatments were incomplete, because of the presence of *Cryptosporidium* oocysts and *Giardia* cysts in the effluent samples (Dungeni & Momba, 2010). In a similar study near Bloemfontein, it was found that the counts of *Escherichia coli* (*E. coli*) and *C. perfringens* at the confluence of the Bloemspruit stream and the Renosterspruit were increased because of effluent discharged into the Renosterspruit by the Sterkwater WWTP (Griesel, 2001).

Pollution from industrial discharges

Industrial discharges also have an impact on stream water quality. Industrial waste water effluent may contain many different chemicals such as ammonia (NH_3), arsenic (As), cadmium (Cd) and lead (Pb), which in turn may cause death of aquatic organisms and pose a health risk to humans and animals that use the water (DWAF, 1996a). Therefore, industrial effluents have been regarded as one of the most important factors that may lead to the deterioration of the quality of water in a stream (Hussain et al., 2011; Kiedrzy´nska et al., 2014). For example, the industrial waste water effluent from the Taloja

industrial belt in Mumbai, India, showed changes in the physico-chemical properties of the Kasardi River. These effluents polluted the water such that at the point of effluent discharge no vegetation or aquatic life could be found (Lokhande et al., 2012). In African countries, such as Nigeria and Malawi, industrial discharges have also been shown to affect the physio-chemical properties in streams, such that the water in the stream was no longer suitable for drinking, domestic activities and recreation (Phiri et al., 2005; Onojake et al., 2011; Osibanjo et al., 2011).

In South Africa, industries often discharge their effluent into neighbouring streams (Wepener et al., 2011). This has contributed to the deterioration of the quality of the water in a number of streams, which often results in the reduction of aquatic populations (Wepener et al., 2011). In Gauteng Province, industrial discharge into the Vaal River has reduced water quality to such an extent that many fish populations have shown dramatic decline (Wepener et al., 2011).

2.4 Effects of polluted stream water

2.4.1 Introduction

Polluted stream water may be detrimental to aquatic organisms, plants, humans and animals that depend on the water. Pollutants from natural sources and anthropogenic activities cause the deterioration of the quality of water in streams, which may in turn impact the survival of sensitive aquatic organisms (Anyona et al., 2014). Furthermore, water of poor quality, which contains pathogenic micro-organisms, may become a medium for transmission of waterborne diseases to humans and animals using the water.

2.4.2 Effects of polluted stream water on aquatic ecosystem

Eutrophication

Worldwide, eutrophication of streams has been identified as one of the most prevalent water quality problems (Walmsley, 2000; De Villiers & Thiart, 2007). The enrichment of streams, particularly by nitrate and phosphate, often result in eutrophication causing excessive growth of algae and macrophytes (De Villiers & Thiart, 2007). This results in the depletion of much needed dissolved oxygen and nutrients for aquatic organisms (Mueller & Helsel, 2013). The reduction of oxygen in the water leads to the decline of aerobic organism populations and many sensitive organisms living in the water (Lehman et al., 2004; Camargo & Alonso, 2006; Nyenje et al., 2010; Mueller & Helsel, 2013). Diminishing aquatic populations consequently cause changes in the community structure and the reduction in taxa richness, which ultimately results in the decline of the biological integrity of a stream (Scott & Watson, 2005; Couceiro et al., 2007; Oberholster et al., 2009 cited in Nyenje et al., 2010).

Severe instances of eutrophication may produce algal blooms and scums on water surfaces, which produce nasty odours (DWAF, 2002b). The scums and odours affect the aesthetic quality of the water and prevent it from being used for recreational activities (DWAF, 2002b). In circumstances where toxin producing blue green algae (cyanobacteria) are present, their toxins may adversely impact aquatic organism populations (Smith, 2003; Smith et al., 2006; Camargo & Alonso, 2006; Van Ginkel, 2011). In South Africa, high levels of metals such as, ammonium, chloride, nitrate, sulphates, phosphate, and fluorides in the Vaal River have caused eutrophication, thereby increasing algal blooms in the river (DWA, 2009). Because the Vaal River water is used for drinking water purposes, high levels of eutrophication have resulted in increased costs of water purification (DWA, 2009; Dzwairo & Otieno, 2012). Furthermore, excessive algal blooms often clog filters, thereby causing additional financial implications in maintenance costs of water treatment equipment (Walmsley, 2000; Smith, 2003).

Acidification and salination

Acidification and salination may also affect the aquatic organisms in the water. Increased levels of nitrate from agricultural activities cause acidification of water of a stream (Camargo & Alonso, 2006; Petrin et al., 2008). The lowering of the pH of the water may threaten the survival of many sensitive aquatic organisms causing ecosystems to become disrupted, which could ultimately result in their death (Camargo & Alonso, 2006). For example, the shellfish populations were severely affected by acidification of the coastal rivers in the USA (Salisbury et al., 2008). Salination, on the other hand, results from increases of the salt concentration in water bodies, because of the surrounding geological composition and anthropogenic activities (Western Cape Government, 2011; Cañedo-Argüelles et al., 2013). Irrigation, industrial discharges and dry land farming cause salts in rocks to be leached into streams (Schulz, 2011). High levels of salts in the water may prevent the growth of plants in the streams, decrease the numbers of salt sensitive species populations and favour the movement of alien species, which can tolerate high levels of salinity (Schulz, 2011; Cañedo-Argüelles et al., 2013).

2.4.3 Effects of polluted stream water on humans and animals

Since the water of most South African streams within urban surroundings are not used directly for drinking, they are mostly used for recreational activities, irrigation of crops and for other domestic activities (RHP, 2005). However, many street children and homeless people depend directly on stream water for their livelihood and are particularly vulnerable to the dangers of polluted water. Ingestion of polluted water by humans may result in a wide range of possible waterborne diseases. Waterborne diseases are caused by enteric pathogens present in faeces of infected mammals (Jagals, 2000). These pathogens, such as bacteria, viruses, and protozoa are introduced into stream water as a result of runoff which carries faeces from informal settlements, WWTP, as well as from leaking sewer lines. When humans ingest such polluted sewage water, they may suffer from diarrhoea, cholera, typhoid fever and dysentery (DWAF, 1996b; DWAF, 2002a; RHP, 2005).

Of all the waterborne diseases in humans, those presenting as diarrhoea are the leading cause of death in children (DWAF, 1998; Wenhold & Faber, 2009). The impact of waterborne diseases in South Africa is reflected in the number of affected people. Diarrhoea as a cause of death in children under the age of five has been ranked number three with 10 786 (10%) of the total number of deaths in 1999 - 2000 (Oberholster, 2010). At the Duzi Canoe Marathon in 2008, 40% of the people that were present in the stadium had contracted diarrhoea as a result of the consumption of water from the Umsunduzi River, which contained high faecal coliform counts of 115 000 per 100 mL of water, which was far above the limit of 150 per 100 mL for drinking in South Africa (Morgan & Swathe, 2008 cited in Morgan & Swathe, 2010). Other effects of contaminated water include skin rashes, throat and ear infections, irritations of eyes and mucous membrane when the skin is exposed to polluted water (DWAF, 1996b; RHP, 2005).

High levels of nitrate are often found in streams flowing through agricultural areas. Cattle framing and the application of fertilisers are particularly responsible for nitrate enrichment of stream water through runoff. Water containing high levels of nitrate may result in Methaemoglobinaemia in infants when consumed and some mucous membrane irritations in adults (DWAF, 1996b; Camargo et al., 2005). In addition, humans may suffer from cancer, endocrine disruption and other reproductive dysfunctions even if the water consumed contains relatively low levels of nitrate (Ward et al., 2005 as cited in Lassalettaa et al., 2009).

Polluted water also has adverse effects on animals. In many countries, farm livestock, wild animals, pets, fish and birds have died as a result of consuming polluted water (Holdsworth, 1991 cited in DWAF, 2002a). For example, dogs died after drinking water from the La Loue River contaminated with anatoxin-a in France (Guggera et al., 2005). In South Africa crocodiles died in the Olifants River as a result of increased pollution caused by anthropogenic activities (De Villiers & Mkwelo, 2009; Ashon, 2010).

2.4.4 Effects of polluted stream water on plants

Farmers in developing countries often use contaminated water from streams to irrigate vegetables. Such contaminated water deposits pathogens such as faecal coliforms onto irrigated vegetables (Gemmell & Schmidt, 2012). In African countries such as Nigeria and Ghana, high levels of faecal coliforms are often present on vegetables irrigated using water that originated from WWTPs (Keraita 2003; Okafo et al., 2003; Qadira et al., 2010). When contaminated vegetables are consumed, humans may suffer from gastrointestinal diseases (DWAF, 1996c). In Sobantu, South Africa, high levels of faecal coliforms were found on irrigated produce, as well as in the water used to irrigate the produce (Gemmell & Schmidt, 2012). Faecal coliform counts of 1.6×10^6 per 100 mL of irrigated water as well as counts of 1.6×10^5 per gram of irrigated produce where recorded, which exceeded limits for irrigation of raw produce as stipulated by the DWA (1996c), and Department of Health in South Africa (Gemmell & Schmidt, 2012).

Irrigation using water contaminated with heavy metals can also prevent germination and growth of plants. When heavy metals such as lead, zinc, chromium, nickel and mercury are present in water that is used for irrigation, the contaminated water can reduce the germination and growth of plants (Pandey et al., 2008). In India, the distillery effluent from the Mohan Meakin distillery plant was used to irrigate seeds and seedlings of rice and maize of a trial plot. The germination of the seeds was poor and the growth of seedlings retarded, even when the water was diluted with tap water (Pandey et al., 2008). The leaves present on some of the seedlings showed signs of nickel toxicity, making the effluent unsuitable for irrigation (Pandey et al., 2008). In addition, the distillery effluent may have also been responsible for adverse effects on aquatic life (Pandey et al., 2008).

2.5 Pollution prevention of stream water

2.5.1 Water pollution prevention legislation and guidelines

In South Africa, the supply of water of good quality is generally managed through the implementation of legislations and guidelines. A number of legislations have been put in place to facilitate the reduction of the pollution of streams. Section 24 of the National Constitution of South Africa (1996) stipulates that everyone has the right to an environment that is not harmful to their health and well-being (National Constitution, of South Africa, 1996). Therefore, according to the National Constitution of South Africa, no one has the right to pollute water sources in the country. In instances where land owners carry out activities that cause pollution, it is expected that these land owners should put measures in place that will either eliminate the source of pollution, or remedy its effects (National Water Act, No. 36 of 1998). Additionally, according to the National Environmental Act (No. 107 of 1998), the costs of pollution remediation is the responsibility of the polluter; supporting the 'polluter pays' principle.

To further ensure that surface water remains fit for use in a sustainable manner, the DWA developed the South African Water Quality Guidelines (SAWQG) for aquatic ecosystems (DWAF, 1996a). These guidelines specify for each water quality property of importance a Target Water Quality Range (TWQR), an Acute Effect Value (AEV) and a Chronic Effect Value (CEV). The TWQR stipulates the ideal range for a particular water quality property. The AEV provides the limit at which a particular property will cause significant health problems after a relatively short time of exposure, while exposure at the CEV limit may result in health problems after extended periods of exposure.

Other guidelines, such as the South African Water Quality Guidelines for recreational (DWA, 1996b), agricultural (DWAF, 1996c), industrial (DWAF, 1996d) and domestic uses (DWAF, 1996e) were developed to ensure that water of acceptable quality is available for different water users. More recently, a drinking water standard, namely the South African National Standard (SANS) was

developed in 2006 (SANS 241, 2006), which was updated in 2011 (SANS 241, 2011). The SANS 241 (2011) specifies the physical, chemical, microbiological and aesthetic properties of safe drinking water.

2.5.2 Water pollution prevention implementation mechanisms

In the social context, various initiatives have been launched to facilitate the protection of streams against polluting agents. Countries around the world have, for example, initiated pollution clean-up activities of streams (Sulaiman et al., 2014). Many of these clean-up activities involve the motivation and the participation of local communities. Non-Governmental Organisations (NGO) also engage school children in fun activities related to the protection of streams, which inspire the learners to become interested in taking care of streams in their environment (Sulaiman et al., 2014).

In South Africa, the DWA attempts to motivate local communities to participate in protecting and managing the water resources of the country (DWA, 2009). One of the initiatives of the DWA is the Adopt-a-River initiative (DWA, 2009). Through the Adopt-a-River initiative, water quality problems are identified, for example, the high salinity and dissolved solids levels, as well as eutrophication of the Vaal River were identified (DWA, 2009). These problems were then addressed using Adopt-a-River activities by involving the collaboration of local authorities, water services providers, industries and local community organisations (DWA, 2009).

Riparian wetlands are natural water purification mechanisms often found near streams. They are often located between agricultural areas and nearby streams and assist in absorbing pollutants such as nitrate, phosphate and organic pollutants in runoff, particularly from agricultural lands (Verhoeven et al., 2006). When pollutants in runoff are absorbed by the wetland vegetation, the pollutants are distributed by the wetland into the ecosystem, so that the pollutant level is reduced before the runoff finally enters a stream. Since riparian wetlands are effective in removing nutrients from water, artificial wetlands, known as ‘treatment wetlands’, have been created to reduce the levels of pollution in streams

(Verhoeven et al., 2006). For example, waste water from a hotel in Tanzania was purified by flowing through a nine compartment wetland planted with mangrove. This 'treatment wetland', successfully removed organic matter, nutrients and pathogens (Penha-Lopes et al., 2012).

2.6 Assessment of the quality of stream water

The Millennium Development Goals (MDG), Goal 7, stipulates that by 2015 the proportion of people without access to safe water sources should be halved (Stats SA, 2013). In South Africa, the DWA is responsible for implementing Goal 7, and particularly target 7C, which is related to access to save water sources. Working towards this target, one aspect addressed by the DWA is to evaluate and ensure that surface water, including stream water, is of good quality (Stats SA, 2013). A variety of properties are used to assess the quality of water in streams. These properties can be grouped into two main categories, namely, water quality properties and ecological quality properties of water.

2.6.1 Water quality properties of stream water

Stream water can be described by its suspended solids (SS) as well the dissolved solids (DS). Suspended solids of stream water may include; leaves, sticks, wood, plastics, papers, which are introduced into the stream as a result of runoff which carry substances from the surrounding stream area and deposit them into the stream (RHP, 2003a). DS, on the other hand, may consist of fine materials of inorganic and organic matter present in the water, which may include a variety of chemicals, metals, pesticides, as well as gases (RHP, 2005).

Under natural conditions, all streams contain a considerable amount of SS and DS substances. However, apart from runoff which increases both the SS and the DS in water, DS may further be increased in water as a result of anthropogenic factors such as, agricultural practices, industrial activities and WWTP (Bilotta & Brazier, 2008). In addition, the surrounding geological composition of the stream bed also influences the DS concentration in the stream (Bilotta & Brazier, 2008). Thus, the

presence of SS and DS in streams has a major effect on the physical, chemical and biological properties of stream water (Bilotta & Brazier, 2008).

Physical properties of stream water

SS and DS in stream water mostly affect the physical properties of turbidity, temperature, pH and electrical conductivity. Turbidity is a measure of the clarity of a body of water which determines the degree for light penetration into the water (CCME, 2008). The turbidity of water is increased by the presence of SS such as silts and debris, as well as by agricultural runoff and industrial and WWTP effluents. High turbidity levels may affect the survival of various aquatic animals in water. For example, the high levels of turbidity in the Lower Komati River of South Africa caused a decrease in the diversity of macro-invertebrates in the river (Dlamini et al., 2010). Furthermore, of all the measured properties, only turbidity revealed a significant relationship ($p < 0.05$) with the diversity of macro-invertebrates in this river (Dlamini et al., 2010). On the other hand, in a laboratory experiment, *Acartiella natalensis* found in the St Lucia estuary of South Africa was exposed to different levels of turbid water. Extreme levels of turbid water, in the order of 2 500 NTU, caused reduced feeding rates as well as high mortality rates of this copepod species. In contrast, at a low turbidity level of 500 NTU, very low mortality and feeding rates of this copepod was observed (Carrasco et al., 2013).

Highly turbid water may cause sensitive organisms to migrate to other locations with reduced turbidity, which may result in the reduction of the populations of these sensitive organisms. Within the Great Fish estuary in South Africa, high turbidity levels above 356 NTU caused the migration of the spotted grunter (*Pomadasy commersonnii*) to habitats with reduced turbidity (Childs et al., 2008). Such abrupt changes in turbidity within this estuarine environment caused a reduction in the population of the spotted grunter, as well as other fish species within the environment (Childs et al., 2008).

The temperature of water is an important property that may affect aquatic organisms living in a stream (Kleynhans et al., 2008). Suspended particles introduced into water by runoff, can absorb heat and

increase the water temperature (Farrell-Poe, 2000). Stream water temperatures can also be increased as a result of various anthropogenic activities such as, discharges of cooling water from power plants, loss of riparian vegetation, inter basin water transfer, and return flows from agriculture (CCME, 2008). Extreme high water temperatures may cause die-off of aquatic organisms (Airas et al., 2008). For example, the South African spotted grunter can only survive at temperatures between 16 to 30 ºC (Childs et al., 2008). Furthermore, because of climate change, streams and dams in the Kruger National Park have become warmer causing the extinction of numerous aquatic species, including fishes and crocodiles (Erasmus et al., 2002).

In the event where water temperatures are much lower than what aquatic organisms can tolerate, growth rates and motility of these organisms may become affected (Bogan et al., 2004). At extremely low water temperatures, a sediment toxicity test of contaminated sediments of the sawmill pool in Eastern Finland revealed reduced growth rate, reproduction and feeding habits of the aquatic Dipteran *Chironomus riparius* and Oligochaetan *Lumbriculus variegatus* (Airas et al., 2008). On the other hand, at water temperatures below 5ºC, a reduced motility of *Daphnia magna* and Japanese Medaka, *Oryzias latipes*, were observed in a constructed biomonitor (Chen et al., 2012).

An increase in the water temperature also increases the rate of chemical reactions in streams, which may in turn affect biological activities of aquatic organisms (Bogan et al., 2004). When the temperature of water increases, it decreases the ability of gasses such as oxygen, hydrogen, nitrogen and carbon dioxide to dissolve in the water (Bogan et al., 2004). In particular, the depletion of dissolved oxygen in water causes aerobic aquatic organisms to become stressed, which in turn may result in their death and ultimately in a decline in population numbers (Graham & Louw, 2008). For example, low dissolved oxygen levels in urban streams in Manaus, Brazil, caused a reduction in the diversity of macro-invertebrate taxa in the streams (Couceiro et al., 2007).

The presence of dissolved chemicals and metals introduced into streams affects the acidity and the alkalinity of the stream water. The pH of streams water is a measure of how acidic or alkaline the water is, which is usually determined by the presence of hydrogen and hydroxyl ions in the water. Water with less dissolved solids usually has a neutral or slightly alkaline pH (Jonnalagadda & Mhere, 2001). In contrast, effluents from industries such as the paper and pulp, tanning, and leather industries, decrease the pH of water in streams, causing the water to become more acidic (CCME, 2008). The acid drainage from the Panasqueeira mine tailings in China caused a substantial drop in pH of the Zezere River, through oxidation of sulphides, to a level below three (Candeias et al., 2013). Such acidic conditions may cause death to aquatic organisms. Acid draining into the Loskop Lake between 2003 and 2008 caused many fishes and crocodiles to die. By 2008, the number of crocodiles had reduced drastically from 30 to six (Paton, 2008 cited in Oberholster et al., 2010).

Increased dissolved solids in water can also affect the concentration of ions and salts in water. The concentration of ions in water, described as the electrical conductivity (EC) of water, is a measure of the total dissolved ions in water. The geology of the surrounding stream area may influence the EC of a stream. Streams that flow over granite rocks have low conductivity, since granite rocks contain materials which do not ionise in water (DWAF, 1996a; USGS, 2012). But streams that receive runoff containing clay particles may demonstrate relatively high levels of electrical conductivity, because clay has minerals that can ionise in water (DWAF, 1996a). Anthropogenic activities, such as the release of industrial effluents, agricultural runoff and spillage from WWTPs, may also add carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium ions to the water, resulting in an increase in the EC levels (CCME, 2008). Both increases and decreases of ions in water can affect the rate of the metabolism of aquatic organisms and may also affect the nutrient cycling process in the stream (CCME, 2008). Thus, changes in ion concentration in water could have a major effect on the survival and adaptation of aquatic species and ultimately cause changes in community structures and ecosystems (DWAF, 1996a).

Chemical properties of stream water

The presence of SS and DS in water affects the chemical composition of streams water. High nitrate levels in streams may originate from anthropogenic sources, such as agricultural runoff, fertilisers, WWTP effluent, industrial effluent and waste (Graham & Louw, 2008). Exposure to high levels of nitrate over a long time can affect fresh water macro-invertebrates, fishes and amphibians (Camargo et al., 2005). For example, an increased nitrate concentration from agricultural activities around Amala and Nyangores tributaries of the Mara River in Kenya resulted in a decline in the macro-invertebrate taxa diversity at downstream sites (Kilonzo et al., 2014). High levels of nitrate in streams may also cause abundant growth of water plants in streams, which may in turn pose adverse effects to macro-invertebrates (Sulaimen et al., 2014). The high level of nitrate in the Loskop Lake caused excessive growth of cyanobacterium microcystis bloom when compared to the lacustrine zone of the lake (Oberholster et al., 2010).

Natural sources of phosphate in streams may arise from weathering of rocks which causes phosphate salts from the rocks to be leached into streams (DWAF, 1996a). Anthropogenic sources of phosphate include runoff, which contains agricultural fertilisers, domestic and industrial effluents containing detergents as well as sewage discharges from WWTPs (Sulaiman et al., 2014). High levels of phosphate contribute to eutrophication, which may in turn have adverse effects on macro-invertebrates. For example in China, high levels of phosphate and nitrate in rivers reduced the diversity of macro-invertebrate families, leaving mostly dominant families such as Tubificidae, Chironomidae and Physidae (Duan et al., 2011).

Under natural conditions ammonia in streams may originate from the biological breakdown of nitrogenous matter. Other sources of ammonia in streams may include runoff from fertilised fields, effluent from fish farms, discharges from manufacturing and cleaning operations in industries (CCME, 2008). An increase in the level of ammonia in water is toxic to aquatic organisms. It may affect the

respiratory system; reduce hatching and growth rates of different aquatic organisms (CCME, 2008). If ammonia is present at a high concentration in water, it may diffuse into the bodies of fishes, and may affect their ionic balance (Eddy, 2003). Such changes subsequently result in convulsions and death of fishes (Eddy, 2003). Long term exposure to ammonia by fishes has been reported to reduce growth rates in fish and have also affected their endocrine systems (Spencer et al., 2008).

Oxygen dissolved in water is important for aerobic respiration in aquatic organisms. The presence of organic matter, fertilisers and suspended materials in water can reduce the concentration of dissolved oxygen in water (Farrell-Poe, 2000; Krumbein & Bellingham, 2010). Low levels of dissolved oxygen in water cause suffocation of aquatic organisms which may ultimately lead to death (Isenhart, 2008). For example, in the Kebena and Akaki rivers in Addis Ababa, Ethiopia, high levels of pollution of the rivers resulted in the reduction of diversity of macro-invertebrates and diatoms, particularly in areas with low dissolved oxygen levels (Beyene et al., 2009). On the other hand, in a study of the oxygen levels and macro-invertebrates biodiversity in the Lake Tana Sub-basin, Ethiopia, it was found that dissolved oxygen significantly impacted the macro-invertebrate biodiversity (Mehari et al., 2014). When aquatic organisms are exposed to low dissolved oxygen levels in water for an extensive time, it may lead to the death of fish and other aerobic organisms and result in ecosystem instability (Palmer et al., 2004; O'Keefe & Day, 2006).

Microbiological properties of stream water

Runoff produced after heavy storms carry faecal matter from informal settlements, WWTP as well as from agricultural areas, and dispose them into streams. This process may introduce pathogenic organisms such as faecal coliforms and *E. coli* into streams (Little et al., 2007; Kim et al., 2010). For example, runoff from Bloemfontein urban area introduced faecal matter into the Renosterspruit, which increased the levels of *E. coli, C. perfringens* as well somatic coliphages in the stream, to such an extent that the water was no longer suitable for irrigation (Griesel & Jagals, 2002). On the other hand,

disposal of inadequately treated sewage from WWTP may add pathogenic organisms such as *Cryptosporidium oocysts* into receiving water bodies (Dungeni & Momba, 2010). Such water may pose health effects such as diarrhoea to humans using the water for different activities (Dungeni & Momba, 2010).

2.6.2 Classification of water quality

Several approaches are used to describe the quality of water in streams. The first attempt to classify water quality was in 1848 in Germany (Steinhart et al., 1981 as cited in Lumb et al., 2011). This system, described as the saprobic index (SI), was based on the extent of pollution (Steinhart et al., 1981 as cited in Lumb et al., 2011). The SI classifies water according to different levels of pollution by indicating whether water is good or poor through the presence or absence of certain indicator organisms (Sarkar & Abbasi, 2006). However, it was found that this system was unreliable and did not provide a comprehensive analysis of water quality. More recently, standards or guidelines for surface water quality were developed (Debels et al., 2005; Kannel et al., 2007; Avvannavar & Shrihari, 2008 cited in Massoud, 2012). In South Africa, the DWA developed different standards or guidelines, which are used to describe the quality of surface water, which includes rivers and streams (DWAF, 1996a). These guidelines specify water quality limits of an acceptable quality for the different water uses. Since a number of water quality properties are used to describe water quality by comparing the values with standards or guideline limits, such comparisons are not easy to interpret making it difficult to conclude the overall quality of the water.

The utilisation of a water quality index (WQI) that expresses the water quality as a single value has gained popularity. A WQI integrates the measurements of a number of water quality properties and presents it as a single number score, which describes the overall quality of the water in a stream over space and time (Wepener et al., 1999; Said et al., 2004; Sarkar & Abbasi, 2006; Kannel et al., 2007; Nikoo et al., 2011; Bharti & Katyal, 2011; Sharma et al., 2013). The first WQI for surface water that

used physical, chemical and microbiological measurements was developed by Horton of the Ohio River Valley Water Sanitation Commission in the United States in 1965 (Horton, 1965). In developing the index, Horton attempted to reduce the cumbersomeness of the index by using only 10 of the most commonly used water quality properties in water quality assessments (Sarkar & Abbasi, 2006). However, the choice of property by Horton was subjective in nature and excluded a number of important properties (Sarkar & Abbasi, 2006; Lumb et al., 2011). To improve on the subjective nature of this index, Brown et al. (1970) developed a general water quality index, which used the Delphi opinion-based information gathering method to assign weights to individual properties (Sarkar & Abbasi, 2006; Bharti & Katyal, 2011).

After several years, a number of countries such as the United States of America, Canada and Malaysia modified the indexes suggested by Horton (1995) and Brown et al. (1970) and develop indexes that were more suited to their particular country (Said et al., 2004). For example, some of these indexes included the US National Sanitation Foundation's Water Quality Index (NSF-WQI) (Brown et al., 2001), Canadian Council of Ministers of the Environment's Water Quality Index (CCME-WQI) (2001), British Columbia Water Quality Index (BCWQI) (Zandbergen & Hall, 1998), Oregon Water Quality Index(OWQI) (Cude, 2001) and the Florida Stream Water Quality Index (FSWQI) (SAFE, 1995).

In South Africa, the use of a WQI to evaluate the quality of water in streams and rivers is in its rudimentary stage. One known index is the Aquatic Toxicity Index developed by Wepener et al. (1992) that was used to evaluate the quality of water in the Olifants River (Wepener et al., 1992). The index provided the toxic effects of a specific property on fish in cases where the threshold level for normal maintenance of aquatic life was exceeded (Wepener et al., 1992). A water quality index for biodiversity was also developed in South Africa. Because it is known that poor water quality results in a loss in biodiversity, this index was used to measure the water quality at different sites in the Orange River drainage basin to determine if the quality of the water was deteriorating (Carr & Rickwood, 2008). In the

Free State Province of South Africa, a pollution index for dairy farm borehole water quality was developed by Esterhuizen et al. (2012), which was used to evaluate the quality of borehole water in relation to drinking water quality standards.

The use of the physical, chemical and microbiological properties to describe the overall quality of stream water in South Africa is restricted (Roux, 1999; Dallas, 2000; Palmer et al., 2004). Because most sampling actions are undertaken on a monthly basis, peak and low events of water pollution is often neglected (Day, 2000; Palmer et al., 2004; Taylor et al., 2007). In addition, the sampling sites are usually selected based on their accessibility (Day, 2000). Since it is impossible to measure all the water quality properties, often, only a few are sampled (Palmer et al., 2004). Furthermore, often highly toxic chemicals may be present in small amounts that cannot be measured with the regular equipment used for water quality assessment (Day, 2000). It can therefore be concluded that the measurement of physico-chemical properties alone to assess the quality of water in streams is usually inadequate since it does not give a good indication of the impact of pollution on aquatic organisms (Roux et al., 1993; Roux, 1999; Dallas, 2000; Day, 2000; Farrell-Poe, 2000; RHP, 2004; Taylor et al., 2007; RHP, 2007). Physico-chemical measurements also do not provide an indication of the human impact, such as, flow alterations, channel modification and deterioration of habitat, which has an effect on the overall health status of a stream (Roux et al., 1993; Masese et al., 2013).

The need has thus been recognised that water quality studies should include ecological assessments to obtain a better understanding of the water quality and the overall health status of a stream (Dallas, 2000; Day, 2000; Palmer et al., 2004). The assessment of water quality using aquatic organisms as indicator organisms is important because these organisms are present in water throughout their aquatic life stages and as such provides a good indication of the deterioration of water quality (Rosenberg & Resh, 1993; Roux et al., 1993; Day, 2000; Todd & Roux, 2000; Gyedu-Ababio & Wyk, 2004; Palmer et al., 2004; RHP, 2004; Masese et al., 2013).

2.6.3 Ecological quality properties of stream water

The assessment of aquatic organisms in streams has become a popular approach to ascertain the overall health of streams and rivers in South Africa (Dickens & Graham, 2002; Western Cape Government, 2011; Masese et al., 2013). The overall health of a stream can be determined by enumerating living organisms such as macro-invertebrates, fish and algae in the stream. The study of the habitats inhabited by macro-invertebrates, such as riparian and in-stream vegetation, also contributes to such ecological assessments (Roux et al., 1993; RHP, 2007; Ollis, 2005).

The measurement of the health status of streams using aquatic organisms has motivated the development of more simple and rapid techniques that can be used to assess the health status of streams. These techniques, described as biotic indices, have been developed in USA, Canada, Australia, South Africa and some countries in Europe (Zhao & Yang, 2009).

The first index used to assess living organisms in rivers to determine the quality of water in the rivers was developed by Kolkwitz & Marsson (1909) and was called the “Saprobien” or Saprobic system (Kolkwitz & Marsson, 1909 cited in Day, 2000). The Saprobic system assesses the presence or absence of particular indicator organisms such as bacteria, algae, and protozoa, as well as certain benthic macro-invertebrates and fish whose tolerance to pollution has been determined (Sandin et al. 2001 cited in Ollis, 2005). From the different indicator organisms, the degree of organic pollution in a river can be determined (Ollis, 2005). A biotic index (BI), which is used to assess pollution of organic matter and its break down products in flowing rivers in South Africa, was developed by Chutter in 1972. This index also describes differences in faunal communities, which are found within clean streams and streams impacted by organic pollution (Chutter, 1972). This BI is limited in that organic pollution is not the only factor that could have a negative impact on aquatic organisms. Other substances such as poisons, pesticides, phenols and heavy metals could also kill some aquatic organisms, which may in turn provide a false BI value (Chutter, 1972). More so, changes in aquatic faunal composition may

occur as a result of several other factors such as river bed modification, flow abstraction and not only as a result of water quality (Chutter, 1972). The BI has not gained much popularity because of these limitations and because it is also rather labour intensive (Dickens & Graham, 2002).

More recently, improved biotic indices have been developed, which assess the ecological health of rivers based on the presence of macro-invertebrates (Ollis, 2005). In the 1990s the BI was revised using information from the British Monitoring Working Party (BMWP) to produce a faster and easier index called the South African Scoring System (SASS) (Chutter, 1990 cited in Dickens & Graham, 2002). SASS determines the degree of pollution of stream water by assessing the presence or absence of different indicator macro-invertebrates in the water (Ollis, 2005). The SASS method exploits the fact that some macro-invertebrates are more sensitive to pollution and changes in habitats than others (Dickens & Graham, 2002). So in cases of heavy pollution, very sensitive macro-invertebrates will disappear rapidly, while the less sensitive organisms will survive (Ollis, 2005). The SASS method has subsequently been widely accepted and used throughout South Africa and is constantly been revised and updated (Dickens & Graham, 2002).

It was recognised at the turn of the century that the latest version of the SASS method used at the time (version 4) had some limitations (Chutter, 1994 cited in Dickens & Graham, 2002). Certain macro-invertebrate taxa were omitted and the tolerance values of some of the taxa needed to be revised. In addition, the macro-invertebrates identified from different biotopes were sometimes combined in single tray during enumeration, while in other cases they were separated (Dickens & Graham, 2002). As a result of these limitations the SASS version in use at the time was updated to develop the latest version; version 5 (Dickens & Graham, 2002).

Although many different organisms are used to determine the health status of rivers and streams in South Africa, macro-invertebrates are the preferred organism (Resh, 2008; Odume & Muller, 2011). Macro-invertebrates are suitable indictors of stream health for several reasons: They are the most

sensitive to changes in water quality in aquatic ecosystems; they are also visible to the naked eye; they are easily identified; they have a short life span which is based on seasons; and they are rather immobile (Dickens & Graham, 2002; Bonada et al., 2006; Duan et al., 2011; Odume & Muller, 2011).

South African Scoring System

Currently in South Africa, version 5 of SASS is mostly in use to determine the health of streams (Dickens & Graham, 2002). This involves the enumeration of macro-invertebrates, the determination of their diversity, abundance and composition in streams. The SASS method provides information about which organisms are present in a stream, which in turn is indicative of the presence or absence of macro-invertebrates that are pollution sensitive or tolerant (Ollis, 2005). Thus, changes in the structure of aquatic macro-invertebrate communities indicate an overall change in the health of a stream (RHP, 2003a; RHP, 2004). Organic pollution of a number of rivers in China from anthropogenic activities has resulted in the reduction of the populations of collector-filters, including scrapers, shredders and predators that cannot survive in polluted waters (Duan et al., 2011). On the contrary, within these same rivers, the populations of collector-gatherers that can thrive in polluted waters remained reasonably high.

Although the assessment of macro-invertebrates within streams gives a good indication of the health status of a stream, it does not portray the actual cause of the problem or differentiate the sources of pollution (Day, 2000). Some of the problems may range from destruction in habitat as a result of flow alteration or certain structural damages (Palmer et al., 2004). Therefore, when assessing macro-invertebrates in a stream, macro-invertebrate habitat structures should also be assessed to ascertain if the absence of certain macro-invertebrates is a result of water quality or a result of other physical impacts (Ollis et al., 2006).

Invertebrate Habitat Assessment

The measurement of macro-invertebrate habitats forms an integral part of the assessment of the health or integrity of a stream's ecosystem (RHP, 2002; Maddock, 1999 cited in Ollis et al., 2006). The Invertebrate Habitat Assessment System (IHAS), developed by McMillan (1998), quantifies the presence and condition of macro-invertebrate habitats (RHP, 2002; Kleynhans et al., 2005). IHAS was developed to be used alongside SASS in order to aid the interpretation of SASS scores, since the quantity and quality of macro-invertebrate habitats determines the diversity, composition and abundance of macro-invertebrates within a given habitat (Dickens & Graham, 2002; Ollis et al., 2006). It can be assumed that because high SASS scores are obtained from reference sites, a linear relationship should exist between SASS scores and IHAS scores (Ollis, 2005; Ollis et al., 2006). Contrary to this assumption, for measurements made in three different rivers in the South Western Cape, no correlation could be established between SASS and IHAS scores for 67% of the measurements (Ollis, 2005; Ollis et al., 2006).

Index of Habitat Integrity

The Index of Habitat Integrity (IHI) is also an important tool used to determine the impact of modifications on macro-invertebrate habitats. This index quantifies the impact of human disturbance factors on macro-invertebrate habitats. Disturbance factors include water abstraction, flow regulation, bed and channel modifications on the macro-invertebrate habitats such as, pools, rapids, sandbanks, stones, on riverbed and vegetation on the river banks, at a sampling site (Kleynhans, 1996; RHP, 2002; RHP, 2003b; RHP, 2004; Kleynhans et al., 2008). For example, macro-invertebrate habitats within the upper ranges of the Waterval River, Vaal River catchment in South Africa, has been greatly degraded by anthropogenic activities such as mining, industry, agriculture, as well as urban and rural settlements (Gyedu-Ababio & Wyk, 2004). The impacts of these factors on the macro-invertebrate habitats were evident by the low SASS scores and low average score per taxa (Gyedu-Ababio & Wyk, 2004).

2.6.4 Classification of ecological quality

The regional reference condition approach is generally used in South Africa to classify the quality of an ecological system. This approach requires the identification of a regional reference condition. Such a reference is identified by selecting a number of physical, chemical and biological properties of a number of sites which are not degraded and used to produce a suitable reference condition for a particular region (Ollis, 2005). When the ecological quality of a stream is assessed, sample data are compared to a relevant reference condition (Ollis, 2005; Kleynhans et al., 2008). This approach reveals any form of deterioration or deviation of a stream from its natural condition when compared to a reference condition (Roux, 1999; Dallas, 2000). In cases where the streams are severely deteriorated, a best attainable site is used as the reference condition or the reference condition is obtained from historical data or ecological models (Dallas, 2000).

2.7 Conclusion

In South Africa, many rivers and stream are showing signs of deterioration and require attention. Severely degraded rivers and streams do not only impact the environment aesthetically, but also the living environment. Besides the decreasing numbers of aquatic organisms and species, anthropogenic activities are also restricted, such as recreational activities, irrigation and the use of water for domestic use by informal dwellers.

Chapter 3

Study Design and Sampling Sites

3.1 Introduction

This study was carried out to assess the water quality of the Bloemspruit stream in Mangaung, Free State Province, South Africa. The water quality of the stream was assessed by measuring physical, chemical and microbiological water quality properties. An ecological assessment of the stream was also conducted because macro-invertebrates living in the stream, including their habitats, provide an indication of degradation of a stream, which might not be evident when only chemical analyses are performed (Dickens & Graham, 2002).

3.2 Study area

The Bloemspruit stream forms part of the Modder River catchment, and is located in the eastern part of the Bloemfontein urban area. The Bloemspruit stream is a narrow and medium flow stream that receives runoff from the Bloemfontein urban area via a municipal drainage system chanels (Scott & Watson, 2005). For most of the year, the flow of the stream is very shallow; however there are often floods during the rainy season and increased flows are encountered. The stream is also fed by small tributaries, particularly the Fonteinspruit that drains the Batho, Heidedal and Oos-einde settlements, as well as small holdings along its course (Figure 3.1). The Bloemspruit stream also joins another tributary a few kilometres from the Bloemfontein industrial area called the Renosterspruit (Figure 3.1). After the confluence with the Renosterspruit, the stream is known as the Renosterspruit, which runs downstream into the Modder River (Figure 3.1). The study area extended from the Bloemfontein industrial area after the train station up to 1 km after the confluence of the Bloemspruit stream and Renosterspruit, which united to become the Renosterspruit (Figure 3.1).

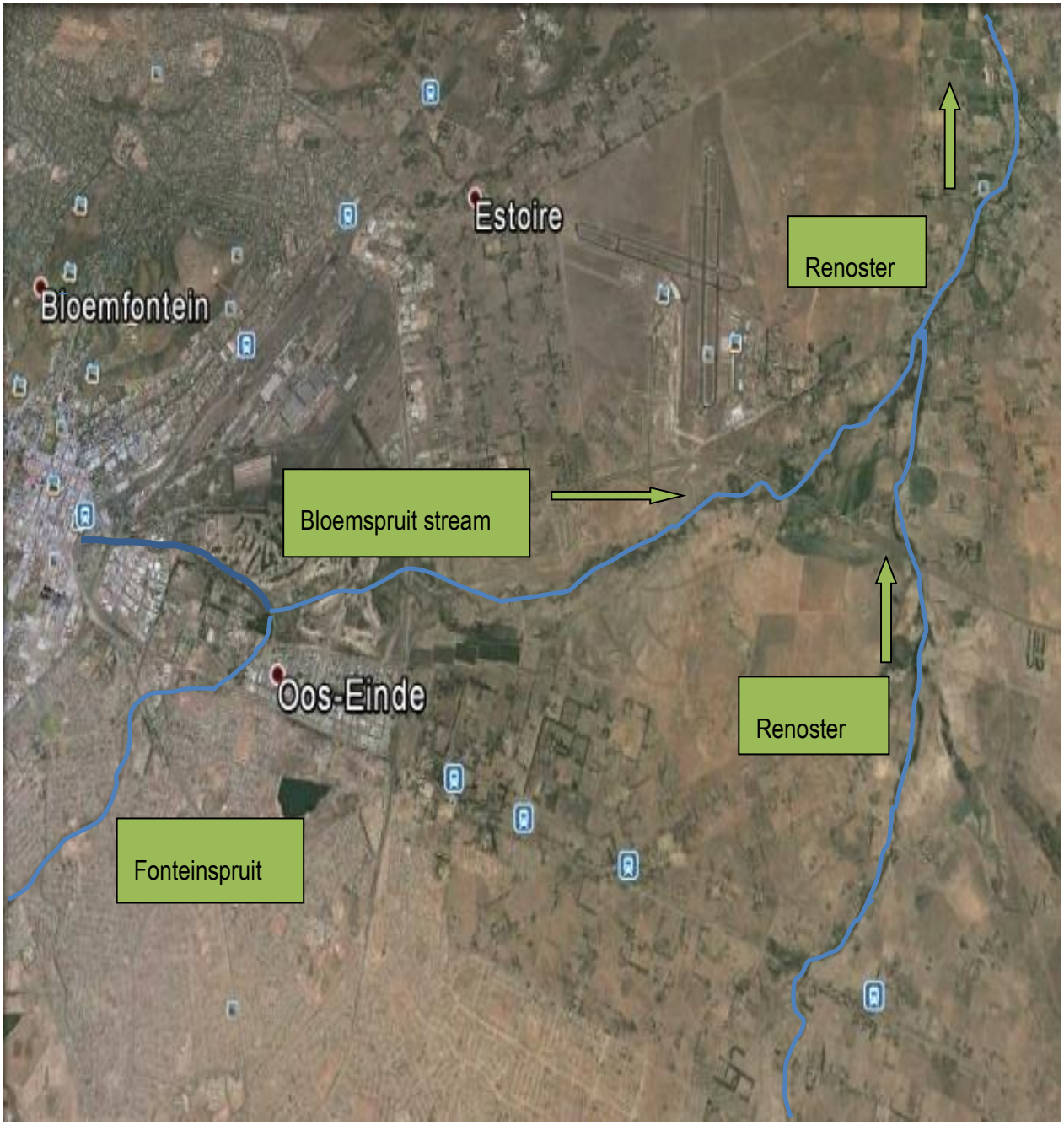


Figure 3.1 Study area of the Bloemspruit stream, tributaries and settlements around the area (adapted from Google Earth Maps, 2014)

Twelve sampling sites were identified and sampled in the study area (Figure 3.2). Each of the sampling sites was selected based on the activities, as well as the polluting agents identified in the area. Such polluting agents included: cattle rearing, small scale crop farming and fishing, industrial activities, an abattoir, a golf course and a waste water treatment plant (WWTP) (Figure 3.2).

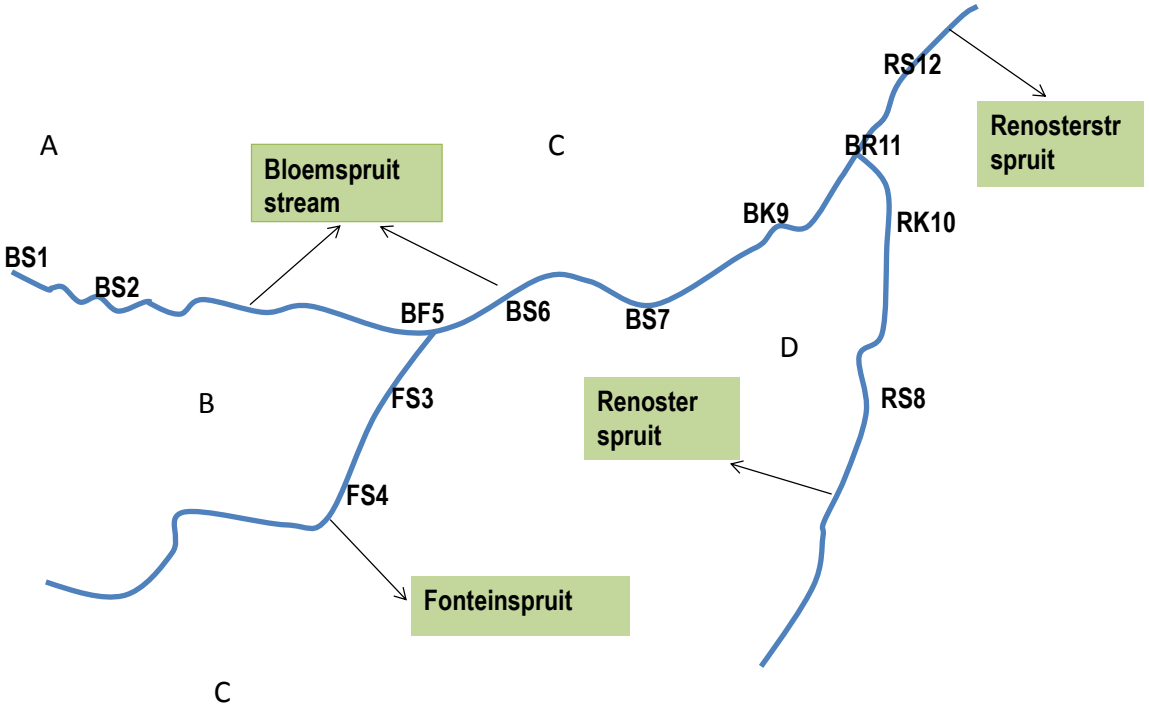


Figure 3.2 Study area of the Bloemspruit stream, sampling sites, tributaries and main activities around the area (A = Bloemfontein industrial area include food processing plants, breweries, petrol stations and car wash; B = WWTP; C = Heidedal industrial area; D = cattle farm; E = events centre)

Supplementary data were also recorded at the twelve sampling sites. These included a description of the general area, coordinates, details of the sampling site and the reason why the sampling site was selected (Table 3.1).

Table 3.1 Sampling sites, references, description of sites and motivation for the choice of the site

Sampling Sites	Area	Coordinates	Description of vicinity	Motivation for choice of site
BS1	Industrial area	29°07'13.35"S 26°13'49.20"E	Part of the Bloemspruit stream situated on Marula Street, under a bridge.	This site was sampled, because this area receives pollutants carried by runoff from the general Bloemfontein urban and industrial area.
BS2	Industrial area	29°07'13.56"S 26°14'09.68"E	Part of the Bloemspruit stream situated on Pine Street next to Laferage plant.	This area receives pollutants from the industrial area and the Buitesig settlement. Sampling at this site isolates the effect of pollutants from this settlement on the stream.
FS3	Batho area	29°07'38.46"S 26°15'04.74"E	Part of the Fonteinspruit located below the Tau Pele WWTP. Pipeline transporting effluent from WWTP cross at this point. Situated next to Mangaung sorghum deport plant.	Leakage of WWTP effluents conveyed in pipes usually occurs at this site. This point was sampled to determine the effect of the Fonteinspruit and the WWTP on the Bloemspruit stream.
FS4	Batho area	29°07'46.85"S 26°14'45.96"E	Part of Fonteinspruit situated below Batho and Heidedal settlements.	This site was sampled to isolate the impact of Fonteinspruit on the Bloemspruit stream.
BF5	WWTP area	29°07'32.24"S 26°15'06.81"E	Confluence of the Bloemspruit stream and Fonteinspruit. Located below Tau Pele WWTP and behind the Bloemfontein Golf Course.	This site was sampled to ascertain which area has a greater impact on the Bloemspruit stream: WWTP, or the Batho area.
BS6	WWTP area	29°07'20.08"S 26°16'02.44"E	Part of Bloemspruit stream located on M10, under a bridge.	This site was sampled to isolate the effect of the WWTP on the Bloemspruit stream.
BS7	Cattle farm area	29°06'39.36"S. 26°18'50.55"E	Part of Bloemspruit stream located on N8, next to a cattle farm.	This site was sampled to ascertain the effect of the cattle farm on the Bloemspruit stream.

RS8	Cattle farm area	29°06'58.27''S 26°19'41.97''E	Part of the Renosterspruit on N8, running through a farm.	This site was sampled to determine if the water quality of the Renosterspruit may have an impact on the Bloemspruit stream.
BK9	Kopano Nokeng area	29°05'58.13''S 26°19'50.90''E	Part of the Bloemspruit stream located next to the Kopano Nokeng area.	This site was sampled to determine the amount of pollutants still present in the Bloemspruit stream from industrial areas, WWTP, Informal settlements and cattle farm.
RK10	Kopano Nokeng area	29°05'55.65''S 26°19'50.58''E	Part of the Renosterspruit located next to the Kopano Nokeng area.	This site was sampled to isolate the impact of the water quality of the Renosterspruit stream on the Bloemspruit stream.
BR11	Kopano Nokeng area	29°05'49.05''S 26°19'54.15''E	Confluence of the Bloemspruit stream and Renosterspruit.	This site was sampled to assess the change in properties of the water at the confluence of the Bloemspruit stream and the Renosterspruit in order to determine which stream has an impact on the other.
RS12	Kopano Nokeng area	29°04'25.68''S 26°20'44.51''E	Renoster situated close to Indaba. About 1 km after the confluence of Renosterspruit and the Bloemspruit stream.	This site was sampled to determine the amount of pollutants still present in the stream after the Bloemspruit stream and the Renosterspruit confluence.

BS = Bloemspruit stream; F= Fonteinspruit; R = Renosterspruit; I = industrial area; W = WWTP; B = Batho area; C = cattle farm area; K = Kopano Nokeng area

Photographs were taken of all the sampling sites during the first, second and third sampling seasons. Figure 3.3 demonstrates the diverse nature of the sampling sites.

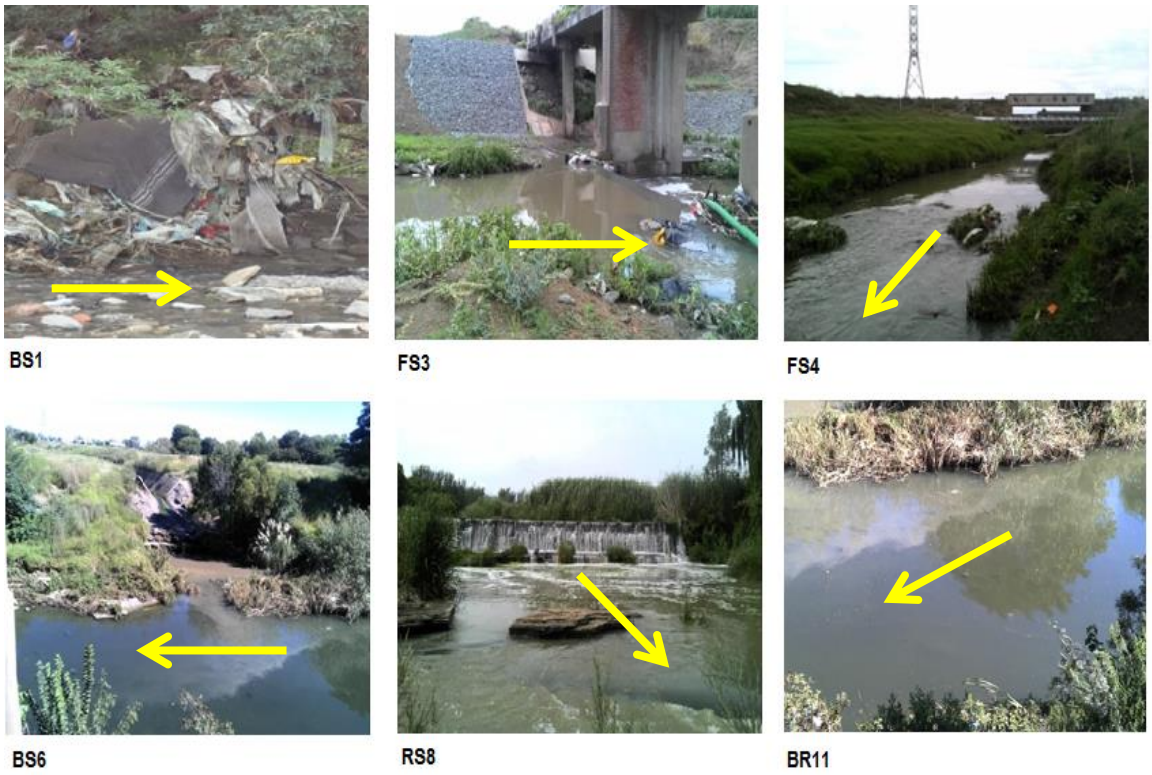


Figure 3.3 Diverse nature of the sampling sites; BS1 shows pollution of solid waste from informal settlements; FS3 is a sampling site under a bridge and where the WWTP effluent pipe crosses at this point; FS4 is a site at Fonteinspruit where a sewage pipe transporting sewage from Heidedal crosses; BS6 shows WWTP effluent being discharged into the Bloemspruit stream; RS8 shows a damp wall at the Renosterspruit stream that increases turbulence; and BR11 shows the confluence of Renosterspruit and the Bloemspruit stream.

3.3 Study design

The Bloemspruit stream, including its tributaries (Fonteinspruit and Renosterspruit), were visited to identify and select suitable sampling sites within the study area. The sampling sites covered the extent of the study area and were positioned downstream from potential polluting agents. Twelve sampling sites were selected for analysis.

Water and ecological samples were collected during three seasons, approximately three months apart, to ascertain whether seasonal effects existed. The seasons were: December to January (summer sampling), March to April (autumn sampling) as well as June to July, which represented the winter sampling season. The study was conducted in three phases which included: the identification of sampling sites, data collection and data analysis (Figure 3.4).

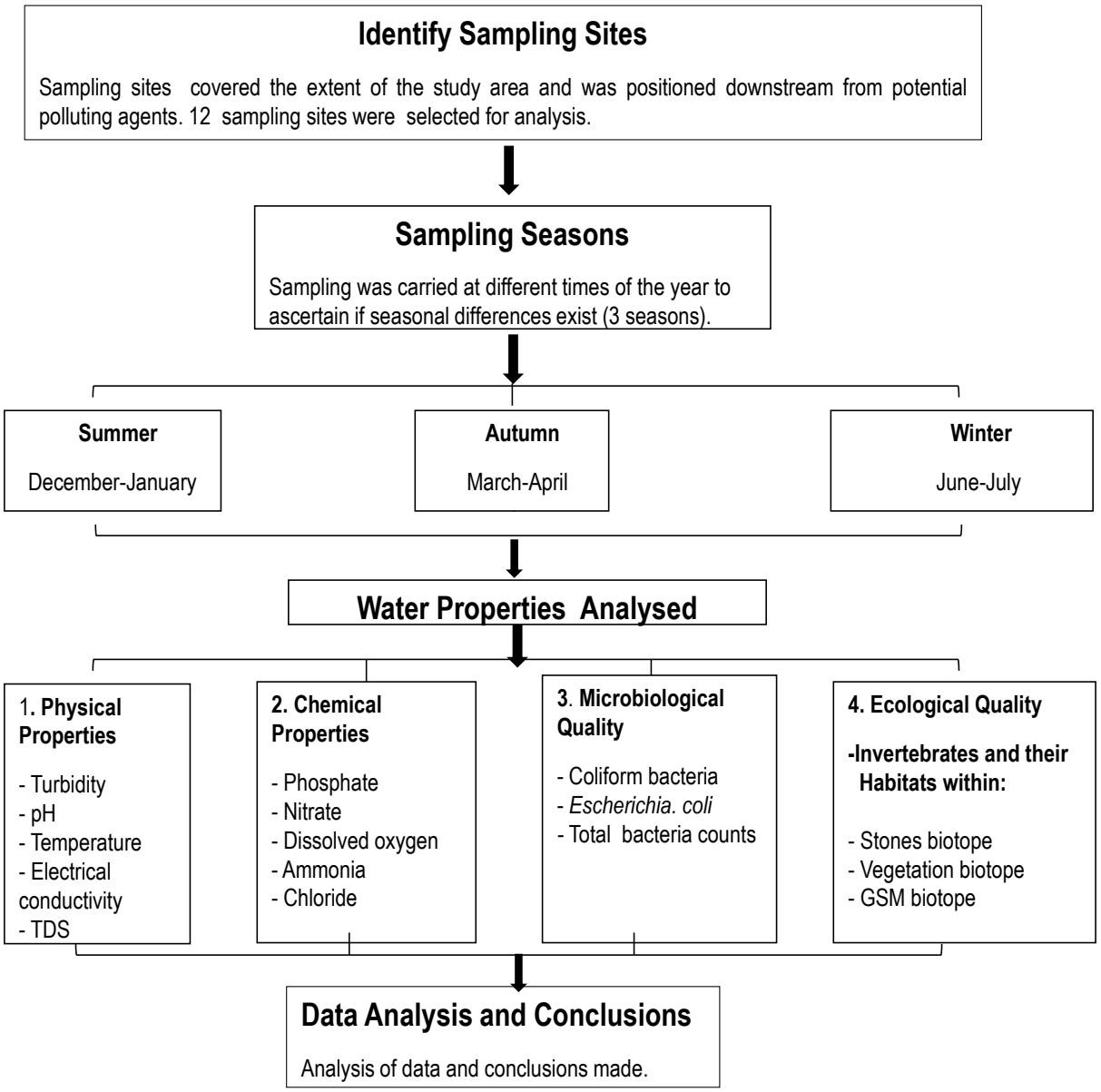


Figure 3.4 Experimental design of the study

Water samples were collected to analyse the physical, chemical and microbiological water quality properties. Four physical properties were analysed on-site: pH, temperature, electrical conductivity (EC) and turbidity. Additionally, total dissolve solids (TDS), in mg/L, were calculated by multiplying the EC in µS/cm by the factor 0.67 (EPA, 2001). Temperature and pH were analysed because they both affect different biological processes within the streams, as well various chemical reactions (Kleynhans et al., 2008). For example, many biological reactions will only take place within narrow pH and temperature ranges (Graham & Louw, 2008; De Kock & Esterhuizen, 2013). The EC of water determines the concentration of dissolved ions and salts in water. Measuring EC provides information about the suitability of water for different uses (industrial, agricultural, domestic and recreational). Turbidity was analysed because it indicates the condition and how productive the system is (De Kock & Esterhuizen, 2013). For example, very murky water prevents light from penetrating into the lower parts, which may affect the survival of water plants and many aquatic species (O'Keefe & Day, 2006; Graham & Louw, 2008). Dissolved oxygen (DO) and chloride were also analysed on-site. DO also facilitate the determination of pollution status of water, since most aquatic organisms depend on oxygen dissolved in water (Graham & Louw, 2008). High levels of chloride are present in sewage and some industrial effluent, thus chloride was measured to determine the degree of pollution of sewage from WWTP and industrial effluent (EPA, 2001). Phosphate, nitrate and ammonia were analysed, because they contribute to the assessment of pollution of streams from different anthropogenic sources such as water treatment plants, agricultural practices and municipal areas.

The occurrence of faecal coliform bacteria and *Escherichia coli* (*E. coli*) are indicative of the degree of pollution of water from faecal matter (EPA, 2001). Total bacteria count (TBC) on the other hand is a measure of the total bacterial load in a water sample and is usually assessed to determine the general hygiene condition or safety of a water source (EPA, 2001).

The ecological analysis was carried out because macro-invertebrates and their habitats reflect environmental stresses and thus will assist in the interpretation of water quality data. For the ecological analyses, macro-invertebrates such as: crustaceans, molluscs, snails, aquatic worms and the immature forms of aquatic insects, such as stonefly and mayfly nymphs were collected from different biotopes (habitats) for analysis in the laboratory. The different biotopes included; the stones biotope (S), vegetation biotope (V) and the gravel, sand and mud biotope (GSM). The habitats occupied by the macro-invertebrates were also analysed visually on-site.

The water quality and ecological data were finally used to describe the overall health status of the Bloemspruit stream. This was achieved by calculating various indexes and scores, including the overall water quality (CCME, 2001), a qualitative assessment, using the macro-invertebrate diversity (Dickens & Graham, 2002) and macro-invertebrate habitat integrity (Kleynhans et al., 2008) to ascertain the overall quality of the sampling sites.

Chapter 4

Water Quality of the Bloemspruit stream

4.1 Introduction

Water quality of a stream can be affected by a number of natural and human factors. Natural factors that may affect the quality of the water of a stream include the weathering of the stream's bedrock that affects the mineral content of the stream water, wind and rainfall runoff that can introduce sediment into a stream, and organic matter and nutrients from the soil that can be leached into the water of a stream (Chidya et al., 2011). Human factors that can degrade the quality of water in a stream include effluent produced by industries and waste water treatment plants (WWTP) in the vicinity, as well as waste water produced from residential and agricultural activities in the surrounding areas (Dabrowski & Klerk, 2013; Bu et al., 2014).

The water quality of the 12 identified sampling sites along the Bloemspruit stream was assessed in terms of the physical, chemical and microbiological properties. Data were collected in three seasons to ascertain whether seasonal effects existed. These data were then compared to aquatic water quality limits proposed for this study.

4.2 Sampling and measurement of water quality

Water samples were collected from the 12 sampling sites to determine the physical, chemical, and microbiological water quality properties. The physical properties of pH, temperature, electrical conductivity (EC), and turbidity were measured on-site as well as chemical properties such as dissolved oxygen (DO) and chloride (Cl). The amount of total dissolved solids (TDS) in mg/L present in the water samples was derived from EC (µS/cm) measurements.

The TDS was calculated as follows (EPA, 2001):

$$TDS\ (mg/L) = electrical\ conductivity\ (\mu S/cm) \times 0.67 \quad (1)$$

The remainder of the chemical properties assessed, namely, phosphate (PO_4), ammonia (NH_3), and nitrate (NO_3), as well as the microbial properties, faecal coliforms, *Escherichia coli* (*E. coli*) and total bacterial counts (TBC), was measured in the water laboratory of the Central University of Technology, Free State.

4.2.1 Sampling of water

At each sampling site some measurements were taken directly in the stream, while other measurements required the collection of a water sample. The properties of pH, temperature, EC, DO and Cl were measured directly in the water, while water samples were collected for the measurement of the remainder of the properties. However, turbidity was determined on-site by collecting water samples using clean water beakers of approximately 500 mL. During the collection of water samples and measurement of on-site properties, protective clothing (wader and gloves) was worn as a precaution against potential health effects that could be caused by the pollutants in the stream. Water samples were collected from the main current of the stream to avoid pollution from the soil of the embankment, whilst facing upstream.

For the laboratory measurements, the following water collection procedure was used:

1. At each site, one 500-mL bottle was filled with water for chemical measurements and one sterile 100-mL bottle for microbial measurements.
2. After opening a sample bottle, a water sample was collected below the surface of the stream approximately one metre from the embankment.
3. All sample bottles were clearly labelled using a permanent marker with site number, time and date.
4. The collected samples were then placed in a cooler box and transported to the laboratory for analysis.

4.2.2 Measurement of water quality properties

Measurement of on-site properties

The pH at each site was measured by placing the probe of a hand-held pH meter directly into the mid-stream water. After swirling the probe a few seconds, the reading was recorded after the reading on the instrument had stabilised.

Temperature, EC, DO and Cl were measured on-site using a battery operated Hach HQd hand-held meter, which uses digital intelliCAL™ probes (Figure 4.1). Specific probes were connected for the different measurements.

Figure 4.1 Hach HQd hand-held meter

Turbidity was measured using a battery operated Hach 2100Q turbidity meter in the following manner (Figure 4.2):

1. A water sample was poured into a clean Hach sample cell and filled to 10 mL mark.
2. The instrument was then switched on and its calibration verified by placing a clean sample cell containing a calibration solution of <100 nephelometric turbidity units (NTU) in the instrument's cell compartment. All sample cells were cleaned with a soft cloth to remove fingerprints and water marks before use.
3. After verifying the calibration of the instrument, the calibration sample cell was removed and replaced with a sample cell containing a water sample.
4. After closing the sample cell compartment, the read button was pressed and the turbidity reading recorded in NTU.

Figure 4.2 Portable Hach 2100Q Turbidity Meter

Measurement of chemical properties

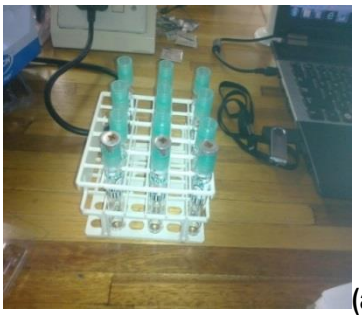
Phosphate, ammonia and nitrate were measured in the laboratory using a Hach DR 3900 Spectrophotometer (Figure 4.3). The Spectrophotometer is arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer, which delivers a voltage signal to a galvanometer. The signal changes as the amount of light absorbed by the liquid changes providing the means to quantify the chemical content.

Figure 4.3 Hach DR 3900 Spectrophotometer

Measurements of phosphate were obtained using the TNTplus™ test in the following manner:

1. The barcode programme for measuring phosphate was selected from the main menu of the instrument.
2. The green DoziCap™ was carefully removed from the TNTplus™ sample cell (Figure 4.4) and 2.0 mL of the water sample was placed into the TNTplus™ sample cell using a pipette.
3. After closing the water sample with the green DoziCap™, the sample was shaken two to three times.

4. The prepared samples were then placed into a Hach DRB 200 Digital Reactor and heated at 100 ºC for one hour. Up to 12 samples were heated at one time (Figure 4.4).
5. After heating, a sample was allowed to cool, after which 0.2 mL of reagent B was pipetted into the cooled sample. The sample was then capped with a grey DoziCap™, and shaken two to three times.
6. Finally, after wiping clean, the sample cells were placed in the Spectrophotometer one-by-one and covered with the light shield.
7. Once the barcode on a sample cell was recognised by the instrument, the amount of phosphate present in the water sample was displayed on the screen, and the reading recorded in mg/L.


(a)

(b)

Figure 4.4 TNTplus™ sample cells (a): TNTplus™ sample cells containing sample solution (b): TNTplus™ sample cells in Hach DRB 200 Digital Reactor

Ammonia and nitrate were measured in the following manner:

1. The appropriate barcode programme for measuring ammonia or nitrate was selected from the main menu of the instrument. For ammonia, the ammonia salic test was used, while for nitrate the high range powder pillow (Nitrate HR PP) test was used.
2. The instrument's calibration was verified by placing a clean sample cell into the instrument's cell compartment. When the instrument displayed a zero, the sample cell was removed.
3. After the ammonia salicylate reagent (for ammonia) and nitrate reagent (for nitrate) was dissolved in separate sample cells containing 10 mL water sample, each of the sample cells was placed into the instrument's cell compartment and covered with a light shield.
4. When the readings of ammonia or nitrate were displayed, they were recorded as mg/L

Measurement of microbiological properties

Faecal coliform bacteria and *E. coli* were counted in the laboratory using the IDEXX (Colilert 18) Quanti-Tray™ method. The Colilert 18 method is a biotechnological detection approach, which uses the

multi-well most probable number (MPN) method. The method incorporates a defined substrate medium which contains θ-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methyumbelliferyl-β-D-glucuronide (MUG). After incubating a sample at 37°C for 18 to 22 hours, coliform bacteria produce a yellow colour due to the production of β-galactosidase and *E. coli* produces blue fluorescence as a result of the action of β-glucuronidase under UV light. The most probable number of *E. coli* in a sample is calculated from the number of positive wells.

The Colilert 18 method used to measure faecal coliforms and *E. coli* bacteria present in the water samples is as follows:

1. Excess water was decanted from a 100-mL water sample bottle and the Colilert 18 medium powder was poured into the bottle and shaken for a few minutes to dissolve.
2. The site name and number were written on the back of the 97-well Colilert 18 Quanti-Tray™2000 tray using a permanent marker.
3. The water sample containing the powder was then poured into a 97-well Colilert 18 Quanti-Tray™2000.
4. Thereafter, the 97-well Colilert 18 Quanti-Tray™2000 trays were heat-sealed one at a time.
5. Twelve 97-wel Colilert 18 Quanti-Tray™2000 trays were then incubated at 37 °C for 20 hours.
6. The presence of coliforms produced yellow coloured wells under natural light (Figure 4.5), while the presence of *E. coli* produced blue florescent wells when placed under UV light (Figure 4.5).

(a)

(b)

Figure 4.5 97-well Quanti-TrayTM 2000 trays (a): 97-well Quanti-Tray™2000 showing the yellow wells indicating the presence of coliforms. (b): 97-well Quanti-Tray™2000 showing blue fluorescent wells indicating the presence of *E. coli*

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

- For faecal coliform bacteria, the number of large yellow wells was matched against the number of small yellow wells. For example; when18 large wells and 14 small wells were counted after incubation of a water sample, the large and small wells were marched on the MPN table as demonstrated in Figure 4.6.
- When this tray was passed under UV light, for example, nine large wells and two small wells fluoresced. These large and small wells were then marched on the MPN table. The MPN for faecal coliform bacteria for this example was 39.8 and for *E. coli* 12.

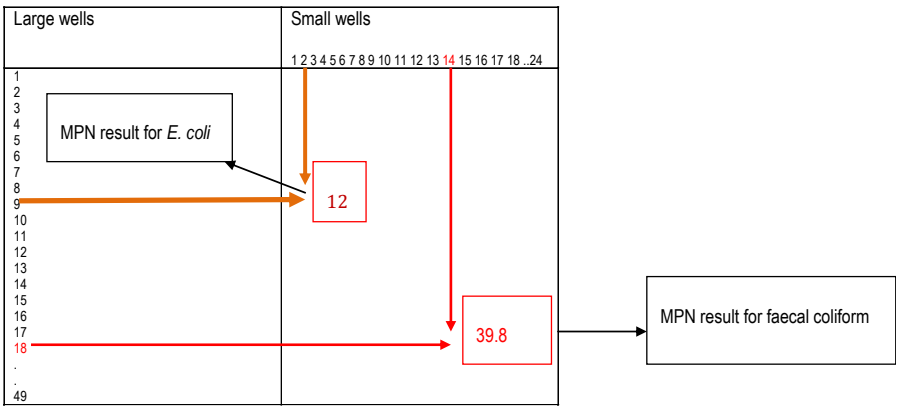


Figure 4.6 Determination of the most probable number (MPN) of faecal coliform bacteria and *E. coli* using the MPN table

Total bacterial counts were obtained using the SimPlate method. This method uses the IDEXX's patented Multiple Enzyme Technology, which uses enzyme substrates and produces blue fluorescence when these substrates are metabolised by waterborne bacteria. After SimPlate medium and sample water were added to a SimPlate and incubated for 48 hours, the wells were then examined under UV light and the number of fluorescent wells counted. The number of fluorescent wells is equal to the Most Probable Number of total bacteria in the water sample.

The SimPlate method used to measure the total bacteria count in a water sample is as follows:

1. A water sample was diluted 100× to be able to count the most probable number of total bacteria present in the water sample.
2. 100 mL of prepared saline solution were transferred into a 200-mL bottle and autoclaved for 30 minutes to sterilise the solution, after which the solution was again decanted into a 100-mL bottle and kept in a refrigerator until use.

3. One millilitre of saline solution was then removed from the 100-mL bottle saline solution and replaced with one mL of water sample, and shaken.
4. Thereafter, 10 mL of the saline-sample solution were transferred into a tube containing powdered SimPlate medium and shaken to dissolve the powder (Figure 4.7).
5. The content of the tube containing medium and saline sample solution was then transferred to the centre of a SimPlate base plate.
6. The SimPlate base plate was then gently swirled to allow the solution to fill all the wells, after which the plate was tilted to drain excess solution onto an absorbent pad (Figure 4.7).
7. The SimPlate base plate was labelled with a site number and then incubated upside down for 48 hours, which was then passed under UV light and the number of fluorescent wells counted.


(a)


(b)

Figure 4.7 Media tubes and plate-base (a): Media tubes containing sample solutions and (b): Sample solutions placed in a plate-base

To determine the MPN of total bacteria present in each water sample, the number of positive wells which corresponded to the MPN table was determined. For example, for a particular sampling site, 17 fluorescence wells were counted on the SimPlate base plate after 48 hours of incubation. This number of positive wells corresponds to 3.8 on the MPN table, which is then multiplied by 100 to give 380 cfu/ mL of total bacteria in the water sample.

4.3 Analysis of data

The water quality data obtained from the 12 sampling sites were entered into Excel spread sheets for analyses. The physical, chemical and microbiological data were statistically analysed by determining the descriptive statistics and by performing inferential tests; which included analyses of variance

(ANOVA) and Scheffe's post hoc tests. Water quality indexes (WQIs) were also calculated for each of the sampling sites to describe the overall water quality condition at each site.

4.3.1 Proposed water quality limits

In South Africa, no specific standards or guidelines exist, that contain all the limits to protect aquatic organisms in streams. An extensive review of the literature was thus undertaken to search for suggested limits for aquatic ecosystems similar to this study. The literature that was sourced did not include microbiological limits that are suitable for the protection of aquatic organisms. Therefore, the microbiological data were compared to guidelines for irrigation (Blumenthal et al., 2000) and recreational waters (DWAF, 1996b) (Table 4.1). On the other hand, no explicit values for water quality limits for temperature could be sourced, as such; a number of sources were used to determine a temperature range suitable to support aquatic organisms (DWAF, 1996a; ANZECC, 2000; Lumb et al., 2006; Le Roux, 2013). For the physical and chemical properties, the review of the literature revealed twenty potentially relevant water quality guidelines for aquatic ecosystems. These water quality guidelines were then assessed for their suitability for this study. Three of these water quality guidelines were selected for further scrutiny based upon the following two selection criteria:

- Guidelines should be for similar water conditions; and
- The limits should be relatively stringent.

Water quality limits for the measured properties were selected from these guidelines only if the limits were similar amongst the three guideline sets. In the event where the limits of some properties were too diverse amongst the three guidelines, scientific studies were consulted that specifically referred to limits that protect aquatic organisms in stream ecosystems. The selected limits obtained from the water quality guidelines and the scientific studies, together with the irrigation and recreational limits for the microbiological properties, were then used to propose the Aquatic Water Quality Limits for Urban Streams (AWQUS) (Table 4.1). The measurements found in this study were compared to these limits.

Table 4.1 Proposed Aquatic Water Quality Limits for Urban Streams

Water quality property	Original purpose of limit	Proposed limit	Reference
Faecal coliform and *E. coli*	Irrigation	0[1] ; ≤200[2] (cfu/100 mL)	Blumenthal et al. (2000)
Faecal coliform and *E. coli*	Recreational	≤1 000 cfu/100 mL	Department of Water Affairs and Forestry (1996b)
Total bacteria count	Irrigation and recreational	≤1 000 cfu/100 mL	World Health Organisation (2001)
pH	Aquatic ecosystem	5.5-9	Environmental Protection Agency (2001)
Turbidity	Aquatic ecosystem	≤5.6 NTU	Australian and New Zealand Environment and Conservation Council (2000)
Electrical conductivity (EC)	Aquatic ecosystem	≤1 000 µS/cm	Environmental Protection Agency (2001)
Total dissolved solids (TDS)	Aquatic ecosystem	≤1000 mg/L	Australian and New Zealand Environment and Conservation Council (2000)
Dissolved oxygen (DO)	Aquatic ecosystem	6.5-9.5 mg/L	Canadian Council of Ministers of the Environment (2008)
Temperature	Aquatic ecosystem	≥5≤25[3]	Department of Water Affairs and Forestry (1996a); Australian and New Zealand Environment and Conservation Council (2000); Lumb et al. (2006); Le Roux (2013)
Nitrate (NO_3)	Aquatic ecosystem	≤2mg/L	Camargo et al. (2005)
Phosphate (PO_3)	Aquatic ecosystem	≤0.7 mg/L	Environmental Protection Agency (2001)
Ammonia (NH_3)	Aquatic ecosystem	≤1.3 mg/L	Lumb et al. (2006)
Chloride (Cl)	Aquatic ecosystem	≤250 mg/L	Environmental Protection Agency (2001)

[1] = crops eaten raw; [2] = commercially processed and fodder crops; [3] = References used to estimate a temperature range for aquatic water quality limit; EC = electrical conductivity; TDS = total dissolved solids; DO = dissolved oxygen

4.3.2 Statistical analysis

Descriptive statistics including means, standard deviations and compliance percentages were calculated to describe and summarise the physical, chemical and microbiological water quality properties. Analysis of variance (ANOVA) tests were performed on the data at a significance level of 0.05 to ascertain if there were any differences between the different seasons. Scheffe's post hoc tests were performed on the data were ANOVA tests were significant.

4.3.3 Application of a water quality index

A water quality index (WQI) integrates a large number of water quality property measurements to produce a single number that describes the overall quality of water at a specific site within the stream (Sarkar & Abbasi, 2006). A review of the literature was undertaken to search for indexes that could be suitable for this study. From the literature search, three indexes were selected, namely, the arithmetic (Brown et al., 1970), weighted arithmetic (SAFE, 1995; Cude, 2001) and the Canadian Council of Ministers of the Environment Water Quality Index (CCME, 2001). The indexes were selected for further scrutiny based upon the following three selection criteria:

- The WQI should be relatively easily to compute;
- The WQI should include all properties used in this study; and
- The WQI should include more than one season.

The CCME was then selected as the most appropriate for this study based upon the fact that it allowed for repeated measurements. Although 13 water quality properties were measured, 10 were selected for the calculation of a WQI, based on known literature about the impact of each property on the macro-invertebrate survival and population diversity (Carr & Rickwood, 2008). The ten selected properties included pH, temperature, EC, turbidity, TDS, DO, Cl, PO_4, NH_3, and NO_3. The proposed AWQUS limits were used in the calculation of each WQI.

The calculation of the CCME WQI values involved calculating three main factors, namely the scope (F_1), the frequency (F_2) and the amplitude (F_3), in the following manner:

1. **Calculation of F_1**: F_1 denotes the number of properties (expressed as a percentage) that did not meet the proposed limits (failed properties):

$$F_1 = \left(\frac{Number\ of\ failed\ properties}{Total\ number\ of\ properties}\right) \times 100 \quad (2)$$

2. **Calculation of F_2**: F_2 denotes number of measurements or test (expressed as a percentage) over all three seasons that did not meet the proposed limits (failed properties):

$$F_2 = \left(\frac{Number\ of\ failed\ \ measurements\ (test)}{Total\ number\ of\ measurements\ over\ all\ three\ seasons}\right) \times 100 \quad (3)$$

3. **Calculation of F_3:** F_3 is a measure of the extent of the failure of all measurements (test) and is calculated in three steps:

   a. **An excursion is calculated for each failed measurement as follows**:

   - Where the measurement must not exceed the limit:

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

   - Where the measurement must not fall below the limit:

$$Excursion_i = \left(\frac{Limit\ of\ property}{Failed\ measurement}\right) - 1 \quad (5)$$

   b. **The normalised sum of all excursions (nse) is calculated as follows**:

$$nse = \frac{\sum_{i=1}^{n} Excursion_i}{\sum_{j=1}^{m} Measurements_j} \quad (6)$$

   Where n = number of failed properties and m = total number of measurements over three seasons

c. **Calculation of F_3:**

$$F_3 = \left(\frac{nse}{0.01nse+0.01}\right) \quad (7)$$

With the three factors in place, the WQI was then calculated in the following manner:

$$CCME\ WQI = 100 - \left(\frac{\sqrt{F_1{}^2 + F_2{}^2 + F_3{}^2}}{1.732}\right) \quad (8)$$

To demonstrate how to calculate the CCME WQI (CCME, 2001), data of the measurements of 10 properties at one site and three repeats have been used to demonstrate the calculation of the index. These data are presented in Table 4.2.

Table 4.2 Example data used to demonstrate the calculation of a WQI for a site

Properties	pH	Temp	EC	TDS	Turbidity	DO	PO_4	NO_3	NH_3	Cl
Limits	5.5-9	≥5≤25	≤1000	≤1000	≤5.6	6.5-9.5	≤0.7	≤2	≤1.3	≤250
Season 1	8.5	20.7	253	170	26.7	6.89	0.8	3.72	0	28.8
Season 2	7.9	19	549	368	12.4	5.48	5.2	2.8	0	269
Season 3	8.1	8.8	572	383	1.93	6.93	0.4	0.4	0	142.2

The demonstration data have been used to calculate CCME WQI (CCME, 2001) in a stepwise fashion. The individual sub-components used in the calculation of the CCME WQI (CCME, 2001) are demonstrated in Table 4.3.

Table 4.3 Step-by-step calculation of a WQI using example data

Scope (F_1)	The number of properties that did not meet the limit is 5 (Turbidity, DO, PO_4 and NO_3), total number of properties is 10. Therefore: $F_1 = \left(\frac{5}{10}\right) \times 100 = 50$
Frequency (F_2)	The number of measurements that did not meet the limit is 8, and the total number of measurements for all seasons is 30. Therefore: $F_2 = \left(\frac{8}{30}\right) \times 100 = 26.6$
Excursion	The excursion, is calculated as follows: $Excursion_i = \left(\frac{26.7}{5.6}\right) - 1$ = 3.76 (e.g. for turbidity, where measurement exceeded the limit). $Excursion_i = \left(\frac{8}{5.48}\right) - 1$ = 0.45 (e.g. for DO where measurement must not fall below the limit). The middle value between 6.5 and 9.5 was used as the limit in the calculation. Sum of excursion = 3.76 + 1.21 + 0.45 + 0.14 + 6.42 + 0.86 + 0.4 + 0.07 =13.31 Total number of measurements = 30
Normalised sum of excursion (*nse*)	The *nse* is calculated as follows: $nse = \frac{13.31}{30}$ = 0.44
Amplitude (F_3)	F_3 is calculated as follows: $F_3 = \left(\frac{0.44}{0.01\,(0.44)+0.01}\right)$ = 30.55
CCME WQI	Finally the CCME WQI is calculated as follows: $(F_1)^2 + (F_2)^2 + (F_3)^2 = (50)^2 + (26.6)^2 + (30.55)^2 = 4140.86$ $100 - \left(\frac{\sqrt{{F_1}^2 + {F_2}^2 + {F_3}^2}}{1.732}\right) = 100 - \frac{\sqrt{4140.86}}{1.732}$ = CCME WQI = 62.85 = 63

After the WQIs were calculated, the water quality condition for each site was classified using five different categories suggested by the CCME (2001). The five categories are based upon the classification of water in relation to how close the water quality is to the natural condition (CCME, 2001). Scores for the CCME WQI (CCME, 2001) range from 0 to 100 (Table 4.4).

Table 4.4 Categories used to rank water quality (CCME, 2001)

CCME WQI	Condition	Description
>94-100	Excellent condition	Water quality is protected with absence of threat. Condition is very close to natural levels.
>79-94	Good	Water quality is protected with minor degree of threat. Condition rarely departs from natural levels.
>64-79	Fair	Water quality is protected but occasionally threatened. Condition sometimes departed from natural levels.
>44-64	Marginal	Water quality is protected but is threatened frequently. Condition always departs from natural levels.
0-44	Poor	Water quality is always threatened. Condition is always departed from natural levels.

4.4 Water quality results

4.4.1 Physical properties

Four of the physical properties, pH, temperature, EC and TDS measured in this study displayed measurements within the proposed AWQUS limits for all three seasons. In contrast, only three of the 36 (8.3%) turbidity measurements were within the AWQUS limit (Table 4.5). Furthermore, the mean overall turbidity recorded in Season 1 was approximately six times greater than the mean turbidity that was recorded for Seasons 2 and 3.

Table 4.5 Measurements and summary statistics of the physical properties for Season 1, 2 and 3

Sample	pH			Temp (°C)			EC (µS/cm)			TDS (mg/L)			Turbidity (NTU)		
Limits	5.5-9.0			≥5≤25			≤1000			≤1000			≤5.6		
Seasons	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
BS1	8.5	7.9	8.1	20.7	19.0	8.8	253	549	572	170	368	383	26.7	12.4	2.0
BS2	8.7	7.9	8.1	21.0	20.0	10.0	277	584	606	186	391	406	30.5	2.3	1.4
FS3	8.7	7.8	7.8	23.8	22.0	13.0	308	675	835	206	452	559	86.4	52.6	74.2
FS4	7.9	7.6	7.9	21.5	23.0	14.0	375	600	793	251	402	531	108.0	35.7	140.0
BF5	8.1	7.5	7.9	21.6	21.0	12.0	382	602	794	256	403	532	96.0	40.7	54.0
BS6	8.0	7.5	7.8	22.1	23.0	7.6	288	610	710	193	409	476	39.3	9.3	6.2
BS7	6.2	8.0	7.7	19.4	24.0	10.0	266	657	778	178	440	521	40.0	14.7	18.7
RS8	7.0	7.7	7.4	20.9	22.0	8.8	587	540	642	393	362	430	63.0	29.0	28.3
BK9	7.5	7.7	7.9	21.9	22.0	10.0	260	540	795	174	362	533	464.0	29.0	12.3
RK10	7.5	7.7	7.9	21.1	22.0	9.1	603	546	622	404	366	417	74.1	31.7	16.6
BR11	7.6	8.0	7.4	20.3	22.0	10.0	373	681	745	250	456	499	299.0	18.8	11.3
RS12	7.5	8.0	8.1	21.9	22.0	9.2	337	641	748	226	429	501	410.0	30.6	9.6
Mean	7.8	7.8	7.8	21.4	22.0	10.2	359	602	720	241	403	482	175.0	25.5	31.2
Median	7.8	7.8	7.9	21.3	22.0	10.0	323	601	747	216	403	500	91.2	29.0	14.4
Minimum	6.2	7.5	7.5	19.4	19.0	7.6	253	540	572	170	362	383	26.7	2.3	1.4
Maximum	8.7	8.0	8.1	23.8	24.0	14.0	603	681	835	404	456	559	464.0	52.6	140.0
SD	0.72	0.18	0.23	1.08	1.33	1.87	119.48	52.34	87.96	79.97	34.89	58.88	154.90	14.43	40.64
% Non-Compliance	0	0	0	0	0	0	0	0	0	0	0	0	100	92	83

EC = electrical conductivity; TDS = total dissolved solids; NTU = nephelometric turbidity units; SD = standard deviation

ANOVA tests were performed to ascertain if any seasonal effects existed. Apart from pH, temperature, EC, TDS and turbidity showed significant differences between the three sampling seasons.

Table 4.6 ANOVA tests of seasonal variation for pH, temperature, EC, TDS and turbidity

Physical properties	df	SS	MS	*f*-Value	*p*-Value
pH	2	0.03	0.02	0.09	0.920
Temp	2	1038.04	519.02	283.41	0.001**
EC	2	812856.70	406428.40	44.99	0.001**
TDS	2	364686.50	182343.30	45.03	0.001**
Turbidity	2	108505.60	54252.81	6.27	0.007*

df = degree of freedom; SS = sum of squares; MS = mean of sum of squares; *f* = variance of the group means; *p* = probability; ** = highly significant ($p < 0.001$); * = significant ($p < 0.05$)

Scheffe's post hoc tests were conducted on the measurements that revealed significant ANOVA tests. The Scheffe's post hoc tests revealed significant results for three season pairs for EC and TDS while for temperature and turbidity, two season pairs demonstrated significant results (Table 4.7).

Table 4.7 Scheffe's post hoc tests for (a) temperature, (b) EC, (c) TDS and (d) turbidity

Seasons	S1	S2	S3
Temp			
S1		-0.4833	11.1416**
S2			11.6250**

S1	S2	S3
EC		
	-243.0000**	-360.9166**
		-117.9166*

TDS		
S1	-162.7500**	-241.7500**
S2		-79.0000*

Turbidity	
119.1833*	113.5333*
	-5.6500

** = highly significant ($p < 0.001$); * = significant ($p < 0.05$); S = seasons

A visual perspective in the form of a histogram shows clearly to what extend the turbidity measurements exceeded the AWQUS limit. For the most, Season 1 exceeded the measurements of both Seasons 2 and 3 (Figure 4.8). When looking at the turbidity measurements of individual sites, a

number of differences could be discerned. For Sites BS1, BS2, BS6, RS8, and RK10, the turbidity measurements were only slightly higher than the AWQUS limit, although the measurements of Season 1 at sites RS8 and RK10 were higher than those of the other two seasons. Sites FS3, FS4 and BF5 demonstrated elevated turbidity measurements in all three seasons. The remainder of sampling sites (BS7, BK9, BR11 and RS12) all showed exceptionally high values for Seasons 1, with relatively low measurements in Seasons 2 and 3.

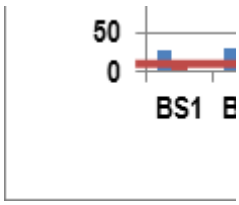


Figure 4.8 Histogram of the turbidity measurements for all three seasons (S1, S2 and S3) (Red horizontal line indicates the AWQUS limit for turbidity)

The relatively low turbidity measurements recorded for BS1, BS2, RS8, and RK10 sites can be mostly attributed to minimal anthropogenic activities in that area. Furthermore, sites BS1 and BS2 are in a built-up area and were not extensively affected by the rains during the sampling of Season 1 (Figure 4.9). The relatively low Season 1 turbidity values at sites RS8, and RK10 can be explained by the fast flowing Renosterspruit tributary after the extensive rains on the day of sampling, which resulted in the dilution of the runoff caused by the rains (Figure 4.9). Site BS6, on the other hand, is located after the confluence of the Bloemspruit stream and the Fonteinspruit, where water flow rate was relative fast because of the two water streams joining, thus accounting for the relatively low turbidity value recorded at this site during Season 1 (Figure 4.9).

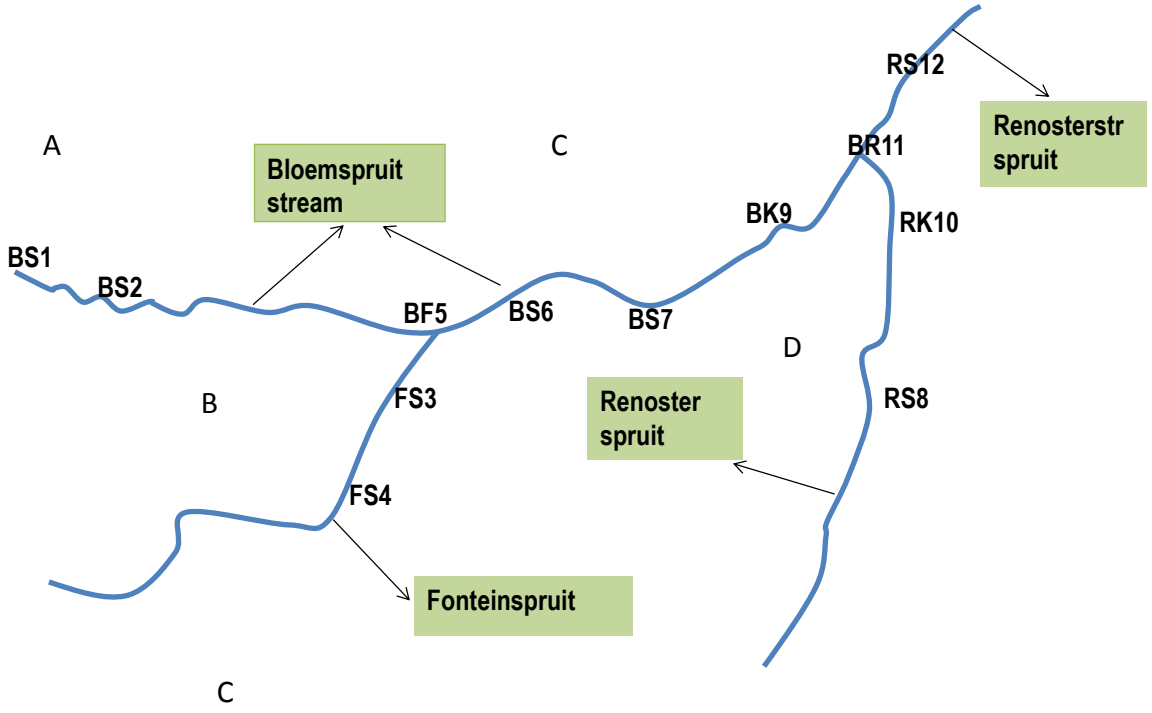


Figure 4.9 Bloemspruit stream, tributaries, sampling sites and main activities around the sampling area (A = Bloemfontein industrial area; B = WWTP; C = Heidedal industrial area; D = cattle farm; E = events centre)

Overall, the turbidity measurements were relatively high in all seasons at sites FS3, FS4 and BF5. Because sites FS3 and FS4 were located in the Fonteinspruit and BF5 at the confluence of the Bloemspruit stream and the Fonteinspruit, they were directly affected by runoff from the immediate environment, which includes a sewage pipe leaking into the Fonteinspruit, extensive industrial activities and a WWTP. The turbidity measurements of Season 1 was not as high as at a number of the other sites, probably because of some dilution of the runoff caused by the excessive rain during the collection of Season 1 measurements.

The turbidity measurements were exceptionally high for Season 1 for the downstream sites, BS7, BK9, BR11, and RS12, when compared to Season 2 and 3. These high levels of turbidity may be attributed to the fact that there was little or no dilution of runoff by the stream on the day of sampling after the heavy rains in Season 1. In addition, the turbidity levels could also have increased as a result of the contribution of the WWTP effluent, and the effect of the Fonteinspruit at sites BS7, BK9, BR11, and

RS12, which are located downstream of the Bloemspruit stream. Furthermore, site BS7 is located close to a cattle post that could contribute large volumes of waste into the stream. Additionally, at sites BR11 and RS12, agricultural runoff from agricultural activities in the vicinity may have contributed to the high turbidity levels recorded in Season 1. Conversely, for Season 2 and 3 the turbidity measurements recorded for sites BS7, BK9, BR11, and RS12 were only slightly higher than the AWQUS limit, probably because there was no rain on the day of sampling of Season 2 and 3, thus no contribution from runoff during these Season's measurements.

4.4.2. Chemical properties

The measurements of the two chemical properties, NH_3 and Cl, for all seasons were fully compliant with the proposed AWQUS limits (Table 4.8). In contrast, many measurements of DO, PO_4 and NO_3 did not fall within the AWQUS limit in all three seasons. For PO_4 the level of non-compliance was greater than 70% in all three season. However, for DO and NO_3 non-compliance was relatively low in Season 3, but more than 65% for DO and 92% for NO_3 for the other two seasons. Furthermore, the mean values for two seasons were outside the range of the proposed limits for DO and NO_3, whereas for PO_4 all the mean values were outside the proposed AWQUS limit.

Table 4.8 Measurement and summary statistics of chemical properties of Seasons 1, 2 and 3

Sample	Dissolve oxygen			Phosphate			Nitrate			Ammonia			Chloride		
Code/ Units	DO (mg/L)			PO_4 (mg/L)			NO_3 (mg/L)			NH_3 (mg/L)			Cl (mg/L)		
Limits	6.5-9.5			≤0.7			≤2			≤1.3			≤250		
Seasons	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BS1	6.9	5.5	6.9	0.8	0.7	0.4	3.7	2.8	0.4	0.00	0.00	0.00	28.8	269.0	142.2
BS2	6.5	7.7	9.4	0.8	0.5	0.4	1.6	2.4	1.0	0.07	0.01	0.00	33.7	165.0	119.2
FS3	3.7	0.2	1.4	0.6	0.3	6.7	11.2	10.9	1.2	0.01	0.11	0.10	25.2	259.0	127.2
FS4	4.5	4.4	2.9	0.1	3.0	5.3	5.8	3.9	3.4	0.01	0.06	0.20	40.4	242.0	123.2
BF5	3.4	0.1	4.4	0.4	3.4	2.0	10.0	4.4	1.8	0.00	0.03	0.08	67.6	174.0	143.2
BS6	4.8	3.1	6.7	1.3	2.6	8.4	9.9	0.5	1.3	0.01	0.01	0.01	26.3	152.0	147.2
BS7	5.9	5.7	6.9	2.3	6.1	8.7	13.3	4.4	1.1	0.07	0.01	0.02	17.1	171.0	111.2
RS8	5.1	6.5	8.0	5.1	4.9	4.6	3.4	8.2	3.1	0.01	0.01	0.08	43.7	86.2	97.2
BK9	5.5	5.2	6.9	2.2	6.6	6.7	2.8	8.4	0.9	0.05	0.06	0.00	16.8	108.0	127.2
RK10	6.0	6.6	9.0	5.9	4.9	7.1	2.08	9.7	3.0	0.01	0.04	0.04	37.8	86.9	121.2
BR11	4.6	5.1	7.1	2.8	6.2	8.1	5.6	6.9	1.8	0.02	0.05	0.01	27.3	119.0	123.2
RS12	2.8	7.2	8.1	4.4	5.8	8.3	5.9	4.8	1.2	0.04	0.04	0.06	16.8	124.0	129.2
Median	5.0	5.4	6.9	1.7	4.1	6.7	5.7	4.6	1.3	0.01	0.04	0.03	28.05	159.0	125.2
Mean	5.0	4.8	6.5	2.2	3.8	5.5	6.3	5.6	1.7	0.03	0.04	0.05	31.8	163.0	126.0
Maximum	6.9	7.7	9.4	5.9	6.6	8.7	13.3	10.9	3.4	0.00	0.11	0.18	67.6	269.0	147.2
Minimum	2.8	0.2	1.4	0.1	0.3	0.4	1.6	0.5	0.4	0.07	0.00	0.00	16.8	86.2	97.2
SD	1.24	2.48	2.39	1.96	2.28	3.07	3.90	3.18	0.97	0.02	0.03	0.05	14.47	64.08	13.99
Range	4.0	7.6	8.0	5.9	6.3	8.3	11.7	10.4	3.0	0.07	0.10	0.20	50.8	183.0	50.0
% Non-Compliance	75	67	25	75	83	83	92	92	25	100	100	100	100	100	100

ANOVA tests were performed on the chemical properties to determine if seasonal effects existed. Four of the five chemical properties assessed; DO, PO_4, NO_3 and Cl showed significant differences between the three sampling seasons (Table 4.9).

Table 4.9 ANOVA tests for seasonal variation of DO, PO_4, NO_3, NH_3 and Cl

Chemical properties	df	SS	MS	*f*-Value	*p*-Value
DO	2	20.72	10.43	5.60	0.010*
PO_4	2	66.83	33.41	11.34	0.001**
NO_3	2	147.66	73.83	7.97	0.002*
NH3	2	0.004	0.002	1.120	0.340
Chloride	2	109827.70	54913.85	40.10	0.001**

df = degree of freedom; SS = sum of squares; MS = mean of sum of squares; f = variance of the group means; p = Probability; ** = highly significant (< 0.001); significant = (p < 0.05)

Scheffe's post hoc tests were conducted on the measurements that revealed significant ANOVA tests. Scheffe's post hoc tests showed significant results for only one season pair for DO and PO_4, while for NO_3, two season pairs demonstrated significant results. However, all three seasons pairs revealed significant differences for Cl (Table 4.10).

Table 4.10 Scheffe's post hoc tests for (a) PO_4, (b) NO_3, (c) NH_3, and (d) Cl

Seasons	S1	S2	S3
DO			
S1		0.2000	-1.5000
S2			-1.7000*
NO_3			
S1		0.6650	4.5900*
S2			3.9250*

S1	S2	S3
PO_4		
S1	-1.5250	-3.3333*
S2		-1.8083
Cl		
S1	-131.2166**	-94.1583*
S2		37.0583*

** = highly significant (< 0.001); * = significant (p < 0.05); S = seasons

To obtain a better perspective of the data, histograms were constructed for the properties of DO, PO_4 and NO_3 that showed non-compliant to the AWQUS limits. For DO, the histogram clearly shows to what extend the DO measurements were mostly below the AWQUS limit, range indicated by the horizontal red lines (Figure 4.10). Although most of the DO measurements were outside the AWQUS limit; for Season 3, 75% of the measurements were within the limits.

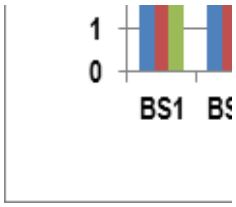


Figure 4.10 DO measurement for Season 1, 2 and 3 (S1, S2, S3) (Red horizontal line indicates AWQUS limit for DO)

The DO measurements that were within the AWQUS limits can be explained by the low winter temperatures at the time of sampling for Season 3. Despite the fact that 75% of the measurements of Season 3 demonstrated compliance with the AWQUS limits, measurements at sites FS3, FS4, FS5 were below the AWQUS limits for all three sampling seasons. These non-compliant values may, to some extent, be attributed to the leakage of a sewage pipe into the Fonteinspruit and the WWTP. This results in an increase in organic matter, including micro-organisms, and consequently an increase in decomposition of organic matter which leads to the reduction in DO (Krumbein & Bellingham, 2010).

The histogram of the PO_4 measurements shows that most measurements exceeded the AWQUS limit. Only six of the 36 PO_4 measurements were compliant with the AWQUS limit (Figure 4.11). In general

terms the relatively high phosphate readings of the sites beyond BS2 could be attributed to the contribution of sewage from the leaking sewage pipe in the Fonteinspruit and the WWTP.

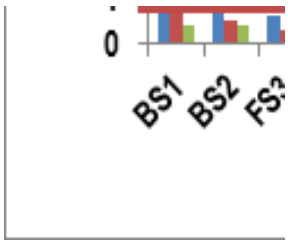


Figure 4.11 Phosphate measurements for Season 1, 2 and 3 (S1, S2, S3) (Red horizontal line indicates AWQUS limit for phosphate)

For NO_3, the histogram clearly shows that most measurements for NO_3 exceeded the AWQUS limit (Figure 4.12). Similarly to PO_4, the relatively high NO_3 measurements at the sites beyond BS2 can also be attributed to contributions from sewage, as well as from the cattle post near site BS7. The high levels of NO_3 found at FS3 and sites beyond FS3 in Season 2 could be directly attributed to a sewage spillage at the WWTP a few days prior to the collection. The reasons for the high levels of NO_3 found at FS3, FS4, BF5, BS6 and BS7 in Season 1, as well as the relatively low levels found for all sites of Season 3 cannot be clearly discerned.

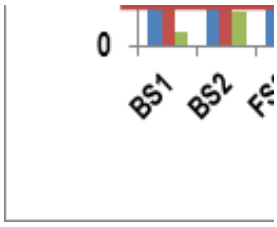


Figure 4.12 Nitrate measurements for Season 1, 2 and 3 (S1, S2, S3) (Red horizontal line indicates the limit for nitrate)

The evidence of the sewage spillage a few days prior to the collection of Season 2 samples were clearly discernible. The water of the Bloemspruit stream was black, a strong pungent odour could be detected and heavy deposits of sludge were found at certain sites (Figure 4.13).

(a)

(b)

Figure 4.13 Sludge on water after the sewage spillage before sampling of Season 2; at (a): site FS3 and (b): at site BF5

4.4.3 Microbiological properties

All three of the microbiological properties measured; faecal coliform, *E. coli* and total bacterial counts, displayed exceptionally high levels above the proposed AWQUS limit for most of the sites in all three seasons (Table 4.11). Also, none of the measurements for coliform bacteria and *E. coli* were within the raw vegetable AWQUS limits. Furthermore, for Season 1, none of the measurements for faecal coliform, *E. coli* and total bacterial counts were within the proposed commercially processed and fodder crops AWQUS limits. In contrast, some measurements for Season 2 and 3 were within the recreational and cooked vegetable AWQUS limits.

Table 4.11 Measurements and statistical summary of microbiological properties

Sample	Coliform / 100 mL			*E. coli* / 100 mL			TBC / 100 mL		
Recreational limit	≤1000 cfu			≤1000 cfu			≤1000 cfu		
Irrigational limit	raw veg = 0[1] cooked veg ≤200[2] cfu			raw veg = 0[1] cooked veg ≤200[2] cfu			≤1000 cfu		
Seasons	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
BS1	>2420*	249	219	>2420	189	178	2760	830	740
BS2	>2420	238	210	>2420	201	195	2480	2480	710
FS3	>2420	>2420	>2420	>2420	>2420	>2420	7380	5550	4700
FS4	>2420	>2420	>2420	>2420	>2420	>2420	7380	5070	4400
BF5	>2420	>2420	>2420	>2420	>2420	>2420	7380	4140	3110
BS6	>2420	>2420	>2420	>2420	>2420	>2420	3110	3280	2660
BS7	>2420	>2420	>2420	>2420	>2420	>2420	3720	3390	3240
RS8	>2420	1733	1986	>2420	>2420	1203	3110	2760	2570
BK9	>2420	>2420	2419	>2420	1417	>2420	2990	2870	2660
RK10	>2420	1553	1733	>2420	1414	980	2570	2480	2480
BR11	>2420	>2420	>2420	>2420	2419	2420	2870	3390	3240
RS12	>2420	>2420	>2420	>2420	>2420	>2420	2870	3240	2870
Median	>2420	2420	969	>2420	2420	2420	3415	3260	2765
Mean	>2420	1928	959	>2420	1882	1826	4555	3290	2782

SD	0.00	841.55	844.10	0	876.03	919.61	2056.65	1237.38	1185.53
Range	0	2182	2210	0	2219	2225	4900	3070	3990
% Non-Compliance									
Recreation	100	83	83	100	83	75	100	92	83
Raw veg	100	100	100	100	100	100	100	100	100
Cooked veg	100	100	100	100	92	83	100	92	92

cfu = coliform forming units; [1] = crops eaten raw; [2] = cooked veg; TBC = Total Bacterial Count; * = maximum reading of the test.

ANOVA tests were performed on the microbiological properties to determine if seasonal effects existed. All three properties measured; faecal coliforms, *E. coli* and total bacteria counts revealed significant differences between the three sampling season (Table 4.12).

Table 4.12 ANOVA test for seasonal variation of faecal coliforms, *E. coli* and total bacteria count

Microbiological properties	df	SS	MS	*f*-Value	*p*-Value
Faecal coliforms	2	1823517.0	911758.5	3.83	0.04*
E. coli	2	2581219.0	1290609.0	4.18	0.03**
HPC	2	9805756.0	4902878.0	7.58	0.003**

df = degree of freedom; SS = sum of squares; MS = mean of sum of squares; f = variance of the group means; p = probability; ** = highly significant ($p < 0.001$); * = significant ($p < 0.05$)

Scheffe's post hoc tests were conducted on the measurements that revealed significant ANOVA tests. These tests showed that for faecal coliform, *E. coli* and total bacteria count, only a single season pair showed significant results (Table 4.13).

Table 4.13 Scheffe's post hoc tests for (a) faecal coliforms, (b) *E. coli*, (c) TBC

Season	S1	S2	S3
Faecal coliform			
S1		492.250*	461.0833
S2			-31.1666

S1	S2	S3
E. coli		
	538.3333	593.6666*
		55.3333

TBC		
S1	761.6666	1270.0000*
S2		508.3333

* = significant ($p < 0.05$)

4.4.4 Water quality index

WQIs were calculated for each sampling site to evaluate the overall quality of the water. These calculations revealed that at none of the sampled sites the water was of good quality. However, at two of the sites the water quality was fair. For the rest of the sampling sites, the water quality was marginal (58%) or poor (17%) (Table 4.14).

Table 4.14 Water quality indexes and water quality ranges for the different sampling sites

Sites	WQI	Condition	Explanation
BS1	63	Fair	Fair water quality at sites BS1 and BS2 might be attributed to fewer anthropogenic activities in the vicinity, whose activities had little effect on the water quality at the sites.
BS2	73	Fair	
FS4	50	Marginal	Marginal water quality condition might be attributed to the fact that the anthropogenic activities might have had a lesser impact on the water quality at this site.
BS6	60	Marginal	The marginal water quality conditions might be attributed to increased flow at this section of the stream that assisted with the dilution of the pollutants.
RS8	55	Marginal	The marginal water quality conditions at sites RS8, RK10 and RS12, might be attributed to reduced anthropogenic activities in the vicinity of the Renosterspruit. At sites BK9 and BR11, the marginal water quality conditions might be attributed to an increased flow at this section of the stream that assisted with the dilution of pollutants.
BK9	55	Marginal	
RK10	54	Marginal	
BR11	48	Marginal	
BS12	47	Marginal	
FS3	44	Poor	Poor water quality at this site might be attributed to the combined effects of the Fonteinspruit and the WWTP sewage on this section of the stream.
BF5	44	Poor	Poor water quality might be associated with the effects of the Fonteinspruit, WWTP sewage as well as the Bloemspruit stream.
BS7	44	Poor	The poor water quality might be attributed to the effects of the cattle post on the sites and the combination of the Bloemspruit stream on this section of the stream.

4.5 Conclusions

Of the thirteen properties investigated approximately 50% demonstrated non-compliance with the proposed AWQUS limits. Of the five physical properties measured, only one property, namely turbidity, was non-compliant, which could be expected for a surface water source. In contrast, some of the chemical properties were non-compliant (DO, PO_4 and NO_3), whereas all the microbiological properties were non-compliant. The relatively high level of non-compliance of all the sites sampled was also reflected in the WQI values that showed only two sites with fair water quality. None of the sites revealed good water quality. Expected strong seasonal effects were also demonstrated by most of the properties. Except for one physical (pH) and one chemical property (NH_3), the remainder of the properties demonstrated significant seasonal differences ($p < 0.05$). These results clearly show that the water quality of the Bloemspruit stream is highly degraded and could pose a risk for aquatic organisms living in the stream, as well as for humans and animals that eat food irrigated by these waters or use it for domestic purposes.

Chapter 5

Ecological Quality of the Bloemspruit stream

5.1 Introduction

At present, the ecological integrity or the health of an ecosystem has been of central concern in most ecological studies (Ollis, 2005). Many different views have been put forward as to the actual meaning of the term ecological integrity or the health of an ecosystem (Ollis, 2005). Ecological integrity or the health of a stream can be described as the ability of a stream ecosystem to be able to support and maintain a community of organisms, which is similar to that of the natural habitat of the region (Ollis, 2005). One of the methods to determine the health status of a stream is by enumerating the biota (living organisms) in the stream; such as fish, algae, diatoms and macro-invertebrates (Dickens & Graham, 2002; Ollis, 2005; RHP, 2007; Munyika et al., 2014). The health status of a stream is further examined by assessing the stream habitat of particularly macro-invertebrate (Ollis et al., 2006; RHP, 2007). The information obtained from such studies provides an estimate of the diversity of the organisms present in the stream, as well as the extent to which the organisms are stressed (Day, 2000). Thus, an assessment of the ecological quality of a stream using living organisms is a good indicator of the health status of a stream (Duan et al., 2011; Masese et al., 2013).

Of all the different organisms that have been used to assess the health status of streams; the study of the diversity and abundance of macro-invertebrates is the most popular method (Dallas, 2000; Palmer et al., 2004; Thirion, 2007; Resh, 2008). Macro-invertebrates are suitable indictors of stream health for several reasons; they are highly sensitive to stressors in an aquatic ecosystem; they are also visible to the naked eye; they are easy to be identified; they have a short life span; and they are relatively immobile (Day, 2000; Dickens & Graham, 2002; Bonada et al., 2006; Duan et al., 2011). In addition, macro-invertebrates are sensitive to changes in their biotope (habitat). For example, in the event of a

chemical spillage macro-invertebrate populations may be adversely affected, and might not be detected by chemical analyses (Thirion, 2007).

Macro-invertebrates do not have a backbone and are large enough to be seen with the naked eye. These organisms live most of their life time in fresh water habitats (Kleynhans et al., 2005). Macro-invertebrates include: crustaceans such as crayfish, molluscs such as clams and snails; aquatic worms and immature forms of aquatic insects, such as stonefly and mayfly nymphs (Kleynhans et al., 2005).

Macro-invertebrates that are regularly used as indictors of stream health include:

- *Stoneflies* (order Plecoptera): Stoneflies have a high affinity for oxygen; hence they are very sensitive to organic pollution (Wenn, 2008).
- *Mayflies* (order Ephemeroptera): Mayflies have been known to show less resistance to different forms of stress in an aquatic environment. However, within the order, the family Beatidae is able to thrive within nutrient rich environments (Wenn, 2008).
- *Caddisflies* (order Trichoptera): Caddisflies have also been known to be sensitive to environmental stress (Wenn, 2008).
- *Chironomidae* (order Diptera): Chironomidae are known indicators of organic pollution. Organisms from this family reproduce rapidly and colonise areas polluted with organic runoff (Mason, 2002).
- *Aquatic worms* (order Oligochaeta): Tubificidae are known to be exceptionally resistant to organic pollution (Mason, 2002).
- Ephemeroptera, Plecoptera and Tripchoptera: Ephemeroptera, Plecoptera and Tripchoptera (EPT) are used as indicators of water quality condition since the mayfly; stonefly and caddisfly are known to be less resistant to organic pollution. As such, sites with high EPT richness indicate good water quality condition (Wenn, 2008).

A macro-invertebrate biotope (habitat) is an area with similar environmental characteristics where an assemblage of macro-invertebrates lives (Thirion, 2007). A biotope is described in terms of a number of physico-chemical and biological features (Kleynhans et al., 2005). Some of the physical features include the velocity and depth of the water, and the composition of the substrate, such as bed rock, cobbles, vegetation, sand, gravel and mud (Kleynhans et al., 2005). Chemical features include chemical composition, turbidity, oxygen concentration, and biological features such as food sources and the presence of predators (Kleynhans et al., 2005).

Different species of macro-invertebrates have different living requirements, therefore, are collected from different biotopes present at a particular stream site (Dickens & Graham, 2002). These include the stones biotope, the vegetation biotope, and the gravel, sand and mud biotope (Figure 5.1).

Figure 5.1 Different biotopes sampled (a): stones (b): vegetation (c): gravel, sand and mud

The stones biotope includes areas in the stream consisting of stones in the stream current or out of current, bedrock and other solid objects (Dickens & Graham, 2002). The vegetation biotope, on the other hand, consists of vegetation submerged in the water described as aquatic vegetation, as well as vegetation hanging into or growing along the stream banks described as marginal vegetation (Dickens & Graham, 2002). The gravel, sand and mud (GSM) biotope comprises of gravel, which consist of small stones <2 cm, while sand is <2 mm and mud, silt and clay are smaller particles <0.06 mm in diameter (Dickens & Graham, 2002).

The ecological health of the Bloemspruit stream was determined by measuring the diversity and abundance of macro-invertebrates within the stream. The South African Scoring System (SASS) developed by Chutter (1998) that was modified by Dickens and Graham (2002) was used to collect and enumerate the macro-invertebrates from the different biotopes. Additionally, the ecological health was assessed by visually quantifying the condition and the quality of the macro-invertebrate habitats on-site (Ollis et al., 2006).

In South Africa, the Invertebrate Habitat Assessment System (IHAS) (Ollis et al., 2006) is the most popular method used to measure the condition and the quality of macro-invertebrate habitats when a SASS assessment is performed (Ollis et al., 2006). Because the IHAS method does not include the physico-chemical properties of the stream water when measuring the condition and quality of macro-invertebrate habitats, the IHAS was modified for this study to include these properties. Therefore, a modified IHAS known as the modified Invertebrate Habitat Assessment System (mIHAS) was developed to incorporate these limitations. The mIHAS was then used to measure the condition and quality of macro-invertebrate habitats at each of the sampling sites. In addition, the effects of disturbance factors such as water abstraction, flow regulation, bed and channel modification on the macro-invertebrate habitats, were also visually quantified on-site using the Index of Habitat Integrity (IHI) (Kleynhans et al., 2008).

5.2 Macro-invertebrates sampling

During the collection of the water samples in each of the three seasons, ecological data were also collected at the 12 sampling sites. Macro-invertebrates were collected from their biotopes and scored using the SASS5 scoring sheet (Dickens & Graham, 2002). However, because of the difficulty of the terrain at site BR11, macro-invertebrates were not collected from this site. A standard SASS net, that consist of 1mm mesh size was used to collect macro-invertebrates from the stones, vegetation, as well as from the gravel, sand and mud (GSM) biotopes (Dickens & Graham, 2002) (Figure 5.2). Other

equipment used during the collection of macro-invertebrates included the use of wader gear and plastic cloves to protect the collector from hazardous pollutants in the water.

Figure 5.2 Standard SASS Net

The macro-invertebrates within the different biotopes were collected in the following manner according to the SASS5 score sheet (Dickens & Graham, 2002):

Stones biotope

1. The net was placed downstream from the stones in the water. After dislodging by kicking and overturning the stones in current (SIC) and bedrock in current for two minutes, as well as the stones out of current (SOOC) and bedrock out of current for one minute; the macro-invertebrates were collected with the net by sweeping through the disturbed water.
2. To dislodge macro-invertebrates on the surfaces of the bedrocks, the surfaces were rubbed by hand and by wader boots and the dislodged macro-invertebrates collected with the net.
3. Macro-invertebrates from all these collections were massed to form a stones biotope sample.

Vegetation biotope

The macro-invertebrates were collected from the marginal and aquatic vegetation in the following manner:

1. To collect macro-invertebrates from the marginal vegetation along the embankment, the vegetation was pushed with the net along areas of approximately two metres in length. For the collection of macro-invertebrates in aquatic vegetation; the net was also prodded in an area of approximately one square metre.
2. While collecting the macro-invertebrates in the vegetation biotopes, the net was kept below the water surface so as to prevent organisms above the water surface to be collected.

3. Macro-invertebrates from both these collections were massed to form a vegetation biotope sample.

Gravel, sand and mud (GSM) biotope

The macro-invertebrates were collected from the GSM biotope in the following manner:

1. To collect macro-invertebrates from the GSM biotope; the gravel, sand and mud were disturbed using the wader boots for one minute.
2. After some of the larger dislodged sediments had settled; the net was swept over the area to collect the dislodged macro-invertebrates.
3. Macro-invertebrates from all these collections were massed to form a GSM biotope sample.

Preparation of macro-invertebrate samples for transportation

After collecting the macro-invertebrates from each biotope at each sampling site, the samples were prepared for transportation in the following manner:

1. A net containing a macro-invertebrate collection also contains some leaves, twigs, debris and stones. The macro-invertebrates, leaves, twigs, debris and stones are first washed to the bottom of the net using water.
2. After inverting the net, it was placed in a 2-L container and the macro-invertebrates, leaves, twigs, debris and stones flushed into the container with water.
3. The large leaves, twigs, debris and stones were then removed from the water and rinsed with the sample to remove all macro-invertebrates clinging to their surfaces. These leaves, twigs, debris and stones were then discarded.
4. The 2-L container with a macro-invertebrate sample was then placed in a cooler box containing ice-packs and transported to the laboratory for analysis. The collected samples were kept in a refrigerator at approximately 4 ^{0}C before analysis commenced.

5.3 Macro-invertebrate measurements

5.3.1 Enumeration of macro-invertebrates

On a sampling day, a variety of different samples were collected. These included samples for water quality analyses, as well as samples for ecological quality analyses. Therefore, the macro-invertebrate samples were enumerated in the laboratory within 72 hours after transportation to the laboratory.

The SASS5 method (Dickens & Graham, 2002) was used to determine the number of different macro-invertebrate taxa present at each sampling site. The SASS method identifies macro-invertebrates to family level. The procedure was performed as follows:

1. A macro-invertebrate sample was removed from the refrigerator 30 minutes before enumeration and placed on a tray containing clean water to allow the macro-invertebrates to become active.
2. A hand-held lens was used to identify the macro-invertebrate families using the Aquatic Invertebrates of South African Rivers Illustration Guide (Gerber & Gabriel, 2002a) and Aquatic Invertebrates of South African Rivers Field Guide (Gerber & Gabriel, 2002b).
3. The macro-invertebrate families were recorded on a simplified SASS5 sheet (Dickens & Graham, 2002). An example of a recording sheet of the macro-invertebrates is presented in Table 5.1.
4. For each sample, the sensitivity scores for each identified family were allocated. The sensitivity scores were obtained from the Aquatic Invertebrates of South African Rivers Field Guide (Gerber & Gabriel, 2002b). These sensitivity scores indicate the degree of tolerance to pollution and range from one to 15 (Dickens & Graham, 2002). For example, families with high scores indicate that they are highly sensitive to pollution, whereas low scores indicate tolerance (Dickens & Graham, 2002) (Table 5.1).

Table 5.1 Example of macro-invertebrates families identified with the sensitivity scores

Order and family	Sensitivity score	S	V	GSM	Total sensitivity score
ANNELIDA					
Oligochaeta	1	5			**1**
Leeches	3			4	**3**
CRUSTACEA					
Potamonautidae	3				
ODONATA					
Coenagrionidae	4				
Gomphidae	6				
DIPTERA			1		**4**
Chironomidae	2				
Syrphidae	1				
Culicidae	1	20		20	**2**

S = Stones; V = Vegetation; GSM = Gravel Sand and Mud biotopes

Three different SASS indices were calculated for each sampling site. These indices included the SASS score (Dickens & Graham, 2002), number of taxa and average score per taxon (ASPT) (Dickens & Graham, 2002). Table 5.2 demonstrates how these indices are calculated using the example information in Table 5.1.

Table 5.2 Calculation of the SASS score, number of taxa and ASPT

SASS score (Dickens & Graham, 2002)	The SASS score is calculated by summing the sensitivity scores of the different macro-invertebrate families found at each sampling site. For example, the SASS score =10.
Number of taxa (Dickens & Graham, 2002)	The number of taxa represents the different macro-invertebrate families found at each sampling site. For example, the number of taxa = 4.
ASPT (average score per taxa) (Dickens & Graham, 2002)	ASPT reflects the overall sensitivity of the macro-invertebrates in a particular site. The ASPT is the SASS score divided by the number of taxa. For example, the ASPT = $\frac{10}{4}$ = 2.5.

5.4 Macro-invertebrate habitat sampling

5.4.1 Development of mIHAS

A number of indexes are available in South Africa, to quantify as well as to assess the condition of macro-invertebrate habitats in streams. These included the Habitat Quality Index (HQI) (Moore & McMillan, 1992), Habitat Assessment Matrix (HAM) (Roux, 1993, cited by Dallas, 2000), Habitat Score Version 1 (HABS1) (Chutter, 1994 cited in Ollis et al., 2006), as well as the Invertebrate Habitat Assessment System (IHAS) (Ollis et al., 2006). It has been shown that when the HQI, HAM and HABS1 are used interchangeably, they do not always produce consistent results (Ollis et al., 2006). Thus the IHAS has become the most popular macro-invertebrate habitat assessment method used in South Africa.

The IHAS (Ollis et al., 2006) measures a number of characteristics of macro-invertebrate habitats for a total score of 100. This score covers characteristics such as the presence of stones, vegetation, gravel, sand and mud. It also includes physical attributes describing the stream, for example, colour of the water, depth, width and velocity. Each of these characteristics are assessed by asking a set of questions and scoring the condition with values from 0 to 5; where 0 indicates a poor condition and 5, a good condition. All the values for a particular site are then summed to provide IHAS score (Ollis et al., 2006).

The IHAS (Ollis et al., 2006) does not include physico-chemical properties of the stream water, such as pH, NH_3 and temperature. Physico-chemical properties often cause direct or indirect deterioration to macro-invertebrates as well as their habitats, especially if the physico-chemical measurements are beyond the required limits for aquatic organisms (Duan et al., 2011). For example, in China, high levels of nitrogen and phosphorus in rivers reduced the diversity of macro-invertebrate families, leaving mostly dominant families such as Tubificidae, Chironomidae and Physidae (Duan et al., 2011).

The IHAS (Ollis et al., 2006) does not include physico-chemical properties of the stream water, although it is known that physico-chemical properties may affect macro-invertebrate populations as well as their habitats (Duan et al., 2011). For this study, therefore, it was decided to include the measurements of physico-chemical properties into the existing IHAS (Ollis et al., 2006) providing a more comprehensive and possibly a more accurate assessment of the condition of macro-invertebrate habitats. This modified Invertebrate Habitat Assessment System was thus named the mIHAS, with ‘m’ indicating that it is a modification of the IHAS (Ollis et al., 2006).

5.4.2 Water quality properties of the mIHAS

In the development of the mIHAS physico-chemical properties measured in this study that were deemed important were included. Nine physico-chemical properties were selected based upon their impact on the macro-invertebrate populations and their habitats (ANZECC, 2000). The properties included pH, temperature, turbidity, electrical conductivity (EC), dissolved oxygen (DO), ammonia (NH_3), nitrate (NO_3), phosphate (PO_4) and total dissolved solids (TDS) (Table 5.3). Two physical attributes of the stream, such as water colour and flow regime were also included, while the rest of the physical attributes of the stream that were not measured in this study, such as the depth, width, velocity and disturbance, were excluded from the mIHAS calculations (Table 5.3).

Table 5.3 Physico-chemical properties included in the mIHAS and reasoning for their selection

Property	Reason for choice of property	Reference
pH	pH alters the ionic and osmotic balance of aquatic macro-invertebrates. Such imbalances lead to loss of energy followed by slow growth, as well as reduced reproduction. Progressive reduction in pH may result in changes in the community structure whereby acid-tolerant organisms replace less tolerant organisms.	Carrasco et al. (2013)
Temperature	Water temperature affects metabolic activity of macro-invertebrates as well as their distribution within a stream. Macro-invertebrates use changes in temperature as a cue to indicate seasonal changes, which cause them to migrate, emerge and spawn. Also, temperature changes affect the rates of most chemical reactions, for example, an increase in temperature leads to an increase in the toxicity of ionised NH_3 in water and also decreases the solubility of oxygen. These changes, in turn, increase the toxicity of the water which may negatively affect macro-invertebrates.	DWAF (1996) Bogan et al. (2004) Graham and Louw (2008) Kleynhans et al. (2008)
Turbidity	Turbid water reduces the visibility and therefore the ability for macro-invertebrates to access food, which may result in starvation and even death under adverse conditions. Sediments can also suffocate aquatic insects, clams and oysters resulting in a stream with only few tolerant species. Murky waters also absorb more sun energy, which in turn increases water	ANZECC (2000) Carrasco et al. (2013)

	temperature. Very turbid water may inhibit sunlight from penetrating to the bottom of the stream, which can reduce the rate of photosynthesis in aquatic plants and other photosynthetic aquatic organisms.	
EC	The electrical conductivity of water is a measure of the dissolved ions in water. Changes in ion concentration lead to changes in the chemical composition of the water, which in turn could affect macro-invertebrate populations adversely and ultimately lead to extinction.	ANZECC (2000) CCME (2008)
Dissolved oxygen (DO)	DO is used by macro-invertebrates in aerobic respiration and thus has an effect on the survival of macro-invertebrates.	ANZECC (2000) Mattson et al. (2007)
NH_3	High levels of ammonia are toxic for macro-invertebrates. Ammonia affects the respiratory passages, hatching process and growth rate of macro-invertebrates. Ammonia can also enhance eutrophication, resulting in increased growth of water plants and subsequent the death of macro-invertebrates.	CCME (2008) Spencer et al. (2008)
Nitrate	At high levels, nitrate is toxic to macro-invertebrates. High nitrate levels in streams can cause tissue damage and death to early life stages of, for example, prawns and may also increase their attraction to diseases. Nitrate can also enhance the growth of aquatic plants leading to eutrophication, resulting in increased growth of water plants and subsequent the death of macro-invertebrates.	ANZECC (2000) Camargo (2005) Kilonzo et al. (2014)
Phosphate	High levels of phosphate can enhance the growth of aquatic plants leading to eutrophication, resulting in increased growth of water plants and subsequent the death of macro-invertebrates.	O'Keefe & Day (2006)
TDS	Dissolved solids in water, comprises of all the compounds that are dissolved in water. Some of the compounds such as salts, carry electrical charges, while other inorganic and organic compounds do not dissociate in water, as such, are not charged. Increases in levels of dissolved solids in water may have long-lasting physical effects on macro-invertebrates by affecting their potential to adapt.	Bilotta & Brazier (2008)

The limits that were used for the nine water quality properties that were included in the mIHAS were based upon the limits as described for AWQUS in Chapter 4. The limits for the nine water quality properties are listed in Table 5.4.

Table 5.4 AWQUS limits used for the nine water quality properties that were included in the mIHAS

Water quality property	Original purpose of limit	Proposed limit	Reference
pH	Aquatic ecosystem	5.5-9	Environmental Protection Agency (2001)
Turbidity	Aquatic ecosystem	≤5.6 NTU	Australian and New Zealand Environment and Conservation Council (2000)
Electrical conductivity (EC)	Aquatic ecosystem	≤1 000 µS/cm	Environmental Protection Agency (2001)
Total dissolved solids (TDS)	Aquatic ecosystem	≤1000 mg/L	Australian and New Zealand Environment and Conservation Council (2000)
Dissolved oxygen (DO)	Aquatic ecosystem	6.5-9.5 mg/L	Canadian Council of Ministers of the Environment (2008)
Temperature	Aquatic ecosystem	≥5≤25[3]	Department of Water Affairs and Forestry (1996a); Australian and New Zealand Environment and Conservation Council (2000); Lumb et al. (2006); Le Roux (2013)
Nitrate (NO_3)	Aquatic ecosystem	≤2mg/L	Camargo et al. (2005)
Phosphate (PO_3)	Aquatic ecosystem	≤0.7 mg/L	Environmental Protection Agency (2001)
Ammonia (NH_3)	Aquatic ecosystem	≤1.3 mg/L	Lumb et al. (2006)

[1]= Four references were used to estimate a temperature range for aquatic water quality limit

The application of the limits of the different water quality properties in the scoring process of the mIHAS also involved a 6-point scoring system. The development of the 6-point scoring system for the different physico-chemical properties was based on the AWQUS limits for the nine water quality properties. A 4-point quality range was further devised, namely; *ideal*, *acceptable*, *tolerable* and *unacceptable*, with a score of 5 for *ideal* and 0 for *unacceptable*. Two additional transitional scoring points were also included

making it a total of a 6-point scoring system as demonstrated for nitrate in Table 5.5.

Table 5.5 Example of a scoring sheet for nitrate using the mIHAS

WQ Property	AWQUS Limit	Score					
		0	1	2	3	4	5
		Unacceptable		Tolerable		Acceptable	Ideal
NO_3	≤2	≥20 mg/L		≥5≤10 mg/L		>2-3 mg/L	≤2 mg/L

5.5 Macro-invertebrate habitat measurements

5.5.1 Macro-invertebrate habitat measurement using mIHAS

The macro-invertebrate habitat scoring process using the mIHAS comprised of two steps. In the first step of the scoring process the habitat was visually inspected, while in the second step the laboratory measurements of the nine physico-chemical properties, as well as two physical attributes of the stream, such as water colour and flow regime were included. Thereafter, a composite macro-invertebrate habitat score was calculated.

The score sheet for the macro-invertebrate sampling habitat comprised of three subsections. The subsections included the stones in current (SIC), vegetation (V) and other (O), where each describes different aspects of the physical habitat (Table 5.6). After visual inspection of a sampling site, the different physical habitat attributes are scored by ticking the appropriate box in the table as demonstrated in red in Table 5.6.

Table 5.6 Example of mIHAS scoring sheet for the sampling habitat of a particular site (Ollis et al., 2006)

Sampling habitat	Habitat scores					
Stones in current	**0**	**1**	**2**	**3**	**4**	**5**
Total length (m) of broken water (riffles/rapids)	none	0-1	>1-2	>2-3	>3-5	>5√
Total length (m) of submerged stones in current (run)	none	0-2	>2-5	>5-10√	>10	
Number of separate SIC areas kicked	0√	1	2-3	4-5	6+	
Average size (cm) of stones kicked (gravel<2; bedrock>20)	none	<2>20	2-10	11-20	2-20	>20√
Amount of stone surface clear (of algae, sediment, silt, etc.) (%)	n/a	0-25√	26-50	51-75	>75	
Protocol: Time (mins) spent actually kicking SIC (gravel/bedrock=0)	0	<1√	>1-2	2	>2-3	>3

Vegetation	**0**	**1**	**2**	**3**	**4**	**5**
Length (m) of marginal vegetation sampled (banks)	none	0-½	>½-1√	>1-2	2	>2
Amount (m^2) of aquatic vegetation/algae sampled	none	0-½√	>½-1	>1		
Marginal vegetation sampled in or out of current	none		In current	Out of current		both√
Type of veg. (% leafy veg. vs. stems/shoots) (aq. veg. only=49)	none	0	1-25	26-50√	51-75	>75

Other habitat	**0**	**1**	**2**	**3**	**4**	**5**
Stones Out Of Current (SOOC) sampled (m^2) (protocol=1m^2)	none√	0-½	>½-1	1	>1	
Sand sampled (mins) (protocol=1min) (under=present below stones)	none√	under	0-½	>½-1	1	>1
Mud sampled (mins) (protocol=½ min) (under=present below stones)	none√	under	0-½	½	>½	
Gravel sampled (mins) (protocol=½ min) (if all, SIC stone size=<2)*	none√	0-½	½	>½*		
Bedrock sampled (all=no SIC/sand/gravel) (if all, SIC stone size=>20)*	none	some			all*√	
Algal presence (1-2m^2=algal bed; rocks=on rocks; isol.=isolated clumps)	>2m^2	rocks√	1-2m^2	<1m^2	Isol.	none

Tray identification (using time as per protocol)		under		correct√		over
HABITAT TOTALS: J K (J=total adjustment [B+E+H]; K=Habitat Total [C+F+I])	**J** Adj=34			Max=55		**K**

J = total adjustment scores {B = adjusted SIC scores to equal 20; E = adjusted Veg scores to equal 15; H = adjusted other habitat scores to equal 20}. K = Habitat total scores {final total for SIC scores; F = final total for Veg scores; I = final total for other habitat scores

Similarly to the scoring of the sampling habitat, the water condition score was determined. This score uses the laboratory measurements of the nine water quality properties as well as physical attributes of the stream, such as water colour and flow regime as demonstrated in Table 5.7.

Table 5.7 Example of a mIHAS scoring sheet for water quality of a particular site

Water condition	**Water condition score**					
	0	**1**	**2**	**3**	**4**	**5**
WQ Properties	**Unacceptable**		**Tolerable**		**Acceptable**	**Ideal**
Water colour (discolour= visibility discoloured but still clearest)	Silty and black		greenish√	discolour		clear
Flow regime	flood		turbulent√	fast		gentle
pH	<3		4-5	≥10>20		5.5-9√
Turbidity (NTU)	≥200		>50<100	25-50√	>6≤10	≤5.6
EC (µS/cm)	2000-3000		≥1500≤2000		>1000≤1500	≤1000√
TDS (mg/L)	2000-3000		≥1500≤2000		>1000≤1500	≤1000√
DO (mg/L)	0-1		2-3		<3<4	6.5-9.5√
NH_3 (mg/L)	>10		5-7		2-3	≤1.3√
NO_3 (mg/L)	≥20		≥5≤10		>2-3	≤2√
PO_4 (mg/L)	≥20		≥5≤10	3-4√	2-3	≤0.7

After completing the scoring process using the respective scoring sheets of the mIHAS, the mIHAS score was calculated. This value represents a composite score indicating the overall quality of a particular macro-invertebrate habitat at a sampling site. The calculation of a mIHAS score using the example data of Tables 5.6 and 5.7 is demonstrated in Table 5.8.

Table 5.8 Example of the calculation of the total mIHAS score for a particular sampling site

Actual mIHAS score	Adjusted mIHAS score	Subsection scores	Description of calculation
26 (SIC) + 18 (V) + 30 (O) = 74	20 (SIC) + 15 (V) + 20 (O) = 55	**Total sampling habitat score**	The habitat total score represents the sum of all the values that were ticked on the score sheet for the sampling habitat for stones in current, vegetation and other habitats. For example: The actual sampling habitat total score in the example (Table 5.6) = 15 (stones in current) + 11 (vegetation) + 8 (other habitat) = 34
50	45	**Total water condition score**	The water condition score represents the sum of all the values that were ticked on the score sheet for water condition. For example: The actual total water condition score = 40
124 (74 + 50)	100 (55 + 45)	**mIHAS score**	The total mIHAS score represents the sum of the total sampling habitat score and the total water condition score expressed as a percentage. For example, the mIHAS score = $\left(\frac{34+40}{124} \times \frac{100}{1}\right) = 59.7$

5.5.2 Macro-invertebrate habitat measurement using Index of Habitat Integrity

The impact of disturbance factors on macro-invertebrate habitats was assessed at each sampling site by calculating the Index of Habitat Integrity (IHI) (Kleynhans, 1996). IHI (Kleynhans, 1996) visually quantifies the impact of different disturbance factors such as water abstraction, flow regulation, bed and channel modifications, on both the *in-stream* zone, as well as *riparian* zone. The *in-stream* zone

represents macro-invertebrate habitats in the main current of a stream, while the *riparian* zone represents macro-invertebrate habitats at the embankments (Dallas, 2005; Kleynhans et al., 2008). For each of these zones a score is calculated which is then summed to produce an IHI score (Kleynhans, 1996).

The impacts of these factors on macro-invertebrate habitats are quantified by completing an IHI scoring sheet, which contains a number of criteria about the *in-stream* and the *riparian zones* (Kleynhans, 1996). The degree of impact of each criterion on habitats was originally rated using a scoring system that ranged from 0 to 5 (Kleynhans, 1996). Later this scoring system was modified to a 26-point score (0 to 25) (Dallas, 2005), where a score of 0 denotes that there is little or no observed impact of disturbance factors on the quality and diversity of a habitat, and a score of 25 indicates that a habitat has been greatly modified, and as such, the quality and diversity of the habitat has been adversely affected at a particular site.

Some of the listed criteria in the original IHI score sheet (Kleynhans, 1996) were not suited to the terrain of this study. These criteria were thus excluded from the calculation of the IHI score (Kleynhans, 1996). The excluded criteria included the extent of inundation, presence of exotic aquatic fauna and presence of exotic macrophytes. The modified IHI score sheet used in this study thus comprised of five criteria for each of the *in-stream* and the *riparian zones.* Because of the modification of the score sheet, the percentage weights allocated to the remainder of the criteria used in this study had to be adjusted to accommodate the exclusion of some criteria.

In the original IHI score sheet the *in-stream* and the *riparian zone* criteria were weighted separately and their contribution calculated separately. The IHI score was calculated by summing the contributions of each of the *in-stream* and the *riparian zone* criteria. In this study, in the calculation of the modified IHI, weights were allocated to the combined criteria of the *in-stream* and the *riparian zones*, and the final IHI

score was calculated in one step. An example of the scoring sheet to calculate an IHI score for one site in this study is demonstrated in Table 5.9.

The proposed weights for this study were calculated in the following manner:

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

Table 5.9 Example of an IHI scoring sheet for impact of disturbance factors on macro-invertebrate habitats showing the modified weights

Criterion	Score	Original weight (%)	Proposed weight (%)	Estimation of impact of criterion
In-stream zone				
Water abstraction: e.g. pumps, irrigation, cultivated lands, settlements, industries	5	14	12	2.48
Water quality: clarity, odour, presence of macrophytes etc. due to untreated sewage, urban, and agricultural runoff	5	14	12	2.48
Flow modifications: relating to effects of abstraction or regulation by impoundments	5	7	7	1.24
Bed modification: Indirect indications of sedimentation are stream bank and catchment erosion	5	13	12	2.30
Solid waste	10	6	5	2.12
Riparian zone				
Water abstraction: presence of pumps, irrigation etc.	5	13	12	2.30
Water quality: clarity, odour, presence of macrophytes etc.	5	13	12	2.30
Flow modifications: This shows the consequence of abstraction or regulation by impoundments	5	7	6	1.24
Channel modification: This results in change in flow which alters the in-stream and riparian habitat	5	12	11	2.12
Bank erosion	10	14	12	4.96
TOTAL		**113**	**100**	**23.54**

The scores of the different criteria were entered in an Excel spread sheet and calculated for each of the sampling sties in all three seasons. Table 5.10 demonstrates how the IHI score was calculated for a particular sampling site using the data in Table 5.9.

Table 5.10 Calculation of an IHI score

1. Criterion scoring	Each of the criteria was scored according to the 26-point scoring system of Dallas (2005).
2. Moderation of impact score	Each criterion score was moderated by multiplying the score with the proposed weight. Thus, the moderated score = assigned score (Step 1) × proposed weight of impact. For example, in the case of water abstraction: Moderated score of a criterion = 5 × 12.
3. Estimated impact of a criterion	Estimation of an impact score for a criterion $= \frac{\text{Moderated score of a criterion (Step 2)}}{\text{Maximum possible value of a score}}$ The maximum value of a score was 25 according to the 26-point scoring system of Dallas (2005). For example, in the case of water abstraction: Estimated impact score for water abstraction = $\frac{5 \times 12}{25}$ = 2.4.
4. IHI score	The IHI score in this study represents the sum of all the estimated impacts scores of all the criteria, expressed as a percentage (Step 3). IHI score for a particular site = $\frac{\text{Sum of estimated impact scores} \times 100}{\text{Number of criteria}}$ For example, in case of the different scores presented in Table 5.9, the total impact score was = 23.54. Because the impact score is in percentage units the IHI is simply 100 minus the impact. IHI score = 100 - 23.54 = 76.46.

5.6 Qualitative assessment of the sampling sites

A qualitative assessment of all the sampling sites was undertaken to ascertain the overall quality of a particular sampling site. This assessment was undertaken using the various indicators of pollution sensitivity of the macro-invertebrate, as well as the macro-invertebrate habitat condition.

This assessment was undertaken by calculating a Quality Assessment Score (QAS) for each sampling site in the following manner:

1. The various indicators were listed in a table, namely, number of macro-invertebrates families observed at each sampling site, mean SASS scores, mean mIHAS scores and mean IHI scores.
2. For each of the indicators a qualitative assessment was performed, classifying them as relatively good, relatively acceptable and relatively poor.
3. Each of these indicators where then classified for each sampling site.
4. The overall quality of a sampling site was then determined by adding the number of quality descriptors.
5. Finally, a sampling site was then classified as being good, acceptable or poor by scoring the overall quality of the site with values ranging from one to six. A score of one or two was indicative of a poor quality site, three and four of an adequate quality site, while a score of five and six indicated a good quality site.

5.7 Analysis of data

5.7.1 Analysis of macro-invertebrate data

The number of different macro-invertebrate families found at each sampling site was determined for the 12 sampling sites. The degree of sensitivity of the different macro-invertebrate families to pollution was also determined by awarding sensitivity scores to each of the families identified (Gerber & Gabriel, 2002b). To determine the pollution condition as well as the diversity condition of the different macro-invertebrate families observed at each sampling site, the SASS scores and the ASPT scores calculated were classified using a modelled reference condition for the Highveld Eco-region (Dallas, 2007). Dallas (2007) developed a classification system incorporating both the SASS score and ASPT calculation

(Dallas, 2007) (Table 5.11). This diversity scoring system comprises of six classes (A to F), which can be used to describe the pollution condition as well as the macro-invertebrate diversity condition at a particular sampling site.

Table 5.11 Categories used to classify SASS and ASPT scores

SASS score	ASPT	Class	Condition	Description
>124	>5.6	A	Unimpaired.	High diversity of taxa with high sensitivity.
83-124	4.8-5.6	B	Slightly impaired.	High diversity of taxa, but with fewer sensitive taxa.
60-82	4.6-4.8	C	Moderately impaired.	Moderate diversity of taxa.
52-59	4.2-4.6	D	Considerably impaired.	Mostly tolerant taxa present.
30-51	<4.2 (Variable)	E	Severely impaired.	Only tolerant taxa present.
<30	Variable	F	Critically impaired.	A few tolerant taxa present.

5.7.2 Analysis of macro-invertebrate habitats

Analysis of the mIHAS scores

The mIHAS scores were calculated for each sampling site and interpreted using the Invertebrate Habitat Assessment System scoring guideline developed by McMillan in 1998 and updated in 2002 (Golder Associates, 2009) (Table 5.12).

Table 5.12 Macro-invertebrate habitat classification

mIHAS Score (%)	Description	Explanation
>65	Good	Highly suited for supporting a diverse aquatic macro-invertebrate community.
55-65	Adequate/Fair	Adequate for supporting a diverse aquatic macro-invertebrate community.
<55	Poor	Inadequate for supporting a diverse aquatic macro-invertebrate community.

Analysis of the IHI scores

The impact of disturbance factors was assessed using IHI (Kleynhans et al., 2008). The IHI scores obtained were interpreted using a rating system developed by Kleynhans et al. (2008), including a small modification of the description of the rating intervals, to describe the impact of disturbance factors on macro-invertebrate habitat integrity (Table 5.13).

Table 5.13 Description of the impact of disturbance factors on habitat intergrity (Kleynhans et al., 2008)

Habitat integrity criteria	Condition	Description	Rating (% of the total)
A	Unmodified	Habitat is largely natural, with negligible modifications.	>90-100
B	Largely natural with few modifications	The flow regime is only slightly modified and pollution is limited to sediment. A small change in habitat might have taken place.	>79-90
C	Moderately modified	Loss and change of natural habitat and biota have occurred, but the basic ecosystem functions are still unchanged.	>59-79

D	Largely modified	Large loss of natural habitat, biota and basic ecosystem functions has occurred.	>39-59
E	Seriously modified	The loss of natural habitat, biota and basic ecosystem functions is extensive.	>19-39
F	Critically, extremely modified	Modifications have reached a critical level and the systems have been modified completely with and almost complete loss of natural habitat.	0-19

5.8 Results of macro-invertebrate analyses

5.8.1 Macro-invertebrate families

A total of 27 macro-invertebrate families were observed at the 12 sites sampled in the Bloemspruit stream. Out of these 27 macro-invertebrate families, eight belonged to the order Diptera, which is able to reproduce rapidly and colonise areas polluted with organic runoff (Table 5.14). The two families Chironomidae and Oligochaeta, which are used as indicators for organic pollution, were found in more than 80% of sampling sites (Kotze, 2002). The numbers of Chironomidae families identified at the different sampling sites were relatively high.

Table 5.14 Macro-invertebrate families for Seasons 1, 2 and 3

Site	BS1			BS2			FS3			FS4			BF5			BS6			BS7			RS8			BK9			RK10			RS12		
Season	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Taxa																																	
ANNELIDA																																	
Oligochaeta	5	2		5	6	3	2			1						4		1				6	3	10		1	3		4	7		1	
Leeches	5	2	6			6										1							12	14				2	2	7		1	
EPHEMEROPTERA																																	
Baetidae																							6	2					3	9		4	1
CRUSTACEA																																	
Potamonautidae				2	3										1								5			11		3	1			3	
ODONATA																																	
Coenagrionidae	1			1		4																						2		2		1	
Aeshnidae				1																													
Gomphidae				10									4			4																	
Libellulidae																			1														
HEMIPTERA																																	
Belostomatidae	1												2						2				1					4			1		
Notonectidae																				1					1								
Corixidae		7		21	3								1							2		3	1	17			7	7	3	18	3		9
DIPTERA																																	
Simuliidae				20		7						1									1	4	2	6					2	11			
Syrphidae			1		16		5	12		8			2	1			1		1				2										
Ceratopogonidae	3	10		20	3	8																2											

Chironomidae	40	18	19	30	6	15	6	3	3	8	6	6	7	10		50	5	4	24	14	16	4	3	13	25	15	25	1	4	15	7	12	8
Culicidae		2	9		3	7	1			1		1	4				6			2				10		6	2		3			4	
Psychodidae		6				1			2						2							1		3						1			
Anthericidae	2																																
Muscidae		2																															
GASTROPODA																																	
Lymnaeidae	22	1	30		3	15																		1									
Physidae	5	5	30	20	3	6	1		1			1				3										1							1
Planorbinae		1																															
Ancylidae																								3						1			
COLEOPTERA																																	
Elmidae																1		1	14				1			1					2		
Hydraenidae																				1													
Hydrophilidae	1	1		1	2	1	1			1			1						13	2					1				2	4		2
Dytiscidae																							1			1					5		
Number of families	10	12	6	11	10	11	6	2	3	4	1	4	7	2	3	5	3	5	6	6	1	6	8	9	3	7	4	6	9	11	6	8	5

BR11 was not assessed because of the difficulty of the terrain

To gain a better perspective of the number of families identified at the respective sampling sites, the overall number of families per site, over the three seasons, was calculated. The histogram of the number of families per sampling site showed that the two sites in the built-up area (BS1 and BS2) demonstrated the highest number of families (Figure 5.3). Furthermore, the sampling sites beyond the confluence of the Bloemspruit stream and the Fonteinspruit, sites BS7 to RS12, also showed relatively high numbers of families, with RS8 site located in the Renosterspruit tributary displaying the same number of families as BS2. In comparison, the sampling sites, FS3 to BS6, demonstrated relatively low numbers of macro-invertebrate families. These sampling sites were probably polluted by the WWTP, cattle post and sewage leakage in their vicinity.

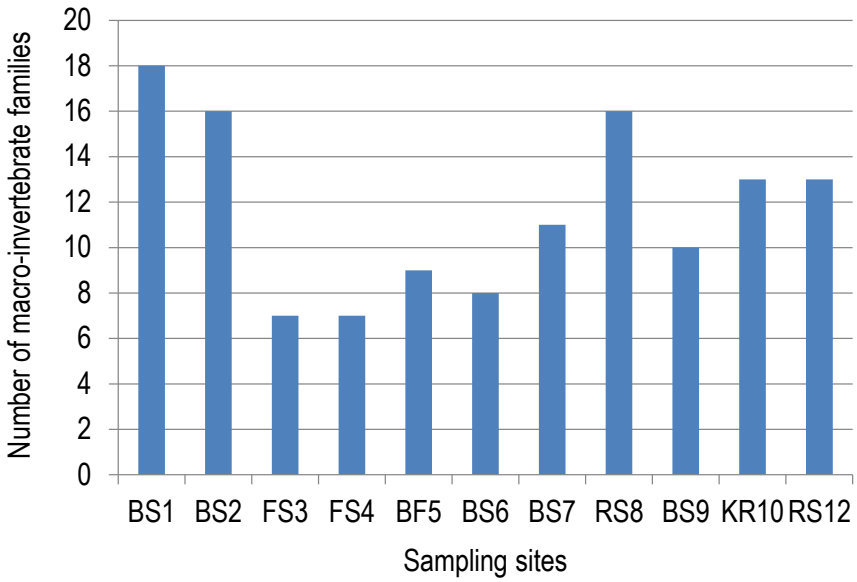


Figure 5.3 Total number of macro-invertebrate families at the 12 sampling sites over the three seasons

5.8.2 Sensitivity classification of macro-invertebrate families

Pollution sensitivity of the different macro-invertebrate families was calculated for each sampling site using the SASS method (Dickens & Graham, 2002). In this study, the 27 macro-invertebrate families that were identified were awarded sensitivity scores (Gerber & Gabriel, 2002b) (Table 5.15). These

scores are indicative of a family's sensitivity to pollution and range from one to 15, with 15 indicating a highly sensitive family.

Table 5.15 Macro-invertebrate family with sensitivity scores

Observed families	Sensitivity scores	Observed families	Sensitivity scores
ANNELIDA		Ceratopogonidae	5
Oligochaeta	1	Chironomidae	2
Leeches	3	Culicidae	1
EPHEMEROPTERA		Psychodidae	1
Baetidae	4	Anthericidae	3
CRUSTACEA		Muscidae	1
Potamonautidae	3	Syrphidae	1
ODONATA		**GASTROPODA**	
Coenagrionidae	4	Lymnaeidae	3
Gomphidae	6	Physidae	3
Libellulidae	4	Planorbinae	3
Aeshnidae	8	Ancylidae	6
HEMIPTERA		**COLEOPTERA**	
Belostomatidae	3	Elmidae	8
Notonectidae	3	Hydrophilidae	5
Corixidae	3	Hydraenidae	8
DIPTERA		Dytiscidae	5
Simuliidae	5		

The two sampling sites in the built-up area (BS1 and BS2) demonstrated the highest numbers of macro-invertebrate families in this study, except for the measurement at BS1, in Season 3 (Table 5.16). At the remainder of the sampling sites fewer macro-invertebrate families were found. Although the

sensitivity scores (SASS scores) were also the highest at these two sampling sites, the overall sensitivity per taxon as indicated by ASPT was similar for all the sampling sites; ranging from 1.3 to 4.3. The means of SASS scores for Seasons 1 and 2 were similar; while the mean SASS scores for Season 3 (cold season) was substantially less.

Table 5.16 Number of taxa, SASS scores and ASPT values for Seasons 1, 2 and 3 at the 12 sampling sites

Indices	Number of taxa			SASS scores			ASPT		
Seasons	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Sites									
BS1	10	13	6	39	32	13	3.9	2.5	2.2
BS2	11	10	11	48	27	35	4.4	2.7	3.2
FS3	6	2	3	13	3	6	2.2	1.5	2.0
FS4	4	1	4	5	2	7	1.3	2.0	1.8
BF5	7	2	3	21	3	5	3.0	1.5	1.7
BS6	5	3	5	17	4	12	3.4	1.3	2.4
BS7	6	6	1	23	24	2	3.8	4.0	2.0
RS8	6	8	9	17	28	25	2.8	3.5	2.8
BK9	3	7	4	10	27	11	3.3	3.9	2.8
RK10	6	9	11	18	34	38	3.0	3.8	3.5
RS12	6	8	5	26	30	14	4.3	3.8	2.8
Mean	6.4	6.3	5.7	21.6	19.5	15.3	3.2	2.8	2.4
Median	6	7	5	18	27	12	3.3	2.7	2.4
Min	3	1	1	5	2	2	1.3	1.3	1.6
Max	11	13	11	48	34	38	4.3	4.0	3.4
SD	2.34	3.85	3.32	12.49	13.31	12.13	0.91	1.04	0.58

BR11 was not assessed because of the difficulty of the terrain; SD = Standard deviation

5.8.3 Classification of the pollution condition

The SASS scores and the ASPT scores were classified using a modelled reference condition for the Highveld Eco-region (Dallas, 2007). The general trend of macro-invertebrates observed showed relatively low SASS scores for all three sampling seasons at most of the sampling sites. The SASS scores and ASPT values for this study fell within the E and F classes. The pollution condition of most of the sampling sites were thus classified as F (critically impaired), while the remainder were classified as E (severely impaired) (Table 5.17). A critically impaired condition reflects SASS values below 30 and only a few tolerant taxa present. On the other hand, a severely impaired condition represents SASS values from 30 to 50 and only tolerant taxa present.

Table 5.17 Classification of the SASS scores and ASPT values obtained for Seasons 1, 2 and 3

	SASS	ASPT	Condition*	SASS	ASPT	Condition*	SASS	ASPT	Condition*
Seasons	**1**	**1**		**2**	**2**		**3**	**3**	
Sites									
BS1	39	3.9	Severely impaired	32	2.5	Severely impaired	13	2.2	Critically impaired
BS2	48	4.4	Severely impaired	27	2.7	Critically impaired	35	3.2	Severely impaired
FS3	13	2.2	Critically impaired	3	1.5	Critically impaired	6	2.0	Critically impaired
FS4	5	1.3	Critically impaired	2	2.0	Critically impaired	7	1.8	Critically impaired
BF5	21	3.0	Critically impaired	3	1.5	Critically impaired	5	1.7	Critically impaired
BS6	17	3.4	Critically impaired	4	1.3	Critically impaired	12	2.4	Critically impaired
BS7	23	3.8	Critically impaired	24	4.0	Critically impaired	2	2.0	Critically impaired
RS8	17	2.8	Critically impaired	28	3.5	Critically impaired	25	2.8	Critically impaired
BK9	10	3.3	Critically impaired	27	3.9	Critically impaired	11	2.8	Critically impaired
RK10	18	3.0	Critically impaired	34	3.8	Severely impaired	38	3.5	Severely impaired
RS12	26	4.3	Critically impaired	30	3.8	Severely impaired	14	2.8	Critically impaired
% Severely impaired	18	18		27	27		18	18	
% Critically impaired	82	82		73	73		82	82	

* = according to Dallas (2007); Orange = Class E; Red = Class F; BR11 was not assessed because of the difficulty of the terrain

5.9 Results of macro-invertebrate habitat analyses

5.9.1 Results of mIHAS scores

The mIHAS scores were calculated to obtain an overall description of the quality and the condition of the macro-invertebrate habitats at each sampling site. These scores indicated that only 17% of the sampling sites demonstrated good enough conditions to support diverse aquatic macro-invertebrate communities, while a few (25%) habitats were poor and thus were too inadequate to support aquatic macro-invertebrate communities effectively. However, the majority of the sampling sites (58%) could only adequately support a diverse aquatic macro-invertebrate community (Table 5.18).

Table 5.18 mIHAS scores (%) calculated for 12 sites over the three seasons

Site	Season			Habitat description
	1	2	3	
BS1	74	73	66	Good
BS2	76	70	72	Good
FS3	47	52	44	Poor
FS4	46	50	44	Poor
BF5	68	55	59	Adequate/fair
BS6	56	59	57	Adequate/fair
BS7	62	59	57	Adequate/fair
BS8	59	61	59	Adequate/fair
BK9	65	65	74	Adequate/fair
RK10	65	65	60	Adequate/fair
BR11	39	40	40	Poor
RS12	65	60	63	Adequate/fair

5.9.2 Results of IHI scores

IHI scores were calculated to determine the impact of disturbance factors on macro-invertebrate habitats at each sampling site. The scores indicated that for all three seasons, only 8.3% of the macro-invertebrate habitats had been largely modified by disturbance factors, while the remainder (91.7%) had been moderately modified, but the basic ecosystem functions were still unchanged (Table 5.19).

Table 5.19 Index of Habitat Integrity (IHI) scores for Seasons 1, 2 and 3

Site	Season			Classification
	1	2	3	
BS1	76	69	75	C
BS2	76	76	77	C
FS3	58	59	57	D
FS4	67	70	65	C
BF5	69	62	73	C
BS6	75	67	77	C
BS7	77	75	76	C
BS8	78	71	76	C
BK9	72	71	74	C
RK10	74	71	78	C
BR11	67	63	67	C
RS12	67	66	67	C

C = Macro-invertebrate habitats have been moderately modified, but the basic ecosystem functions are still unchanged.
D = Large loss of natural macro-invertebrate habitat have occurred and basic ecosystem functions has changed.

5.10 Overall discussion and conclusions

A qualitative assessment of all the sampling sites was undertaken to ascertain the overall quality of a particular sampling site. This assessment took into account the quality of the macro-invertebrate communities, as well as the quality of the macro-invertebrate habitats. Overall, only sites BS1, BS2 and BK9 could be classified as being relatively good (Figure 5.4). This could probably be attributed to the

locality of these sampling sites. Sampling sites BS1 and BS2 are located in a build-up area, with few anthropogenic activities and thus limited polluting possibilities. Furthermore, because stones, vegetation as well as gravel, sand and mud macro-invertebrate habitats were represented at these sites; a variety of macro-invertebrate families were identified. On the other hand, the good condition of site BK9 could be attributed to the relatively fast flow of the stream at this point, thereby diluting pollutants that entered upstream. Also, all the different macro-invertebrate habitats were represented at this site. Additionally, the sampling site BK9 is located close to a conference centre Kopano Nokeng, who makes an effort to keep this area of the Bloemspruit stream clean and in good condition; mostly because of its recreational value. Also, at this point the stream embankments have been raised with stones to reduce erosion and prohibit pollution from the surrounding areas. These stones therefore prevent the riparian vegetation from being damaged, thus encouraging the survival of macro-invertebrates that thrive within the vegetation macro-invertebrate habitat.

The condition of the four sites, BS7, RS8, RK10 and RS12, could be classified as being relatively acceptable (Figure 5.4). Although BS7 is located in a high pollution region near a cattle post, its acceptable classification can be attributed to the fast water flow at this section of the stream, which assists in diluting the excessive pollutants. Sampling sites RS8, RK10 and RS12 are located in the Renosterspruit where minimal anthropogenic activities could be identified, thus limiting polluting possibilities (Figure 5.4). Additionally, the impact of disturbance factors on macro-inveterate habitats was relatively low.

The condition of the remaining sampling sites, FS3, FS4, BF5, BS6, BR11, were classified as being poor. The four sampling sites upstream of BR11, namely, FS3, FS4, BF5 and BS6, are located in an area largely affected by various polluting agents. These include a leaking sewage pipe into the Fonteinspruit, food processing plants, breweries, and a WWTP. Because of the sluggish flow of the water through these sites, dilution of pollutants is limited. Sampling site BR11, on the other hand, is

located at the confluence of the Renosterspruit and Bloemspruit stream and has been subjected to extensive erosion of the embankment, thereby destroying the macro-invertebrate habitats.

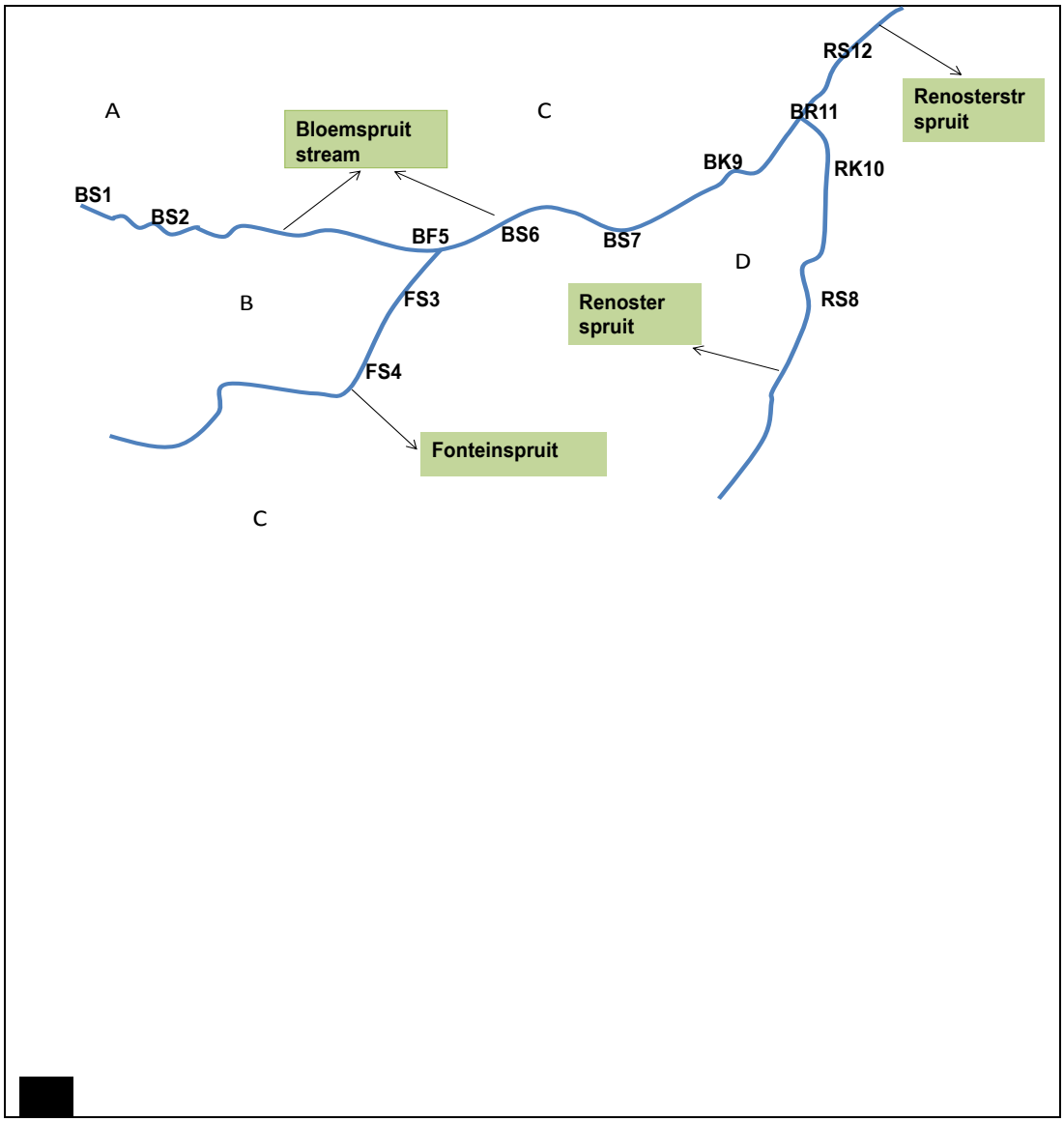


Figure 5.4 Qualitative assessment of the overall condition of the sampling sites

This study suggests that the overall ecological health of the Bloemspruit stream and its tributaries shows extensive degradation using macro-inveterate family and habitat indicators. This conclusion is supported by the low SASS scores and low ASPT values. Likewise, the presence of pollution tolerant macro-inveterate families at most sites and with sensitive families represented only at a few sites

proposes that this stream and tributaries need extensive consideration. Thus, without some intervention, aquatic life in the Bloemspruit stream and its tributaries can eventually be totally destroyed.

Chapter 6

Discussion and conclusion

6.1 Introduction

This study measured the water quality as well as the ecological quality at 12 sampling sites in the Bloemspruit stream flowing through parts of Bloemfontein and its outskirts in Mangaung. The Bloemspruit stream, which originates from the city of Bloemfontein meanders through the eastern side of the city towards the airport. The water quality of the stream was measured because the stream drains a large part of the Bloemfontein urban area, and at the periphery of the city, industries, a waste water treatment plant (WWTP), and informal dwellers dump their effluent and waste into the stream. In addition, the stream receives runoff from informal settlements and agricultural practices in its vicinity. Pollutants from these sources degrade the quality of water in the stream, causing temporal and spatial changes in the water quality. The ecological quality of the stream was also assessed using macro-invertebrates and their habitats as indicators of overall stream health (Ollis et al., 2006).

Water quality is usually described in terms of a range of physical, chemical and microbiological properties making it difficult to deduce what is the overall quality of water at a specific site (Sarkar & Abbasi, 2006; Lumb et al., 2011). Therefore, many different indices have been formulated incorporating a variety of properties into a single value. In this study the Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI) (CCME, 2001) was calculated and revealed that, except for two sites that displayed fair water quality (highest value of 73), the remainder presented with water of relatively poor quality (lowest value of 44). The macro-invertebrate family and macro-invertebrate habitat indicators also supported this outcome. The SASS scores, indicating the degree of sensitivity of macro-inveterate families to pollution, and average score per taxon (ASPT) were relatively low, ranging from 2 and 1.3 to 48 and 4.4 respectively. The overall condition and quality of the macro-invertebrate

habitats were also relatively poor with mIHAS values ranging from 39 to 76. The impact of disturbance factors further supported the notion that many of the sites in the Bloemspruit stream were degrading by revealing values ranging from 57 to 78. This indicated that although some sites were moderately modified, others were severely modified with a loss of the natural habitat and impaired ecosystem functions. Overall, at only two sites in this study the water and ecological quality were reasonable, probably because these sites are located upstream in the build-up area with few anthropogenic activities in their immediate vicinity.

6.2 Water quality of the Bloemspruit stream

Of the thirteen water quality properties measured in this study, seven demonstrated a large number of measurements that were beyond the proposed AQWUS limits. These properties included turbidity, nitrate, phosphate, dissolved oxygen, faecal coliforms, *E. coli*, and total bacterial counts. When the level of compliance was determined for these properties in the three sampling seasons, the percentage of non-compliance was found to be relatively high. These high levels could be strongly linked to the weather at the time of sampling. Intense thunder storms and runoff on the day or prior to the day of sampling in the first season resulted in high seasonal values that related to high non-compliant percentages for most of the properties. Although rains were encountered about a week prior to the collection of the Season 2 samples, they were not as high as those in Season 1. The samples of Season 3 were sampled during a relatively dry period, which is reflected in more compliant scores than the other two seasons.

Not all water properties with values outside the proposed AQWUS limits have a direct influence on the survival of macro-invertebrates in the stream (Palmer et al., 2004). For example, high levels of faecal coliforms, *E. coli*, and total bacterial counts do not affect macro-invertebrates directly (Palmer et al., 2004). However, the remainder of the properties assessed in this study may impact macro-invertebrate survival directly.

High turbidity measurements, which ranged between 1.4 NTU to 463 NTU, were recorded for most of the sites. The highest of these values were recorded after a rainfall event, which introduced runoff into the stream, from the Bloemfontein urban area, informal settlements, agricultural lands, industrial and WWTP effluents. This finding concurred with a similar study of the Modder River, which lies in the same catchment area as the Bloemspruit stream. High turbidity values, in the order of 800 NTU, were recorded after rains as opposed to values as low as 20 NTU during the drier times (Nadene, 2007). Similarly, domestic sewage water and effluents from industries in Ghaziabad, India, increased the turbidity of the Hindon River from 29 NTU at sites located upstream from industries to 109 NTU at sites in the industrial area (Suthar et al., 2010).

High turbidity levels in water may have adverse effects on macro-invertebrates. For example, the high levels of turbidity in the Lower Komati River of South Africa caused a decrease in the diversity of macro-invertebrates in the river (Dlamini et al., 2010). Furthermore, of all the measured properties, only turbidity revealed a significant relationship with the diversity of macro-invertebrates in this river (Dlamini et al., 2010).

In the Bloemspruit stream nitrate measurements ranging from 0.4 mg/L to 13.3 mg/L were recorded for most sites. High measurements were recorded especially at sites located downstream of the WWTP, the cattle post and other farming activities. Consistent with this study, Suthar et al. (2010) also measured high mean nitrate readings in the Hindon River in India of 245 mg/L, which exceeded the Indian surface water standards limits. These high readings could also be attributed to urban runoff and sewage from point sources. In contrast, the mean nitrate values were relatively low at downstream sites where pollution influences were lower. In a similar study conducted in Nigeria, the Alaro River demonstrated high mean nitrate levels because of effluent received from commercial farms and agricultural industries; whereas the mean nitrate levels were substantially lower in the Ona River that is not in close proximity to any similar polluting agents (Osibanjo et al., 2011). In South Africa, Oberholster

et al., (2010) reported high nitrate levels in Lake Loskop caused by runoff during the rainy season.

Exposure to high levels of nitrate over an extended period of time can affect fresh water macro-invertebrates, fishes and amphibians (Camargo et al., 2005). For example, increased nitrate concentrations from agricultural activities around the Amala and Nyangores tributaries of the Mara River in Kenya resulted in a decline of macro-invertebrate taxa diversity at downstream sites (Kilonzo et al., 2014). High levels of nitrate in streams may also cause abundant water plant growth in streams, which may in turn result in a decline in the macro-invertebrate diversity (Sulaimen et al., 2014).

Similar to the nitrate findings in this study, high phosphate readings were also recorded for most of the sites within the Bloemspruit stream, ranging from 0.1 mg/L to 8.7 mg/L. The phosphate may have originated from runoff which contains agricultural fertilisers, domestic and industrial effluents containing detergents, as well as sewage discharges from waste water treatment plants. In the Berg River in Western Cape, over the past 20 years anthropogenic activities have resulted in a 10 times increase of phosphorus and nitrogen levels (De Villiers, 2007). In a study in China high levels of phosphate and nitrate in rivers reduced the diversity of macro-invertebrate families, leaving mostly dominant families such as Tubificidae, Chironomidae and Physidae (Duan et al., 2011).

In the current study low dissolved oxygen levels were also recorded at most sites; ranging from 0.1 mg/L to 9.4 mg/L. These low levels can mostly be attributed to nutrient enrichment and organic pollution by sewage and effluent from WWTP. This finding is similar to what was found for a study on water quality of the Alaro River in Nigeria. The low levels of dissolved oxygen in the Alaro River were as a result organic pollution from industries (Osibanjo et al., 2011). However, in the absence of organic pollution sources at the vicinity upstream, the levels of dissolved oxygen were higher. In streams and rivers where low dissolved oxygen levels are encountered, the survival of macro-invertebrate families is directly affected. For example, low dissolved oxygen levels in urban streams in Brazil resulted in the reduction of the diversity of macro-invertebrate taxa (Couceiro et al., 2007).

6.3 Impact of Bloemspruit stream water on aquatic environment

To obtain an idea of the health of the water of the Bloemspruit stream, the macro-invertebrates were enumerated and their habitats assessed (Masese et al., 2013). The numbers of macro-invertebrate families identified at each sampling site were used to calculate a SASS score and an average score per taxon (ASPT) to determine the pollution condition, as well as the diversity of the macro-invertebrates for each sampling site (Dickens & Graham, 2002). When the SASS scores and ASPT obtained in this study were classified according to a reference condition for the Highveld Eco-region (Dallas, 2007), it was found that none of the sampling sites displayed conditions that can be deemed acceptable. The majority of the sites sampled were critically impaired, while a few were severely impaired. The presence of mainly tolerant macro-invertebrate families and the absence of sensitive families at most sites were indicative of the poor state of these sites.

The decline in the macro-invertebrate families was evident by the presence of tolerant macro-invertebrate taxa; particularly the Chironomidae, belonging to the order Diptera, as well as Oligochaeta of the order Annelida. Consistent with this study, high numbers of Chironomidae and Oligocheata families were also recorded in the Klip River of South Africa, particularly at a site close to the residential area of Lenacia (Kotze, 2002). Another study in Tawi, India, confirmed that the dominant families Chironomidae and Oligocheata were observed at sites polluted by sewage effluent and other anthropogenic stressors, while the sensitive taxa Plecoptera and Ephemeroptera were absent at these sites (Sharma & Chowdhary, 2011). Similar to these studies, the presence of pollution tolerant macro-invertebrate families in the Bloemspruit stream could be explained by pollution from the immediate environment as a result of de-oxygenation caused by the breakdown of organic matter by bacteria introduced by a leaking sewage pipe, extensive industrial activities and a WWTP (Wenn, 2008).

Several indexes are available to measure the condition of the macro-invertebrate habitats. However, most of these indexes have some or other limitation, thus producing relatively inaccurate assessments (Duan et al, 2011). For this reason a modified Invertebrate Habitat Assessment System (mIHAS) was developed. The mIHAS describes the condition of a macro-invertebrate habitat with a single number using different habitat attributes, as well as water quality properties. In this study the low SASS scores and mIHAS scores were indicative that most of the macro-inveterate habits were not able to support diverse aquatic macro-invertebrate families. Consistent with this finding, low SASS scores and IHAS scores were also obtained for the Sand River tributary, mainly because of absence of certain macro-invertebrate habitats that can support macro-invertebrates communities (Venter, 2013).

Relatively low Index of Habitat Integrity (IHI) scores were obtained for the different sampling sites in this study. This was mainly attributable to the impact of human factors such as bed modifications from sewage and organic pollution, flow modification from industrial and WWTP effluent, as well as bank erosion due to increased water flows. Therefore, it can be concluded that the decline of macro-invertebrates within the Bloemspruit stream is as a result of poor water quality and inadequate macro-invertebrate habitat conditions.

6.4 Conclusion

Overall, the results obtained from this study have revealed that the health of the Bloemspruit stream has been degraded mostly by its immediate environment, which include WWTP, informal human settlements, as well as extensive industrial activities along is stream banks. There are a number of anthropogenic activities along the Bloemspruit stream that could be directly influenced by the poor condition of the stream. Many people from informal settlements (particularly the homeless) use the water for domestic purposes and fish in the stream for food. Because of the high levels of faecal coliforms and *E. coli* in the water, people from these informal settlements are at risk of being exposed to pathogens, which may result in skin rashes, throat and ear infections, irritations of eyes and mucous

membrane (DWAF, 1996b; DWAF, 1996e; RHP, 2005). In addition, these people may also suffer from gastrointestinal diseases such as diarrhoea, cholera, typhoid fever and dysentery if the water is accidentally ingested in large quantities (Wade et al., 2003). The water of the Bloemspruit stream is also used extensively for the irrigation of the vegetables grown in its vicinity. The danger is that faecal coliforms and *E. coli* may be transferred onto irrigated vegetables. And, when such contaminated vegetables are consumed raw, humans may suffer from gastrointestinal diseases such as diarrhoea, cholera, typhoid fever and dysentery (Gemmell et al., 2012). Besides humans being affected by the poor state of the Bloemspruit stream, animals may also contract diseases transferred from the water.

From these data, it has been confirmed that the water quality of the Bloemspruit stream is dire. If local authorities do not recognise this situation and implement an emergency solution, the quality of water in this stream will progressively deteriorate. Thus, aquatic organisms in the stream are therefore threatened as a result of the extensive polluting activities in the immediate vicinity of the Bloemspruit stream.

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