BIOLOGICAL MONITORING OF MINING POLLUTION IN TRIBUTARIES OF THE OLIFANTS RIVER IN THE SEKHUKHUNE AREA

By

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DISSERTATION

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DECLARATION

“I declare that the dissertation hereby submitted to the University of Limpopo, for the degree of Masters of Science in Zoology has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged”.

Makwarela

Mr M Makwarela

Date

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EXTENDED ABSTRACT

Water pollution has been one of the major concerns all over the world for at least the past two decades. In South Africa, the Olifants River System is one of the most polluted river systems. Anthropogenic activities being carried out within the Olifants River Catchment area pose threats to the aquatic ecosystem. The Upper and Middle catchments are being characterised by intensive mining, industries, agricultural practices and inadequate sewage treatments. Thus, the aim of this study was to investigate the influence of mining activities on the water quality and health status of the Steelpoort River, a tributary of the Olifants River System. This was achieved through assessing the quality of water by analysing physico-chemical parameters, macro-nutrients and metals at four selected sites, determining the response of macro-invertebrate assemblages to water quality using the South African Scoring System (SASS) version 5 and determining the diversity of fish using the Fish Response Assessment Index (FRAI).

Sampling of water, sediment, macro-invertebrates and fish was done seasonally (August 2017 – May 2018) at four selected sites. Site 1 and site 2 were located upstream while site 3 and site 4 were located midstream and downstream respectively. Water and sediment samples were analysed by WATERLAB (PTY) LTD by means of Inductively Coupled Plasma Optical Spectrometry (ICP-OES). Macro-invertebrates were sampled following the SASS protocol. Macro-invertebrates were identified, counted and recorded then released back to the river. Fish were sampled following the FRAI index protocol.

The results obtained indicated that the system variables; pH, water temperature, dissolved oxygen and total dissolved solids fell within the target water quality range (TWQR) for aquatic ecosystems. However, some concentrations of macro-nutrients recorded were above the TWQR. These include ammonium at site 3 and nitrogen at sites 2, 3 and 4 which indicated that there was a variation in the influx of macro-nutrients into the river at different river sections. The higher concentrations of ammonium and nitrogen may adversely affect the functioning and survival of biological communities. The metal results indicated that most metals (As, B, Ba, Cd, Cu, Pb, Ni and Cr) fell within the recommended water and sediment quality guidelines (DWAF 1996c; CCME 2012). Chromium concentrations recorded in sediment were above the sediment quality guideline at all the selected sites (CCME 2012). Iron and Zn were also above the guideline values at all selected sites. In terms of physico-chemical parameters and metal concentrations indicated that the water quality of the Steelpoort River was fairly good.

The macro-invertebrates were also analysed; their abundance, distribution and family richness indicated that there was a deterioration of water quality from upstream to downstream which may be an indication of increase in influx of pollutants and modifications in the stream such as flow, cover and microhabitat. The highest macro-invertebrate abundance and richness was at site 1 while the lowest was at sites 3 and 4. Site 3 was highly modified while site 1 was the least modified site. The Ephemeroptera, Plecoptera and Trichoptera index (EPT) and Ephemeroptera, Plecoptera and Trichoptera/Chironomidae ratio (EPT/C ratio) analysis also confirmed that the water quality of the Steelpoort River is deteriorating from upstream to downstream. Site 1 had the highest value of EPT while site 2 had the highest value of EPT/C. Site 3 had the lowest value for both EPT and EPT/C ratio. The higher EPT and EPT/C ratio indicate the presence of highly sensitive taxa. The Canonical Correspondence Analysis (CCA) indicated a strong correlation between metals (Pb, Cr, Mg, As and Se) and macro-invertebrates (Pleidae, Ecnomidae, Athericidae, Synlestidae, Lestidae and Pyralidae). The SASS 5 results also indicated deterioration of water quality from upstream to downstream with the highest values of SASS score and Average Score Per Taxon (ASPT) being recorded at site 1 followed by site 2 while the lowest SASS score and ASPT were at site 3 followed by site 4.

The use of fish as biological indicator also supported the same pattern of water quality deterioration and influx of pollutants which was previously indicated as increasing from upstream to downstream of the Steelpoort River. Site 1 had the highest fish abundance, while site 4 had the lowest fish abundance. However, the fish species richness was highest at site 4 while the lowest species richness was at site 1. The Shannon Weiner Diversity Index also supported that site 4 had the highest fish species richness while site 1 had the lowest species richness. The FRAI results indicated that different sites were in different Ecological Categories (EC). The ECs showed a trend from higher EC category upstream to lower EC category downstream. Site 1 had an EC of C followed by site 2 with an EC of C/D, site 4 with EC of D and then site 3 with the lowest EC of D/E. This might serve as an indication of decrease in habitat availability, increase of pollutants input and increase in stream modification. The CCA showed a weak correlation system variables and fish species. However, a strong correlation was

observed between most metals and most fish species with the exception of *Mesobola brevianalis* Boulenger, 1908, *Chiloglanis pretoriae* Van Der Horst, 1931, *Labeobarbus marequensis* Smith, 1841 and *Enteromius neefi* (Greenwood, 1962).

In conclusion, the water in the Steelpoort River is still in relatively good condition. However, increasing mining, industrial and agricultural practices in the catchment area results in increase of pollutants input into the river system. All the four selected sites were contaminated to some degree, with site 3 being the most affected site and site 1 being the least affected site. For this reason, it is important to continuously monitor the health status of the Steelpoort River and to educate the nearby communities who rely on this river for water supply about the quality of the water from the Steelpoort River.

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LIST OF ABBREVIATIONS

AEV	Acute Effect Values
ASPT	Average Score Per Taxon
BDI	Biological Diatom Index
BMWP	Biological Monitoring Working Party
CEV	Chronic Effect Values
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
FRAI	Fish Response Assessment Index
GSM	Gravel, Sand and Mud biotopes
HAI	Health Assessment Index
ICP-OES	Inductively Coupled Plasma Optical Spectrometry
KNP	Kruger National Park
MV	Marginal Vegetation
MVegIC	Marginal Vegetation in Current
MvegOOC	Marginal Vegetation out of Current
NH4	Ammonium
NO2-N	Nitrite-nitrogen
NO3-N	Nitrate-nitrogen
pH	Potential of Hydrogen
PO4-P	Phosphate as Phosphorus
RHP	River Health Programme
RVI	Riparian Vegetation Index
S	Stone and rock Biotopes
SASS	South African Scoring System
SAWQG	South African Water Quality Guidelines
SIC	Stone in Current
SOOC	Stone out of Current
SV	Submerged Vegetation
TDS	Total Dissolved Substances
TWQR	Target Water Quantity Range
VEG	Vegetation Biotopes

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Water is the one of the most valued resources on land and is the medium in which all living processes occur. Water is essential for a broad diversity of life. However, only a small percentage of the total water on earth is available as freshwater (Sharip 2016). Water shapes the earth's surface and moderates our climate. As global population growth continues to increase, and with rapid industrialisation, urbanisation and escalating demand for food, it consequently leads to over utilisation of natural resources (Mgbemene et al. 2016).

The Millennium Ecosystem Assessment (2005) reported that industrialisation has led to a huge increase in the discharge of toxic chemicals into freshwater bodies over the past half century all over the world. The Africa region, with many developing countries, is no exception to the high demand of water and high level of anthropogenic activities which poses threats to the limited water resources (Awoke et al. 2016). In the southern region of Africa lies South Africa, which is a semi-arid country with a high population increase rate and inadequate water bodies (Arthington et al. 2010; Du Plessis 2017). In South Africa, freshwater ecosystems are the most threatened ecosystems (Edokpayi et al. 2017). The ability of freshwater bodies to maintain a good biodiversity and provide clean reliable sources of water is severely impacted by alterations (Ishiyama et al. 2016). Such alterations include bank and channel modifications which lead to habitat loss and thus ecological degradation. In addition, anthropogenic activities such as industrialisation, mining and agriculture has led to pollution of river systems (Wilson et al. 2019). Together all these activities had caused a decline of the water quality and availability and altered the aquatic biota structure and its distribution (Souto et al. 2019).

Sustainability and prosperity of human communities are dependent on freshwater, and the presence of a safe and reliable source of water is a crucial prerequisite for the establishment of a stable community. However, most developing countries are facing huge challenges in wastewater and pollution (Du Plessis 2017). In South Africa, most local governments have been failing to maintain urban effluents to acceptable standards

(Edokpayi et al. 2017). As a developing country with rapid population growth and increasing urbanisation, the problem continues to exist thus leading to deterioration of health of many rivers. The supply of freshwater has also been one of the major problems in the country, and such limited water supply results in low economic growth (Tissington et al. 2008; Zhang et al. 2016).

Water has been identified not only as one of the most limited natural resources in South Africa, but also as one of the most polluted natural resources (Du Plessis 2017). Water pollution is defined as direct or indirect discharge of organic and inorganic pollutants into water bodies. Such discharges are the main cause of decline in the quality of water and the number of aquatic organisms and may impose threats to humans who utilise these water sources (Kenney et al. 2009). Both organic and inorganic pollution can be caused by anthropogenic activities or can occur naturally. Emeka et al. (2009) reported that in most African countries, there has been serious health and environmental concerns noted in areas close to rivers that pass through industrial, mining and agricultural areas since they tend to be highly polluted. The quality of water refers to the measure of water condition with respect to its chemical, physical and biological characteristics relative to the requirements of biotic species or any intended purpose (Dalu and Froneman 2016). The water quality suitable for one purpose may not be acceptable for another, since the quality of water varies greatly with respect to its various uses (Liao et al. 2018). It is thus important to monitor freshwater systems to detect any deterioration in water quality.

1.2 BIOLOGICAL MONITORING

Traditionally, monitoring of water resources was based on the analysis of chemical and physical properties of water to determine the water quality (Grigore et al. 2018). This method can accurately quantitate the amount of individual substances in the water. However, this type of monitoring has some major limitations. The results found tend to reflect water conditions only at sampling time, it is almost impossible to measure all different chemical substances that could be available in the water and it is difficult to determine interactive and antagonistic effects resulting from different stressors (Dallas 2008; Grigore et al. 2018). To get a broader picture on the condition of a river, a wide variety of alternative methods have been used worldwide.

These alternative methods are based on the use of selected aquatic organisms. Such organisms are referred to as bio-indicators and monitoring using these organisms is referred to as biological monitoring or biomonitoring (Ojija and Laizer 2016). Organisms mostly used for bio-monitoring have varying sensitivity and resistance to different levels of pollutants and they also respond differently to such levels of pollutants. Biological monitoring methods have progressively turned out to be the best alternative tool for monitoring the quality of water bodies (Keck et al. 2017). The crucial distinction between biological monitoring and traditional monitoring is that living organisms show pollution effects that are undermined by the chemical and physical measurements which are occurring in nature (Keck et al. 2017). Due to these, biological monitoring therefore consequently gives a more extended and incorporated perspective of the ecological state and the water quality of a water body (Rak et al. 2017).

Fish and benthic macro-invertebrates and diatoms are mainly used in biomonitoring because their assemblages are influenced by chemical and physical stresses imposed in their habitat (Souto et al. 2019). Community structures and health of these organisms give a good indication of the health status of the river (Everall et al. 2017). In South Africa, the River Health Programme (RHP) was introduced in 1994 by the then Department of Water Affairs and Forestry (DWAF) now known as the Department of Water and Sanitation. The RHP monitor the river health status mainly using biological indicators such as benthic macro-invertebrates, fish and diatoms (Kleynhans et al. 2005). Guidelines were produced for different water use with specific guidelines outlining water quality criteria for safeguarding freshwater ecosystems in South Africa (DWAF 1996c). The water quality criteria include the Target Water Quality Range (TWQR), the Chronic Effect Values (CEV) and the Acute Effect Values (AEV). All of these are used to evaluate specific water quality constituents, however, the TWQR is commonly used.

In addition, a variety of biomonitoring indices have been developed and used in South Africa to monitor the health of aquatic ecosystems, for example: Fish Response Assessment Index (FRAI), Riparian Vegetation Index (RVI), Health Assessment Index (HAI), South African Scoring System (SASS) and Biological Diatom Index (BDI) (Chutter 1998; De la Rey et al. 2008). Some of the indices such as SASS and BDI are good indicators of short-term effects, whereas FRAI is a good indicator of long-term effects. Currently, version 5 of SASS is used (Dickens and Graham 2002; Bellingan et al. 2015).

In this study, SASS 5 in conjunction with FRAI were used. This is because unlike the HAI, the two indices are designed to assess the health of the river rather than the health of the organisms themselves (Jooste et al. 2013).

1.3 THE SOUTH AFRICAN SCORING SYSTEM

The SASS is a biotic index developed by Chutter (1994) for use in riverine ecosystems. SASS was developed based on the Biological Monitoring Working Party (BMWP) method developed in the United Kingdom. It is based on benthic macro-invertebrates, where each taxon (family) is assigned a score based on its sensitivity or tolerance to water quality impairment. SASS has undergone several improvements resulting in the current South African Scoring System version 5 (SASS 5) (Dickens and Graham 2002). SASS 5 results are normally expressed in two ways which include SASS score and Average Score Per Taxon (ASPT). It has been thoroughly tested and is widely accepted in South Africa as a river health assessment tool (Dallas 1997; Dickens and Graham 1998; Fouché and Vlok 2010). It is easy and simple to carry out SASS 5 and it does not require sophisticated equipment. The method is generally cheap, sampling is generally non-destructive except where representative collections are required, and results are easy to interpret. SASS 5 provides a clear view of biological status of rivers in terms of environmental water quality (Gordon et al. 2015). However, SASS 5 as a rapid bioassessment method has some limitations which include masking ecological information from all levels of the ecosystem since it is based only on family level and inability to detect changes in species composition, abundance and distribution within a family in relation to the change in water quality over time (Jackson et al. 2016). Therefore, these limitations bring a need for the exploration and possible application of other intensive methods such as FRAI and Biological Diatom Index (BDI) that could be used along with SASS 5 in the assessment of water quality of a waterbody. The use of a multimetric approach is better than using a single biotic index since multimetric approaches tend to provide more ecological information needed for the management of water resource (Sanchez-Montoya et al. 2010; Arman et al. 2019)

1.4 FISH RESPONSE ASSESSMENT INDEX

The FRAI was developed by Kleynhans (2007) in South Africa and is used in EcoClassification and EcoStatus. This index is based on the environmental intolerances, preferences of fish assemblage and the response of species of the assemblage with respect to environmental drivers. FRAI provide a habitat-based cause and effect, which helps to interpret the deviation of fish assemblages from the reference assemblage conditions (Kleynhans 2007). The number of species expected for the reference condition is compared with the observed (sampled) species. The metric assessed in the FRAI include velocity depth preferences, flow requirements, physico-chemical preferences, introduced species and cover preferences. Unlike other indices FRAI ratings adopted for FRAI are based on the increase or decrease from reference conditions, however rankings and weighting system adopted for this index is the same as for other indices for EcoClassification and EcoStatus (Kleynhans and Louw 2007).

1.5 OVERVIEW OF THE OLIFANTS RIVER CATCHMENT

The Olifants River catchment is comprised of four sub-catchments namely; Lower, Steelpoort, Middle (upper and lower Middle) and Upper sub-catchments. The river itself originates from east Gauteng and flows to the northeast and passes through the Kruger National Park (KNP) and joins the Limpopo River in Mozambique (Heath et al. 2010; Morokong et al. 2016). The Olifants River is one of the South African rivers subjected to prolonged ecological stress and is one of the hardest working rivers in the country (Morokong et al. 2016).

The Upper sub-catchment of the river is characterised mostly by intensive anthropogenic activities which include mining, agriculture and coal power plants which causes dramatic decline of the water quality of the river (Jooste et al. 2013). The Middle Olifants sub-catchment is about 300 km long and comprises of the area between Loskop Dam and the junction of the Olifants and Steelpoort rivers. The sub-catchment has about 28,800 hectares of agricultural area, mining areas and highly erodible soil in Sekhukhuneland which contribute to high loads of pollutants to the Olifants River in this region (Heath et al. 2010).

The Steelpoort sub-catchment comprised of intensive mining and small-scale agricultural activities. Intensive mining in this area includes platinum, alluvial gold, coal, vanadium, ferrochrome, magnesite and granite mines (Jooste et al. 2013).

The Lower Olifants sub-catchment stretches from Drakensberg through the KNP to Massingir Dam in Mozambique. This area is mainly characterised by game farms and industrial activities alongside the border of the KNP in Phalaborwa town (Njiraini et al. 2016). The industrial activities have become a major concern to conservationists after a report of fish kills that occurred downstream of the Phalaborwa Barrage (Warner et al. 2016). However, this area has very little agricultural activities.

Huchzermeyer et al. (2017) reported a fish die-off that occurred in Loskop Dam of the Olifants River, which has affected most of the indigenous fish species. Koelmel et al. (2019) and Warner et al. (2016) also reported major crocodile die-offs in the Olifants River in the KNP. All these die-offs were believed to be associated with the anthropogenic activities occurring at upper and middle Olifants River catchment (Biggs et al. 2017). These could be evident of pollution in the Steelpoort River catchment.

1.6 PURPOSE OF THE STUDY

Water scarcity and pollution have become one of the major problems in 21st century. Although many rivers are being monitored, it remains an important task to monitor the impact of specific human activities, such as mining. This is because monitoring of water quality provides empirical evidence to support decision making on health and environmental issues. Communities living in the Olifants River basin are dependent on the water from the Olifants River and its tributaries for livelihood activities, such as agriculture, bathing, washing and drinking. However, agricultural, industrial and mining (coal, platinum, phosphate and copper) activities concentrated at the upper Olifants River sub-catchment are believed to negatively affect the Olifants River and its tributaries thus, consequently leading to poor water quality.

1.6.1 AIM

The aim of the study was to investigate the influence of mining activities on the water quality and health status at four sites of the Steelpoort River in the Sekhukhune area using SASS 5 and FRAI indices during different seasons (summer, autumn, winter and spring).

1.6.2 OBJECTIVES

The objectives of this study were to:

i. assess the quality of water by analysing physico-chemical parameters, macro-nutrients and metals.
ii. determine the response of invertebrate assemblages to water quality using SASS 5.
iii. determine the diversity of fish using FRAI.

1.7 DISSERTATION LAYOUT

Chapter 1: General introduction – Introduces the study, includes a literature review, brief description of the Olifants River catchment, outlines the purpose of the study, aims and objectives.

Chapter 2: Method and materials – Contains a description of the study area and the selected sites. A description of the methods and materials used for water analysis and sampling of fish and aquatic macro-invertebrates is also included.

Chapter 3: Water and sediment quality – Focuses on water and sediment quality and other selected water variables determined during seasonal surveys at all four selected sites. This chapter includes results in form of graphs and tables and discussion of such results. Conclusion on water and sediment quality is included in this chapter.

Chapter 4: Macro-invertebrates as bioindicators – Contains background on aquatic macro-invertebrates. This chapter contains SASS 5 results in the form of graphs and tables, statistical analysis, discussion of the results and possible influence of the results and conclusion on SASS 5 findings.

Chapter 5: Fish as bioindicators– Contains background on fish and the results obtained from FRAI. Statistical analysis and discussion of the FRAI results and possible influence of the results and conclusion drawn are included in this chapter.

Chapter 6: Results obtained is summarised and conclusions are drawn. This chapter also includes management and conservation recommendations of the river studied.

The journal of African Zoology referencing format is followed.

CHAPTER 2

STUDY AREA

2.1 STUDY AREA

The Steelpoort river sub-catchment is one of the four sub-catchments of the Olifants River catchment and covers an area of about 7,139 km^2 (Stimie et al. 2001; Morokong et al. 2016). The Steelpoort sub-catchment consists of three sub-catchments, namely: the Upper Steelpoort, Central Steelpoort and Lower Steelpoort sub-catchments. The mean annual rainfall of the area ranges between 630 and 1000 mm. Rainfall occurs predominantly in summer months, with January generally experiencing heaviest rain with associated low infiltration of the soil which result in soil erosion (Njiraini et al. 2016).

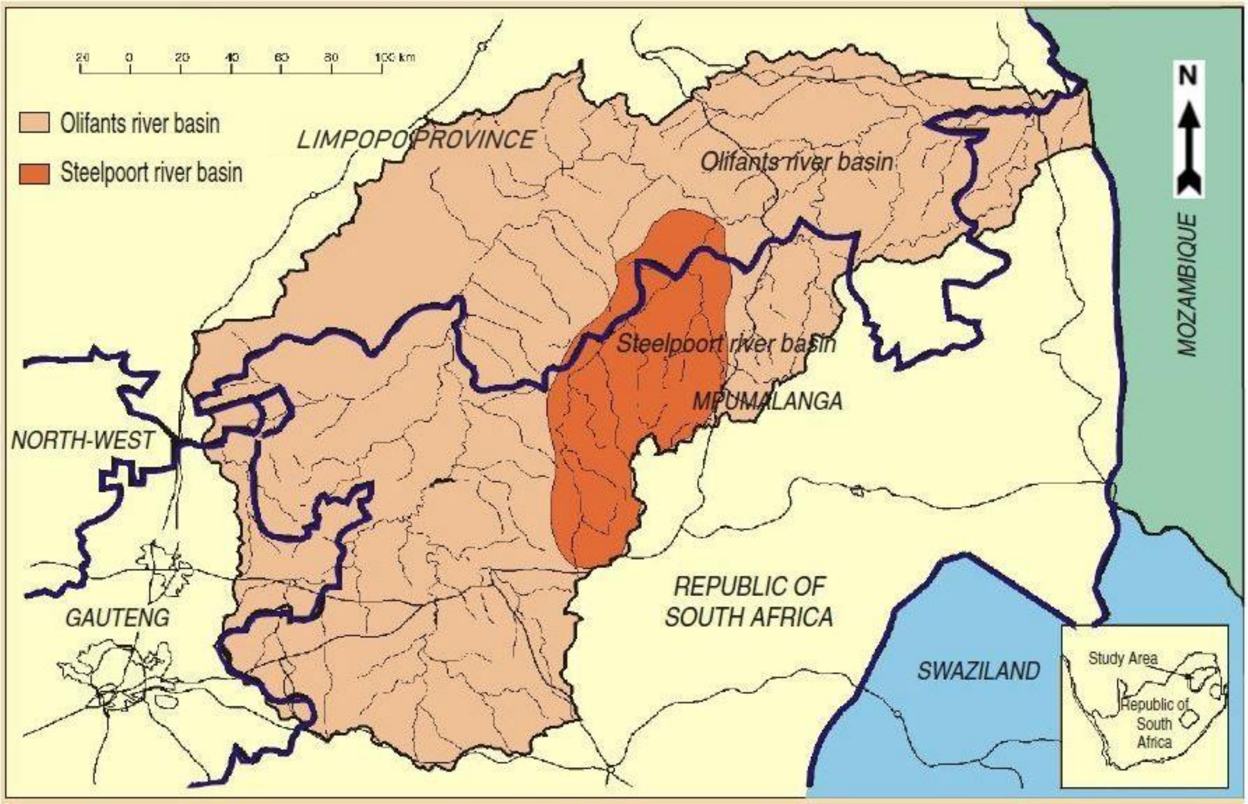

Figure 2.1: Olifants and Steelpoort river catchment areas (Stimie et al. 2001).

A study done by DWAF (1995) on water quality of the Middle Steelpoort sub-catchment revealed that both surface and ground water in this sub-catchment are being further threatened by an increasing level of contaminations from mining, industrial, agricultural and residential sources. There is a high concentration of mines in the Steelpoort River basin (DWAF 2004). According Mativenga and Marnewick (2018) the Olifants River basin was estimated to have around 62 mines which include chrome, granite, coal, platinum, magnesite, alluvial gold, vanadium and mines for construction material, such as bricks and sand.

2.2 SAMPLING SITES

Four sites (sites 1, 2, 3 and 4) were selected along the Steelpoort River to assess the influence of anthropogenic activities (mainly mining) on the water quality, benthic macro-invertebrate assemblages and fish diversity. For each sampling site, four surveys were undertaken, the surveys were conducted in August 2017 (winter), November 2017 (spring), February 2018 (summer) and May 2018 (autumn).

Figure 2.2: Satellite image of all selected sites in the Steelpoort River (From: Google Earth).

SITE 1

Site 1 (25° 6'20.87S, 29°51'7.40E) is situated a few kilometres upstream of De Hoop Dam (Figure 2.3, Figure 2.4). It is situated under a bridge of a gravel road, the riparian area alongside this site is characterised by high density of vegetation and it is surrounded by natural vegetation where there are little anthropogenic activities taking place, however there is small-scale livestock farming in the area. The gravel road near this site plays a role in depositing soil into the stream.

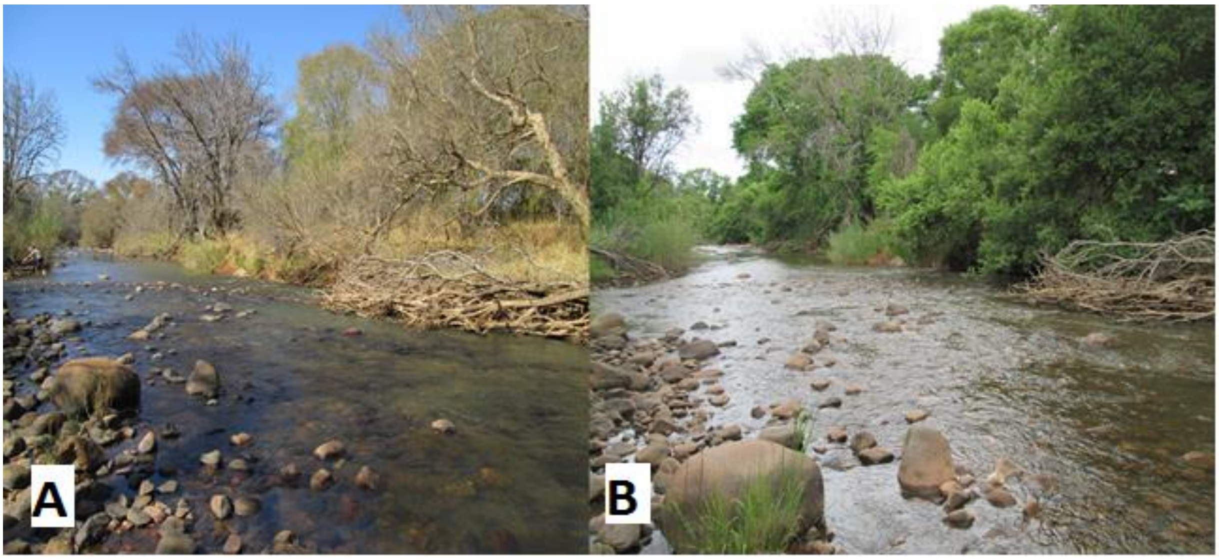

Figure 2.3: Site 1 (A+B), Upstream of De Hoop Dam.

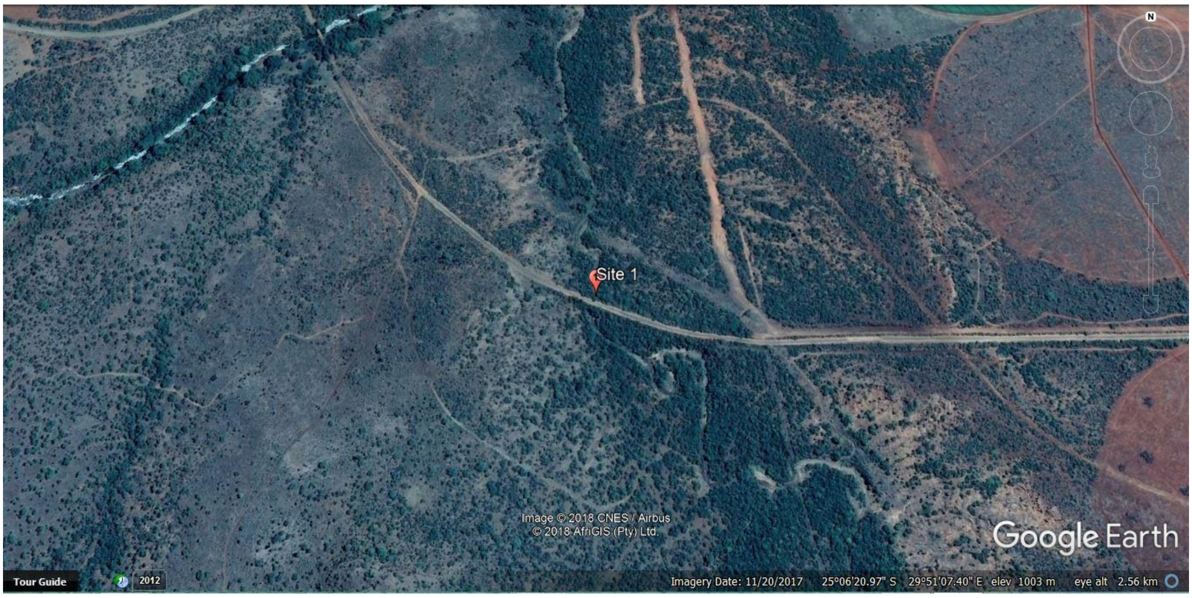

Figure 2.4: Satellite image of site 1 in the Steelpoort River (From: Google Earth).

SITE 2

Site 2 (24°49'49.69S, 30° 4'44.60E) is located at the confluence of Steelpoort River and Dwars River and is near a settlement area (Figure 2.6). There is a small-scale agricultural practice occurring near this site. The residence of the nearby settlement uses water directly from this site and extract sand from the stream. The riparian area is characterised by grasses and shrubs (Figure 2.5).

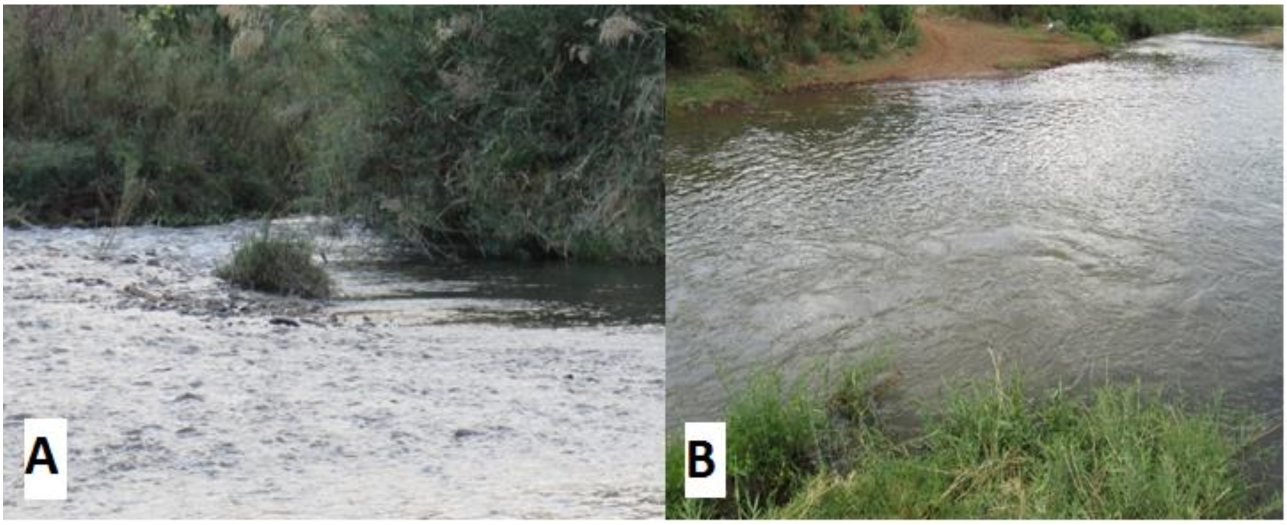

Figure 2.5: Site 2 (A+B), Dwars and Steelpoort confluence.

Figure 2.6: Satellite image of site 2 in the Steelpoort River (From: Google Earth).

SITE 3

Site 3 (24°43'4.70S, 30°12'3.40E) is situated downstream of a bridge on a road from Matholeng before entering Steelpoort town (Figure 2.7). The southern riparian area has little soil cover with more sand exposed to the surface which is easily eroded into the stream during rainfall, however the northern riparian area has a relatively rich soil cover characterised by grasses, shrubs and trees (Figure 2.7). This site is located near mining areas, residential area and a dumping site which has a potential negative effect on the water quality at the site (Figure 2.8).

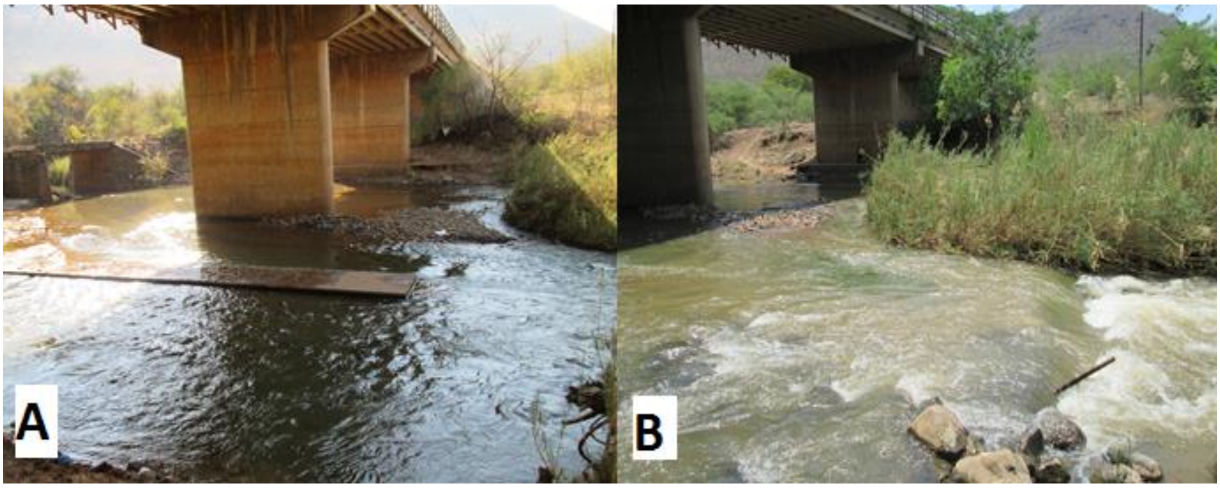

Figure 2.7: Site 3 (A+B), Steelpoort Bridge.

Figure 2.8: Satellite image of site 3 in the Steelpoort River (From: Google Earth).

SITE 4

Site 4 (24°39'33.60S, 30°18'7.20E) is situated in Burgersfort town, downstream of R37 road entering the town (Figures 2.9 and 2.10). There is a dumping site upstream from site 4. Sand is washed into the stream during heavy rains. The riparian area has a soil cover comprised of scattered shrubs and small grasses on an open field on the right side of the river (Figure 2.10). This site is downstream of all other sites and most mining areas.

Figure 2.9: Site 4 (A+B), Burgersfort Bridge.

Figure 2.10: Satellite image of site 4 in the Steelpoort River (From: Google Earth).

CHAPTER 3

WATER AND SEDIMENT QUALITY

3.1 INTRODUCTION

3.1.1 WATER QUALITY

Water is a vital natural resource and fundamental to any form of life, however it is one of scares natural resources. Although water occupies about 70% of the earth's surface, only about 2.5% of that water is fresh with the remainder being mostly salty seawater. About 70% of the freshwater on earth is not available for use, it is frozen as polar ice caps. Only about 0.01% of the water is available in streams, a huge amount of the remaining freshwater is hidden away as ground water (Aremu et al. 2017). Despite the little amount of water available for use, anthropogenic activities have been negatively affecting the water quality globally. Water quality is a term used to express the suitability of water to sustain various uses or purposes (i.e. agricultural, domestic, industrial, recreation) (Du Plessis 2017). The quality of water is dependent on the constituents that are either dissolved or suspended in the water (Bangma et al. 2017).

Each particular water use has certain requirements of physical, chemical or biological characteristics of water (Van Vliet et al. 2017). According to Bangma et al. (2017), the quality of water differs from one continent to another, and from one region to another due to varieties in geomorphology, climate, soil and geology, biotic composition and anthropogenic factors. To ensure sustainable utilisation of water in South Africa, the then DWAF has developed a series of water quality guidelines for different water uses. These guidelines are referred to as South African Water Quality Guidelines (SAWQG). Scientific and technical information for a particular water constituent and the potential effects on aquatic ecosystem health are described in these water quality guidelines (DWAF 1996c). As discussed in chapter one, specific water quality criteria for South African water guidelines include TWQR, CEV and AEV. These criteria indicate various categories of fitness of water for a wide range of water uses (DWAF 1996c). Target water quantity range (TWQR) criteria ensures aquatic ecosystem protection. This criterion encompasses a wide range of concentrations within which no measurable adverse effects are expected on the health of aquatic ecosystems. The AEV is defined as that concentration of a constituent above which there is expected to be a significant probability of acute toxic effects to up to 5% of the species in the aquatic community (DWAF 1996c). The CEV is the concentration of a constituent at which there is expected to be a significant probability of measurable chronic effects to up to 5% of the species in the aquatic community.

The then DWAF (1996a) further divided water quality constituents into four categories. This was done based on the effects that the constituents may have on the aquatic biota. The four categories are System variables, Macro-nutrients, Non-toxic constituents and Toxic constituents. System variables are constituents that regulate essential ecosystem processes such as migration of aquatic biota; such constituents include; temperature, pH and DO. Macro-nutrients are constituents such as nitrite (NO_2), nitrate (NO_3), ammonium (NH_4), sulphate (SO_4) and phosphate (PO_4). Macro-nutrients are generally non-toxic, however they speedup eutrophication if present in excess (Edokpayi et al. 2016). Non-toxic constituents can cause toxic effects when present in high concentrations, these constituents include salinity, water hardness, EC and TDS. Toxic constituents are mainly present in highly impacted streams and include Al, Fe, Zn, Cu, Mn and Pb (Souto et al. 2019).

Inland water bodies including river systems use biological processes to dilute and stabilise pollutants that are dissolved in the water, however these biological processes are unable to degrade toxic pollution resulting from metal contamination (Tiwari et al. 2017). Although metals are important elements for the normal functioning of aquatic ecosystems, they tend to accumulate in high concentrations in aquatic environment and have adverse effects on the water quality and the aquatic ecosystem (Intamat et al. 2016).

3.1.2 SEDIMENT QUALITY

Sediment is an essential part of the aquatic ecosystem in which metals accumulate, thus sediment help to determine the overall assessment of metals. Metals occur as natural constituent of the earth crust and exist as ores in rocks where they are generally recovered as minerals (Robinson et al. 2015). The term “heavy metals” is a term used to describe any metallic element that has a relatively high density and is poisonous or toxic even at low concentrations, these metals include Pb, Hg, As, Ag, Cu, Cd, Zn, Fe, Cr and the platinum group elements (Ali and Khan 2018).

Heavy metals are generally recovered from their ores because of mineral processing mainly in mining areas. These metals can be emitted into the environment by both natural and anthropogenic factors. However, studies have found that the main cause of emission of these metals is due to anthropogenic specifically mining operations (Ali et al. 2016; Chen et al. 2016). Metals tend to be left behind scattered in open or partially covered pits during mining processes and are carried into streams by wind, and runoff after rainfalls thus creating a variety of environmental stresses (Rajeshkumar et al. 2018).

In streams, metals are transported as either dissolved species in water or as an integral part of suspended sediment, thus it is important to use sediment to determine the level of concentration of metals (Edokpayi et al. 2016). Sediment absorbs metals, macro-nutrients and organic chemicals which are directly and indirectly discharged into the water body and release them back into the water slowly (Souto et al. 2019). Metals absorbed by sediment have the potential to seep into underground water, thus contaminating water from the underground sources such as wells (Aremu et al. 2017). Ashton and Haasbroek (2001) reported that the current patterns of water-use and rate of pollution will have significant negative effects on water supply with increased demand in the near future (Kumar et al. 2017). The high demand of freshwater for domestic and industrial use, may have negative effect the aquatic biota. Therefore, monitoring of inland water bodies have become a priority in South Africa for the past few decades (Ashton and Haasbroek 2001). In this study, water quality was analysed by determining a wide range of physico-chemical parameters and metals. In conjunction to this, SASS and FRAI indices were also used to determine the macro-invertebrates and fish assemblages.

3.2 METHODS AND MATERIALS

Water and sediment samples were collected seasonally (August 2017 – May 2018) from the four selected sites selected (Figure: 2.2).

The *in situ* physico-chemical parameters of water were determined using a handheld YSI meter (Model 554 Datalogger with a 4 m multiprobe) during each survey at each sampling site. Water quality parameters determined include total dissolved solids (TDS), pH, temperature, dissolved oxygen (DO) and electrical conductivity (EC). Water and sediment samples were collected into acid pre-treated polypropylene plastic bottles 1ℓ and 500 mℓ bottles respectively. The water samples were then stored in a cooler box with ice and the sediment samples were frozen. Both water and sediment samples were sent to an accredited laboratory (WATERLAB (PTY) LTD) in Pretoria for the analysis of macro-nutrients and metals using Inductively Coupled Plasma Optical Spectrometry (ICP-OES) scan. Water samples were analysed for macro-nutrients and metals while sediment samples were analysed for metals only. Macro-nutrients analysed include, phosphate (PO_4-P), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and ammonium (NH_4) while the metals analysed include, zinc (Zn), chromium (Cr), cobalt (Co), iron (Fe), nickel (Ni), copper (Cu), alkalinity, antimony (Sb), arsenic (As), calcium (Ca), chloride (Cl) lead (Pb), manganese (Mn), sodium (Na), sulphate, and total hardness. Several more metals where analysed reported in Appendix Table 2, however they are not discussed because of limited information in the water guidelines available. Water quality parameter results obtained from the WATERLAB (PTY) LTD were summarised and compared to the SAWQG recommended at outlined by DWAF (1996a, b, c). In case were the are no available data, the British Columbia Environmental Protection Division Water Quality Guidelines, Canadian Council of Ministers of the Environment Water Quality Guidelines and United States Environmental Protection Agency Water Quality Guidelines were used (BCEPD 2006, CCME 2012, USEPA 2012). Analysis of Variance (ANOVA) was performed using SPSS statistics 25 to determine the variation of system variables among the sites with the significance different of $p<0.05$ considered.

3.3 RESULTS AND DISCUSSION

DWAF (1996a) suggested that in South Africa, water quality variables that are primarily measured include macro-nutrients such as nitrogen as nitrite (NO_2-N), nitrogen as nitrate (NO_3-N), ammonium (NH_4) and phosphorus as phosphates (PO_4^{3-}), inorganic salt in elemental form such as chloride, magnesium, sulphate and physical variables such as oxygen, pH, temperature, TDS, etc. The physical and chemical variables of water play a major role in regulation of ecosystem processes such as fish migration. These variables are constantly changing with the influence of climatic fluctuations which may occur on daily to seasonal basis, however significant alteration of these variables may result in severe disruption to the aquatic ecosystems.

Summarised seasonal water quality data for the Steelpoort River are presented in

Appendix A: Table 1. Site average values for the water quality parameters were compared and are presented in Table 3.1. These averages were compared with the water quality guidelines with pH values presented as a range.

3.3.1 PHYSICO-CHEMICAL PARAMETERS

Water temperature

Water temperature is an important system variable because it affects bio-chemical reactions in aquatic organisms and the solubility of gases into the water (Liao et al. 2018). High temperatures result in reduced oxygen solubility while low temperatures result in increased oxygen solubility thus, more DO in the water. High temperatures are associated with low level of DO and may pose stress to aquatic organism, thus making them less resilient to other stressors (Grigore et al. 2018). Inland water bodies experience variation in temperature due to climatic fluctuations which may occur seasonally or on daily basis (Bangma et al. 2017). Surface water temperature is mainly influenced by air circulation, time of the day, flow rate and depth of the water body (DWAF 1996c).

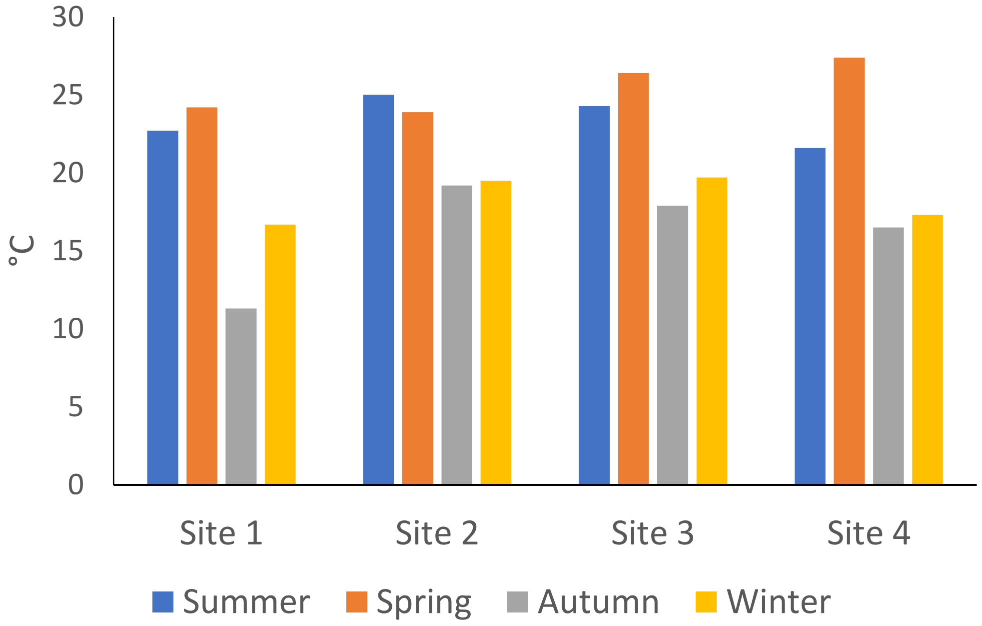

Figure 3.1: Temperature recorded at Steelpoort River during four surveys at each site (August 2017 – May 2018).

The lowest water temperature value (11.3°C) was recorded at site 1 during autumn, while the highest value (27.4°C) was recorded at site 4 during spring (Appendix A: Table 1; Figure 3.1). The lowest mean value of water temperature (18.73°C) was recorded at site 1, while the highest mean value (22.08°C) was recorded at site 3 (Table 3.1). According to DWAF (1996a), the temperature of the inland water bodies generally ranges from 5 – 30°C. This temperature range is ideal for maintaining optimal growth and reproduction for many organisms including fish and macro-invertebrates (Palmer et al. 2004; Humphrey et al. 2018). All temperature values recorded for the duration of the study fell within the ranges accepted in water quality guidelines for aquatic ecosystems (DWAF 1996c). Statistically, there was no significant difference for water temperature between the four sites ($p>0.05$) (Table 3.1).

Table 3.1: Mean seasonal physico-chemical values for the water at four sites in the Steelpoort River (August 2017 – May 2018).

Water quality parameters	Sampling Sites								p	Water Quality Guidelines
	Site 1		Site 2		Site 3		Site 4			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Water Temperature (°C)	18.73	±5.92	21.90	±2.98	22.08	±3.95	20.70	±5.00	0.721	Temperature should not vary more than 10% from normal (natural) value[2]
Dissolved Oxygen (mg/ℓ O_2)	8.98	±0.72	7.80	±1.29	8.35	±1.52	7.96	±2.36	0.727	*
Dissolved Oxygen (%)	91.85	±12.83	89.08	±17.92	96.05	±20.49	88.68	±26.81	0.950	80% – 120% of saturation[2]
pH	8.13-8.92	-	7.56- 8.89	-	7.7- 8.7	-	8.13- 8.93	-	0.66	Should not vary by > 5%[2]
Conductivity (EC) mS/m	217.98	±40.46	232.38	±16.72	293.33	±47.87	315.30	±54.76	0.019	No criteria available
TDS mg/ℓ	122.23	±24.32	129.70	±32.37	144.25	±26.55	170.65	±31.17	0.141	TDS should not change by >15% from normal cycles[2]

Notes:

1 - DWAF (1996a) – South African Water Quality Guidelines: Volume1: Domestic use.

2 - DWAF (1996c) – South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.

2 - BCEPD (2006) – British Columbia Environmental Protection Division: Water Quality Guidelines

3 - *- No guidelines available

Dissolved oxygen

DO refers to the amount of oxygen that is dissolved in water at a given time, temperature, atmospheric pressure and salinity (Dallas and Day 2004; Ultsch et al. 2019). Dallas and Day (2004) reported that the amount of DO increases with an increase in atmospheric pressure, decrease in water temperature and salinity (DWAF 1996a). Several other factors known to reduce concentration of DO in the water body include the presence of oxidisable organic matter together with high concentrations of suspended material which affects the DO saturation concentration (DWAF 1996a). DO in inland water bodies comes mainly from plants and phytoplankton that undergo photosynthesis, thus the DO concentrations decline through the night when the process of photosynthesis diminishes and rise mid-day when photosynthesis is at its peak (Jallet et al. 2016). DO concentration increases with increase in turbulence as natural diffusion of gaseous oxygen from the atmosphere into water is highly influenced by turbulent flowing water (Tian et al. 2019). Aquatic organisms are dependent on the DO for survival and a decline in the amount of DO will negatively affect respiration of aquatic organisms. If the concentration of DO falls below 5 mg/ℓ, most aquatic community start to experience stress, most fish species die at DO less than 2 mg/ℓ (DWAF 1996c)

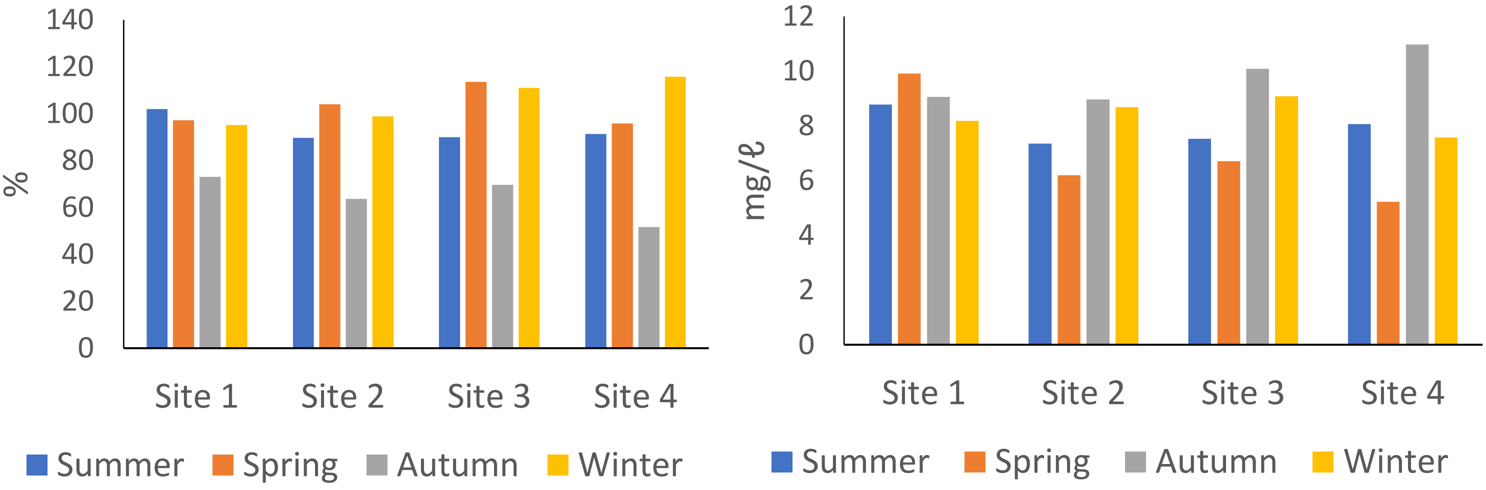

Figure 3.2: Dissolved oxygen in % (left) and mg/ℓ (right) recorded at Steelpoort River during each survey at each site (August 2017 – May 2018).

The lowest DO value (5.23 mg/ℓ) i.e. 51.7% was recorded at site 4 during autumn, while the highest DO value (10.97 mg/ℓ) i.e. 115.7% was recorded at site 4 during winter

(Appendix A: Table 1; Figure 3.2). The lowest mean value of DO (7.80 mg/ℓ) i.e. 89.08% was recorded at site 2 while the highest mean value for DO (8.98 mg/ℓ) i.e. 91.85% was recorded at site 1 (Table 3.2). The concentrations of DO recorded during winter, spring and summer at all sites respectively were within the TWQR of aquatic ecosystems (80 – 120%), however during autumn, DO concentration values recorded at all sites were below the TWQR of aquatic ecosystems (Appendix A: Table 1). The low DO concentrations recorded during autumn may be due to many factors such as turnover of suspended anoxic sediments, dredging activities, increase in oxidizable organic materials and reduced atmospheric aeration. There was no significant difference ($p>0.05$) among the four sites for DO (Table 3.1). Although there was no significant difference among the four sites, DO level varies in different temperatures. High DO concentrations recorded during winter maybe associated with low winter temperatures. All recorded DO concentrations were above 5 mg/ℓ, thus these concentrations may not have adverse effects on aquatic species (DWAF 1996c; Brooks and Haeusler 2016).

pH

The pH refers to the measure of the concentration of hydrogen ions in the water which indicate the acidity or alkalinity of the water. In South Africa most natural inland waters have a pH range between 6.0 and 9.0 (DWAF 1996c). However, pH is affected by deposition of acid forming substances and geological activities as such variation in pH values occurs within a stream and between different streams. The pH of the water body is important as it may influence the toxicity and presence of non-metallic ions as well as trace metals (Dallas and Day 2004). According to DWAF (1996a), gradual change in pH has a higher potential of changing the community structure which may result in tolerant species of both fish and macro-invertebrates replacing the intolerant species.

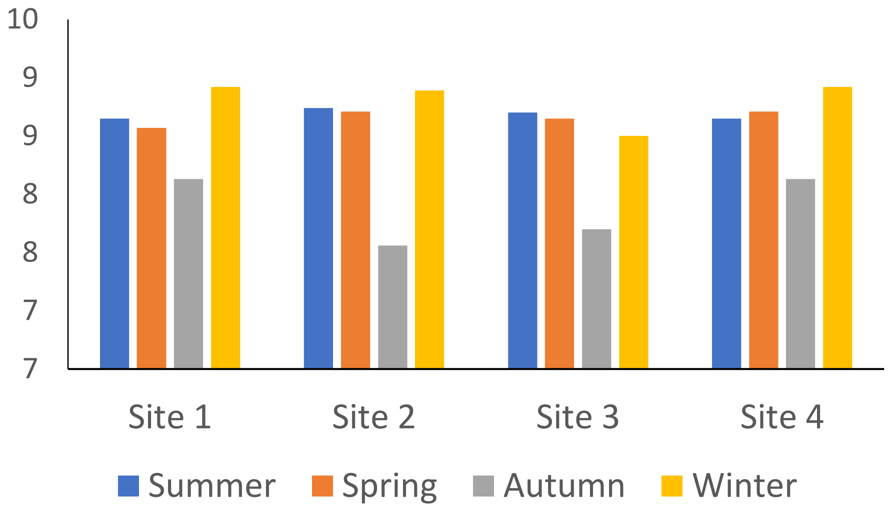

Figure 3.3: pH recorded at Steelpoort River during each survey at each site (August 2017 – May 2018).

The pH values recorded during the study period ranged from 7.56 to 8.93, with the lowest pH value recorded at site 2 during autumn and the highest pH value was recorded at site 1 and 4 during winter (Appendix A: Table 1). There was no significant difference ($p>0.05$) among the sites (Table 3.1, Figure 3.3). All recorded pH values were in alkaline range. Metals such as aluminium tend to be nontoxic in pH values above 7 (DWAF 1996c).

Electrical conductivity

EC refers to the measure of the water ability to conduct electrical current and are measured in mS/m (milli-siemens per meter) (DWAF 1996a). The availability, nature and concentration of ions which carries electric charge has an effect on the EC. These ions include sulphate (SO_4^{2-}), nitrate (NO^{3-}), sodium (Na^+), magnesium (Mg^{2+}), etc. The concentration of dissolved salts in the water body also affect the EC value, where increase in dissolved salt concentration result in an increase in EC (DWAF 1996a).

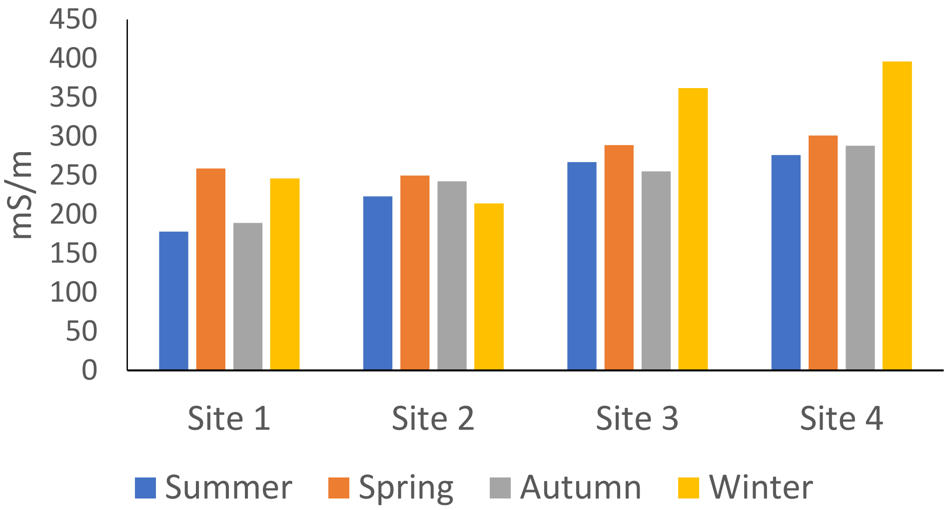

Figure 3.4: Electrical Conductivity recorded at Steelpoort River during each survey at each site (August 2017 – May 2018).

The EC values recorded ranged between 178 and 396 mS/m where the lowest EC value (178 mS/m) was recorded at site 1 during summer and the highest value (396 mS/m) was recorded at site 4 during winter (Appendix A: Table 1; Figure 3.4). The lower EC values at site 1 may be attributed to less impact of anthropogenic activities upstream of this sampling site while the higher EC values at site 4 may be attributed to effluent from mines, agricultural fields and Steelpoort town upstream of site 3 and site 4 which contribute to the addition of inorganic salts such as chloride, aluminium cations and nitrates sodium (Paul and Sen 2012). There was a significant difference ($p<0.05$) in EC values between the four selected sites (Table 3.1). There are no SAWQG for EC for aquatic ecosystems. However, EC is discussed under TDS guidelines because EC is an indirect measure of dissolved constituents (DWAF 1996a).

Total Dissolved Solids

TDS refer to the natural constituents (minerals, salts, cations and anions) in the water body which include both charged inorganic and organic matters. TDS measures the amount of soluble matters in the water and should not be confused with total dissolved salts which measures the quantity of dissolved compounds carrying an electric charge (Davies and Day 1998). Natural systems carry varying quantities of TDS because the dissolution of minerals in rocks, soils and decomposing plant materials differs from one system to the other (DWAF 1996a).

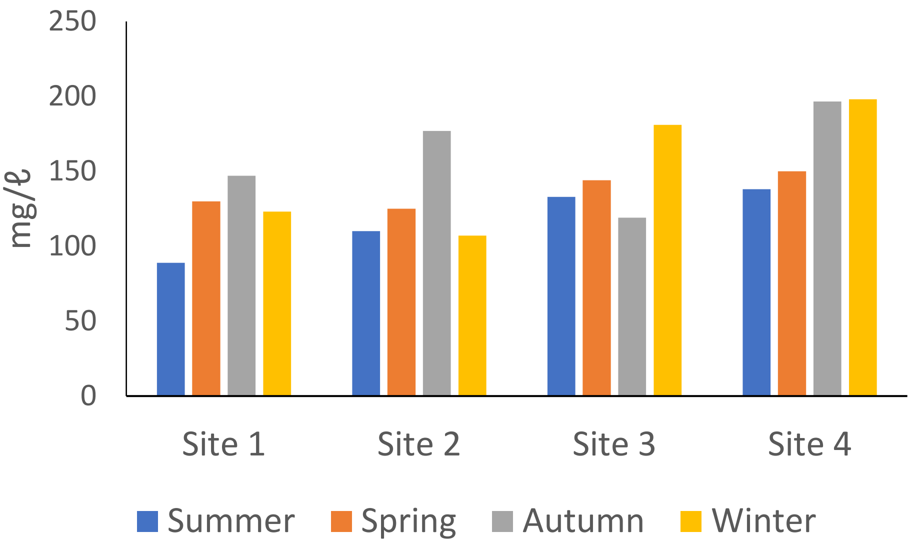

Figure 3.5: Total Dissolved Solids recorded at Steelpoort River during each survey at each site (August 2017 – May 2018).

The TDS concentrations recorded ranged from 89 to 198 mg/ℓ. The lowest value (89 mg/ℓ) was recorded at site 1 during summer, while the highest value (198 mg/ℓ) was recorded at site 4 during winter (Appendix A: Table 1, Figure 3.5). Statistically there were no significant differences ($p>0.05$) among the sites. DWAF (1996c) reported that concentrations of TDS in all inland's waters should not be changed by more than 15% from the water body normal cycle. However, there was no published baseline study with TDS values for the chosen sites to compare the recorded TDS values as to determining if the percentage change is less or higher than 15%. Davies and Day (1998), reported that high concentrations of major ions such as potassium, sulphate, chloride, sodium, magnesium and calcium cations in the water increases the concentration of TDS. Higher concentrations of some of these ions including sulphate, calcium and magnesium were recorded at site 4 which may attribute to the higher concentrations of TDS recorded at this site. Furthermore, these high concentrations of TDS may be attributed to domestic and industrial discharges and surface runoff and the mining activities taking place upstream near Steelpoort town. According to DWAF (1996a), extremely high concentrations of TDS may affect the growth of an organism and may even lead to death.

3.3.2 MACRO-NUTRIENTS

Macro-nutrients refer to major inorganic nitrogen compounds such as nitrogen and phosphorus (Sun et al. 2018). Generally, macro-nutrients are not toxic, however if present at an excessive amount they can stimulate eutrophication (DWAF 1996b). Inorganic nitrogen, phosphorus and sulphate are the primary representatives of macro-nutrients in inland waters. Nitrogen and phosphorus are essential for the survival of aquatic biota where nitrogen is important in the production of plants and animal tissue, phosphorus is important in converting sunlight into usable energy, and essential to cellular growth and reproduction (DWAF 1996b). Sulphate as sulphur is an essential component of proteins for most aquatic species (Dallas and Day 2004).

Table 3.2: Mean macro-nutrients values for the water at four sites in the Steelpoort River (August 2017 – May 2018).

Macro-Nutrients (mg/ℓ)	Site 1		Site 2		Site 3		Site 4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Nitrate (NO_3-N)	0.83	-	0.67	-	1.24	1.04	1.17	0.66
Nitrite (NO_2-N)	0.03	-	0.04	-	0.07	0.04	0.05	-
Ammonium (NH_4^+)	0.07	0.04	0.07	-	0.12	-	0.07	-
Total Nitrogen	0.50	0.57	0.78	-	1.36	1.09	1.23	0.74
Phosphate (PO_4^-)	0.07	-	0.28	0.32	0.13	-	0.04	-
Sulphates (SO_4^{2-})	14.49	9.17	15.67	5.18	22.44	19.00	22.72	13.74

Total nitrogen

Total nitrogen includes major inorganic nitrogen components (nitrite, nitrate, ammonia and ammonium). Although nitrogen occurs in abundant levels naturally, the main sources of inorganic nitrogen that enters inland water bodies are sewage discharges, runoff from agricultural land which includes both crops and livestock farming. Effluents from industries are also nitrogen-containing waste which are released into inland water bodies (DWAF 1996a). Unimpacted inland water bodies tend to have low nitrogen concentrations that are below 0.50 mg/ℓ. Higher concentration of nitrogen (>5 mg/ℓ) can cause rapid algal and aquatic plant growth which may result in toxic blue-green algal blooms (DWAF 1996c). The lowest total nitrogen value (0.1 mg/ℓ) was recorded during summer while the highest value (2.13 mg/ℓ) was recorded during winter (Appendix A: Table 1).

The lowest total nitrogen mean value (0.5 mg/ℓ) was recorded at site 1 while the highest total nitrogen mean value (1.36 mg/ℓ) was recorded at site 3 (Table 3.2). Site 1 and site 2 had relatively low concentrations of total nitrogen as compared to site 3 and site 4. This is evidence that the level of nitrogen in the water system is mostly influenced by the anthropogenic activities taking place in the area. As mentioned previously there is little to no anthropogenic activities taking place upstream of site 1 and small-scale farming and residential area upstream of site 2 which may be the reason for low concentrations of macro-nutrients at these two sites. However, highly intensified mining and the presence of a town upstream of sites 3 and 4 may be attributed to higher levels of total nitrogen at sites 3 and 4 (DWAF 1996c).

Nitrite

Nitrogen occurs in nature in different forms (N, NO_2 and NO_3). Nitrite (NO2) is one form of nitrogen which is an intermediate product of inorganic oxidation, nitrification and denitrification processes (DWAF 1996a). According to DWAF (1996c) nitrite is usually present in inland waters in low concentrations (less than 0.1 mg/ℓ), this is because it is readily oxidised to nitrate or reduced to ammonia. Higher concentration of nitrite may result in acute anoxia and consequently death for many fish species (DWAF 1996a). Inland water bodies subjected to frequent nitrogen-containing waste may have high nitrite concentrations (DWAF 1996c). DWAF (1996b) has provided TWQR for aquaculture for nitrite concentrations which is considered safe for sub-tropic fish species ranging from 0.06 to 0.25 mg/ℓ. The lowest value (0.03 mg/ℓ) was recorded at site 1 during winter while the highest value (0.09 mg/ℓ) was recorded at site 3 during autumn (Appendix A: Table 1). The lowest mean average value (0.03 mg/ℓ) was recorded at site 1 and the highest mean average (0.07 mg/ℓ) was recorded at site 3 (Table 3.2). All recorded values are within the TWQR for aquaculture which suggest that nitrite pose very little to no threat to life of the organisms living in this section of the river (DWAF 1996b).

Nitrate

Nitrate is the end product of the previously mentioned processes i.e. inorganic oxidation, nitrification and denitrification processes (DWAF 1996a). Nitrate co-exist in nature with nitrite. However, nitrate has been reported to be more abundant in inland water bodies because it is reduced chemically and converted by microbes into atmospheric nitrogen (N_2) (Davies and Day 1998). Like nitrite, nitrate concentration is heavily affected by sewage discharge and agricultural runoff, thus it stimulates the growth of algae (Dallas and Day 2004). There are no TWQR for nitrate in aquatic ecosystems, however for domestic use it is 0 to 6 mg/ℓ (DWAF 1996a) and for aquaculture is <300 mg/ℓ (DWAF 1996b). Nitrate is considered the least toxic of the inorganic nitrogen compounds, however if its concentration is 10 mg/ℓ or more, it may indicate pollution in water although such concentrations will be non-toxic. The lowest value (0.5 mg/ℓ) was recorded at site 1 during autumn while the highest value (1.97 mg/ℓ) was recorded at site 3 during winter (Appendix A: Table 1). The lowest mean average value (0.67 mg/ℓ) of nitrate was recorded at site 2 while the highest mean average value (1.24 mg/ℓ) was recorded at site 3. All the recorded values of nitrate were within the TWQR for domestic use and for aquaculture (DWAF 1996a; b).

Ammonia

Ammonia naturally exist in two forms, as ionised ammonium ion (NH_4^+) and un-ionised (NH_3) form. According to Palmer et al. (2004) water temperature, pH and DO influence the ratio between the two forms of ammonia and its toxicity. The toxicity of ammonia increases with the increase in temperature and pH and decrease in DO (DWAF 1996c). Ammonia toxicity is directly related to un-ionised form (NH_3) concentration. Ammonium ion has little or no toxicity to aquatic organisms, but it contributes to eutrophication. Dallas and Day (2004) reported that natural inland waters contain ammonia and ammonium concentrations less than 0.1 mg/ℓ. The lowest ammonium value (0.05 mg/ℓ) was recorded at site 1 during winter while the highest ammonium value (0.12 mg/ℓ) was recorded at site 3 during winter (Appendix A: Table 1). The lowest mean ammonium value of 0.07 mg/ℓ was recorded at sites 1, 2 and 4 and the highest mean value (0.12 mg/ℓ) was recorded at site 3 (Table 3.2).

Some of the values recorded were higher than TWQR for aquatic ecosystems (DWAF 1996c). These higher NH_4^+ values were recorded during summer at site 1 (0.1 mg/) and during winter at site 3. The high concentrations of NH_4^+ at these sites may be attributed to sewage discharges, explosives in mining and irrigation waters entering aquatic system which carry these nitrogen compounds. Furthermore, these high concentrations may be attributed to natural atmospheric deposition of ammonia from combustion and distillation of coals (DWAF 1996c). Excess concentrations of NH_4^+ may cause inhibition of cellular metabolism and reduces oxygen permeability into cell membranes (DWAF 1996c, Dallas and Day 2004). Consequently, aquatic organism experience reduction in growth rate and morphological development (DWAF 1996c).

Phosphorus

Phosphorus (P) is in an elemental form and does not occur in the natural environment. Phosphorus exist in nature as inorganic phosphate (PO_4), orthophosphate, polyphosphates, metaphosphates or pyrophosphates (DWAF 1996c). The main anthropogenic source of phosphate that enters inland water bodies include untreated sewage discharges, urban runoff, domestic and industrial effluents. Phosphorus may enter the water body naturally as a result of rock weathering, decomposition of organic matter or leaching of phosphate salts (DWAF 1996a). Phosphorus was measured as phosphate (PO_4^-); both the lowest value (0.04 mg/ℓ) and the highest value (0.13 mg/ℓ) were recorded during winter at site 4 and site 3 respectively (Appendix A: Table 1). The lowest mean value (0.04 mg/ℓ) of phosphorus was recorded at site 4, however the highest mean value (0.28 mg/ℓ) was recorded at site 2 (Table 3.2). Phosphorus concentrations should not be changed by >15% from that of the water body under local, unimpacted conditions at any time of the year (DWAF 1996c). The percentage change was not calculated due to lack of baseline or reference data for these sites.

Sulphate

Sulphate ion (SO_4^{2-}) is one of the most abundant anions in earth’s crust (Dallas and Day 2004). Sulphate enters natural waters mainly from weathering of sedimentary rock with sulphide minerals such as pyrite, acid mine wastes and other industrial processes that use sulphate or sulphuric acids (DWAF 1996c). According to DWAF (1996a), many forms of sulphate such as calcium sulphate are more soluble in water and when added to water they accumulate and increase sulphate concentrations in water. However, in natural waters sulphate occur in lower concentration of 5 mg/ℓ or less, but when sulphate rich effluents are released to the water body the concentration of sulphate may rise to several hundred mg/ℓ. There are no TWQR for sulphate in freshwater aquatic ecosystem, therefore TWQR for domestic use which ranges between 0 – 200 mg/ℓ was used (DWAF 1996a). The lowest SO_4^{2-} value (8 mg/ℓ) was recorded at site 1 during summer while the highest SO_4^{2-} value (35.87 mg/ℓ) was recorded at site 3 during winter (Appendix A: Table 1). The lowest mean average value (14.49 mg/ℓ) was recorded at site 1 while the highest mean average value (22.72 mg/ℓ) was recorded at site 4 (Table 3.2). All the recorded SO_4^{2-} values fell within TWQR for domestic use (DWAF 1996a). Although all SO_4^{2-} values fell within TWQR, the higher concentrations of at site 4 may be attributed to mine and industrial discharges from upstream of site 4 near Steelpoort town. Excess concentrations of SO_4^{2-} may form sulphuric acid which may have negative effects on aquatic ecosystems (Dallas and Day 2004).

3.3.3 METAL AND METALLOIDS CONCENTRATIONS

Full seasonal data for metal concentrations recorded from both water column and sediment are presented in Appendix A: Table 2 and Table 3. The average water column and sediment metal concentrations for Steelpoort River are summarised in Table 3.4 and 3.5.

Table 3.3: Water quality and sediment quality guidelines for metals and the references used.

Metals	Water Quality Guidelines (mg/ℓ)	Sediment Quality Guidelines (mg/kg)
Aluminium	0.1[3]	*
Arsenic	0.01[1]; 0.005[3]	5.9 (dry weight)[3]
Antimony	0.01[4]	*
Barium	0.7[4]	*
Boron	1.2[2]; 1.5[3]	*
Cadmium	0.00015 - 0.004[1]	0.6 (dry weight)[3]
Calcium	*	*
Chromium	Cr III: 0.012**[1]	37.3 (dry weight)[3]
Cobalt	*	*
Copper	0.0003 – 0.0014[1]	35.7 (dry weight)[3]
Iron	Fe vary <10% background concentration.[1]	0.3[3]
Lead	0.0002-0.0012[1]	35.0[3]
Magnesium	*	*

Table 3.3: Continued.

Potassium	*	*
Nickel	< 0.47[4]	*
Selenium	0.002[1]; 0.001[3]	*
Zinc	0.002[1]	123.0 (dry weight)[3]

Notes:

** – pH dependent.

* No guidelines available

References:

1 - DWAF (1996c) South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.

2 - BCEPD (2006), – British Columbia Environmental Protection Division: Water Quality Guidelines.

3 - CCME (2012) – Canadian Council of Ministers of the Environment: Water Quality Guidelines – Aquatic Life.

4 - USEPA (2012) – United States Environmental Protection Agency: Water Quality Guidelines –Aquatic Life.

Table 3.4: Metal concentrations recorded from water column at four sites in the Steelpoort River (August 2017 – May 2018).

	Site 1		Site 2		Site 3		Site 4		
Metals (mg/ℓ)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
Al	-	-	0.76	-	0.25	0.10	0.67	0.65	-
As	-	-	-	-	-	-	-	-	-
B	-	-	0.01	-	0.01	0.01	0.01	0.004	-
Ba	0.03	0.01	0.03	0.01	0.03	0.01	0.03	0.02	0.94
Cd	-	-	-	-	-	-	-	-	-
Ca	21.28	2.98	25.03	1.72	26.41	2.30	27.44	2.02	0.01
Cr	0.01	-	0.004	-	0.01	-	0.004	-	-
Co	-	-	-	-	0.001	-	-	-	-
Cu	-	-	-	-	-	-	-	-	-
Fe	0.16	0.08	0.29	0.47	0.21	0.13	0.42	0.57	1.00
Pb	-	-	-	-	-	-	-	-	-
Mg	10.76	1.17	12.33	1.30	13.84	2.38	17.43	2.78	0.003
K	1.21	0.10	1.71	0.10	1.71	0.06	1.73	0.07	0.02
Ni	0.001	-	-	-	0.001	-	-	-	-
Se	-	-	-	-	-	-	-	-	-
Zn	0.03	0.02	0.04	0.02	0.03	0.02	0.03	0.02	1.00

Note:

- Undetected

Table 3.5: Metal concentrations recorded from sediment at four sites in the Steelpoort River (August 2017 – May 2018).

Metals (mg/kg)	Site 1		Site 2		Site 3		Site 4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P
Al	44808.1	11571.9	38106.6	9337.2	52516.6	12410.8	50512.3	7293.0	0.39
As	1.4	0.7	1.3	0.3	1.6	0.8	1.0	0.1	0.47
B	18.0	36.0	8.8	17.5	11.8	23.5	14.8	29.5	0.32
Ba	161.6	26.3	264.2	104.5	345.9	119.1	335.6	148.9	0.02
Cd	0.1	0.1	-	-	0.1	0.2	-	-	0.22
Ca	33025.6	8613.5	30882.1	10885.5	43102.6	6482.7	39115.4	4425.8	0.39
Cr	76.1	18.0	343.6	261.9	479.3	111.0	927.0	396.6	0.02
Co	94.4	10.0	82.9	21.4	59.2	21.5	59.2	15.2	0.02
Cu	49.8	3.8	45.8	8.5	27.9	8.0	25.1	4.7	0.02
Fe	215351.3	37876.2	177607.3	65197.5	102383.5	55533.0	93076.7	35368.6	0.02
Pb	4.1	1.6	5.6	4.3	4.5	1.2	4.7	0.9	0.25
Mg	16550.7	3120.9	17328.0	1773.3	25395.4	4312.7	28969.3	5680.7	0.02
K	2957.8	641.2	6398.7	2741.3	7825.8	2201.5	8088.1	1870.0	0.02
Ni	374.1	678.6	270.5	325.9	243.1	228.3	297.3	286.6	0.25
Se	-	-	0.1	0.2	1.6	2.1	-	-	0.32
Zn	149.1	111.4	107.1	111.2	60.8	70.2	59.8	67.5	0.31

Note:

- Undetected

Aluminium

Aluminium (Al) exist in many forms in nature and some forms may be soluble in water while some may be insoluble in water. The solubility of Al is more dependent on pH where low or acidic pH increases the solubility and toxicity of aluminium and higher or alkaline pH decreases the solubility and toxicity of aluminium (DWAF 1996a). Higher concentrations of soluble Al found in inland water bodies are mainly as a results of acid rain and acid mine drainage (Dallas and Day 2004). According to Dallas and Day (2004), soluble ionised aluminium (Al^{3+}) is potentially a more toxic metal which can cause death for many fish and invertebrate species.

In water, the lowest Al concentration (0.18 mg/ℓ) was recorded at site 3 during spring while the highest value (1.13 mg/ℓ) was recorded at site 4 during summer (Appendix A: Table 2). Aluminium was detected at least at one site during each survey. However, it was never detected at site 1 throughout the study period. All the recorded values for Al in the water column were above the TWQR of aquatic ecosystems and aquatic life (CCME 2012; DWAF 1996c; Table 3.3). These concentrations are of concern as they might be toxic to a wide variety of organisms because they have exceeded the AEV (0.15 mg/ℓ at pH>6.5) range recommended by for aquatic ecosystem (CCME 2012; DWAF 1996c). However, all pH values recorded were slightly alkaline thus the Al was partially soluble and biologically unavailable and the aquatic biota may not be affected (Dickson 1983). In sediment, the lowest Al value (28148.90 mg/kg) was recorded at site 2 during spring while the highest Al value (66971.21 mg/kg) was recorded at site 3 during autumn (Appendix A: Table 3). The lowest mean average value (38106.6 mg/kg) for Al was recorded at site 2 while the highest mean average value (52516.6 mg/kg) for Al was recorded at site 3 (Table 3.5). There was no significant difference (p>0.05) among the sites. No available sediment quality guidelines for aluminium are available. However, according to DWAF (1996c), elevated Al concentrations are normally associated with acid mine drainage or acid rain which may explain the higher concentrations recorded at site 3.

Arsenic

According to the USEPA (2007) arsenic (As) is an Endocrine Disruptive Metal (EDM) and is a metalloid element which is toxic to aquatic life. The form and toxicity of As in freshwater is determined by the pH levels (DWAF 1996c). However, arsenic is absorbed readily to suspended materials, thus the presence of sediment, suspended solids and dissolved organic matter is important (Foata et al. 2009). Grosse et al. (2002) reported that As can be released into the environment from natural or man-made sources, where natural sources include ground water, geothermal processes and volcanoes while man-made sources include agricultural, industrial and mining activities, A water body that is subjected to industrial pollution with arsenic wastes or arsenal compounds such as fertilisers and pesticides may have higher concentrations of As (Foata et al. 2009).

High concentrations of As in water may have adverse physiological effects on the health of aquatic biota including fish and macro-invertebrates (Fu et al. 2010; Solomon 2008). Arsenic was undetected in water column throughout the study. In sediment, the lowest As value (0.78 mg/kg) was recorded at site 3 during autumn while the highest value (2.4 mg/kg) was also recorded at site 3 but during summer (Appendix A: Table 3). The lowest mean value (1.0 mg/kg) was recorded at site 4 while highest mean value (1.6 mg/kg) was recorded at site 3 (Table 3.5). There was no significant difference (p>0.05) among the sites. However, all values recorded were within the sediment quality range for aquatic life (Table 3.3; CCME 2012). This suggests that As may not have negative effect on the health of the aquatic biota within the Steelpoort River.

Boron

Boron (B) is a metalloid that normally occurs in nature in combined forms such as borax and may not be harmful to many aquatic species even in higher concentrations (10 mg/ℓ) (USEPA 2008). Boron is released into aquatic environment mainly from anthropogenic sources such as mine drainage agricultural runoff and urban wastes (BC-EPD 2003). In water, the lowest B value (0.004 mg/ℓ) was recorded at site 3 during winter while the highest B value (0.015 mg/ℓ) was also recorded at site 3 but during spring (Appendix A: Table 2). The lowest mean value (0.008 mg/ℓ) for B was recorded at site 3 while the highest mean value (0.01 mg/ℓ) was recorded at site 4 (Table 3.4). Although there is intensive mining in the catchment area, all the recorded values for B in the water column are within the TWQR for aquatic life (Table 3.3; BCEPD 2006; CCME (2012). There was no significant difference ($p>0.05$) among the sites. According to Lambert (2011), if the concentration of B exceeds the recommended levels for aquatic life, disrupts growth and appears to cause photo-oxidative stress in aquatic biota. In sediment, both the lowest B value (35 mg/kg) and the highest value (72 mg/kg) were recorded during winter (Appendix A: Table 3). The lowest mean average value (8.8 mg/kg) was recorded at site 2 and the highest mean average (18 mg/kg) was recorded at site 1 (Table 3.5). No sediment guidelines for boron are available to make comparison.

Barium

Barium (Ba) does not exist freely in nature, it is usually found in compounds such as barium sulfate (barite) and barium carbonate (witherite) (USEPA 1985). Like Al, Ba is pH dependant. The solubility of Ba increases with the increase in pH (USEPA 1985). In water, both the lowest (0.02 mg/ℓ) and highest (0.05 mg/ℓ) Ba values was recorded at site 4, the lowest value was recorded during winter while the highest value was recorded during summer (Appendix A: Table 2). The mean average values for Ba were equal at all sites, hence there was no significant difference ($p>0.05$) among the sites (Table 3.4). Ba values recorded in the water column throughout the study fall within the accepted water quality range for aquatic life (Table 3.3; USEPA 2012). In sediment, the lowest Ba value (123.4 mg/kg) was recorded at site 1 during summer while the highest Ba value (385 mg/kg) was recorded at site 2 during winter (Appendix A: Table 3). The lowest mean average value (161.6 mg/kg) was recorded at site 1 while the highest mean value (345.9 mg/kg) was recorded at site 3 (Table 3.5). There are no sediment guidelines available for Ba, thus no comparison could be made. However, there was a significant difference ($p<0.05$) among the sites.

Cadmium

Cadmium (Cd) is one of the EDM most toxic metal widely used in aquaculture (USEPA 2007). Cadmium is also used in industries such that manufactures fertilisers, pesticides, plastics, paints and batteries (DWAF 1996c). Natural weathering processes also has an effect on the increasing concentration of Cd in inland water bodies. However, industrial activities and agricultural practices has been found to be the main influence of increasing Cd levels in river systems (DWAF 1996b). The solubility of Cd in water is dependent on pH and it increases with the decrease in pH (CCME 1999). The presence of Cd is mostly associated with copper sulphide, lead and zinc. If cadmium is present in high concentrations it may hinder aquatic plant growth which result in disruption of ecosystem as plants are at the bottom of most food chains (Solomon 2008).

Cadmium was undetected in the water column. However, in sediment both the lowest value (0.03 mg/kg) at site 4 and the highest value (0.16 mg/kg) at site 3 were recorded during winter (Appendix A: Table 3). The mean average values (0.1 mg/kg) recorded at site 1 and site 3 were the same (Table 3.5). There was no significant difference ($p>0.05$) among the sites. All recorded Cd values were within the accepted sediment quality guidelines for aquatic life and may not have an adverse effect on the health status of the Steelpoort River (CCME 2012).

Calcium

Calcium (Ca) is an alkaline earth metal which is essential element in living organisms (DWAF 1996c). It is found in structural materials of almost all organism as it is found in bones, shells of molluscs and crustacean exoskeletons. The solubility of Ca is highly dependent on pH and temperature (DWAF 1996c). Calcium dissolved from a wide range of different rock types (Ballance and Bartram 2002). Calcium concentrations above 10 mg/ℓ may be found in waters of granite or siliceous sandy areas (Jooste et al. 2013).

In water, the lowest Ca value (18 mg/ℓ) was recorded at site 1 during summer and the highest Ca value (30 mg/ℓ) was recorded during summer at site 4 and winter at site 3

(Appendix A: Table 2). The lowest mean value (21.28 mg/ℓ) for Ca was recorded at site 1 while the highest mean value (27.44 mg/ℓ) was recorded at site 4 (Table 3.4). There was a significant difference ($p<0.05$) among the sites. In sediment, the lowest Ca value (18740.52 mg/kg) was recorded at site 2 during spring while the highest value (50158.74 mg/kg) was recorded at site 3 during autumn (Appendix A: Table 3). The lowest mean value (30882.1 mg/kg) for Ca was recorded at site 2 while the highest mean value (43102.6 mg/kg) was recorded at site 3. There was no significant difference ($p>0.05$) among the sites. The high Ca concentrations recorded at site 4 may be associated with the tailings upstream of site 4 near Steelpoort town. There are no water and sediment guidelines available for Ca for aquatic ecosystems. According to DWAF (1996c), concentrations (80 mg/ℓ 250 mg/ℓ) of Ca may still be accepted for aquatic ecosystems and for domestic use.

Chromium

Chromium (Cr) is one of the essential trace nutrients crucial in metabolism of carbohydrates and lipids. It occurs naturally in low concentrations in organisms and in the environment (DWAF 1996c). In aquatic ecosystems, chromium is usually very low and may occur in different forms which include chromium (II)–chromous ion (Cr^{2+}), Chromium (III)–chromic ion (Cr^{3+}, trivalent), chromium (III)–chromite ion (CrO_3^{3-}, trivalent), chromium (VI)–chromate ion (CrO_4^{2-}, hexavalent), chromium (VI)–dichromate ion ($Cr_2O_7^{2-}$, hexavalent). According to ATSDR (2008), Cr compounds are classified as human carcinogens. In water, Cr was only detected during winter with the lowest value of 0.004 mg/ℓ was recorded at site 2 while the highest value of 0.012 mg/ℓ was recorded at site 1 (Appendix A: Table 2). The Cr values recorded in the water column fall within the TWQR for aquatic ecosystems (DWAF 1996c). In sediment, the lowest Cr value (69.59 mg/kg) was recorded at site 1 during autumn while the highest Cr value (1460.68 mg/kg) was recorded at site 4 during spring (Appendix A: Table 3). The lowest mean value (76.1 mg/kg) was recorded at site 1 while the highest mean value (927.0 mg/kg) was recorded at site 4 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. All the Cr values recorded throughout the study were above the acceptable concentrations of sediment quality guidelines for aquatic life (Table 3.3; CCME 2012). Although these Cr concentrations may affect the health of aquatic biota and result in death of many organisms, during the study there were no observed effects of Cr such as abnormal macro-invertebrate movements patterns. Sources of high concentrations of Cr in the Steelpoort River may include industries and mine discharges. According to DWAF (1996c), low concentrations of Cr may temporarily reduce the growth phase for young fish. Furthermore, USEPA (2012) have reported that high concentrations of Cr may have adverse effects on aquatic organisms including reduced fecundity, morphological changes, reduced diseased resistance and abnormal movement patterns in benthic invertebrates.

Cobalt

Cobalt (Co) naturally occur in soil, rocks, water, air, plants and animals and has similar properties (DWAF 1996a). High concentration of cobalt may be found in areas contaminated by industrial pollution. Cobalt was undetected in the water column. In sediment, the lowest Co value (37.94 mg/kg) was recorded at site 3 during autumn while the highest Co value (105 mg/kg) was recorded at site 2 during winter (Appendix A: Table 3). The lowest mean value (59.2 mg/kg) for Co was recorded at site 3 and site 4 while the highest value (94.4 mg/kg) was recorded at site 1 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. No sediment quality guidelines are available for cobalt.

Copper

Copper (Cu) enters the aquatic environment naturally mainly as a result of weathering processes (DWAF 1996a). The toxicity of Cu is dependent on its solubility and oxidation state in water. The pH also plays a role, with low pH values associated with high mobility and solubility, thus more toxic. Three oxidation state of Cu includes metallic copper (0), cuprous copper (I) and cupric copper (II) with free cupric copper ions (Cu^{2+}) being the most toxic (DWAF 1996b). Copper was also not detected in water column. However, in sediment Cu was detected with the lowest value (21 mg/kg) recorded at site 4 during autumn and winter and the highest value (57.19 mg/kg) recorded at site 2 during spring (Appendix A: Table 3). The mean value for Cu ranged from 25.1 mg/kg to 49.8 mg/kg with the lowest value being recorded at site 4 and the highest value at site 1 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. The highest value recorded was above the acceptable concentrations of sediment quality guidelines for aquatic life (Table 3.3; CCME 2012). Copper can be toxic to some aquatic organisms even at low concentrations, thus the values recorded on this study show that copper may be highly toxic and may have negative effects on most aquatic species (Dallas and Day 2004).

High Cu concentrations recorded at site 2 may be associated with the runoffs from agricultural land upstream of this site. However, there could be more sources of Cu entering site 2 which may also include mining activities carried out upstream of De Hoop dam.

Iron

According to DWAF (1996c) iron (Fe) is the fourth most abundant element in earth's crust. Iron is an essential nutrient in organisms, however at high concentrations it can be toxic. In less or unpolluted water bodies, its concentration ranges from 0.001 to 0.5 mg/ℓ (DWAF 1996c). Iron in natural systems may come from weathering of sulphide ores, metamorphic rock or sedimentary rock. High concentrations of Fe in surface waters are associated with burning of coal, acid mine drainage, mineral processing and corrosion of iron and steel.

In water, both the lowest Fe value (0.03 mg/ℓ) and the highest Fe value (0.99 mg/ℓ) were recorded at site 2, with the lowest value being recorded during spring while the highest value was recorded during summer (Appendix A: Table 2). The lowest mean value (0.16 mg/ℓ) for Fe was recorded at site 1 while the highest mean value (0.42 mg/ℓ) was recorded at site 4 (Table 3.4). There was no significant difference ($p>0.05$) among the sites. According to DWAF (1996c), the concentration of iron in water should not vary by more than 10% from the normal local concentration, however, there were no reference data for the normal local concentration to make a comparison. In sediment, the lowest Fe value (49199.60 mg/kg) was recorded at site 4 during autumn and the highest Fe value (240527.74 mg/kg) was recorded at site 2 during spring (Appendix A: Table 3). The lowest mean (93076.70 mg/kg) for Fe was recorded at site 4 while the highest mean value (215351.30 mg/kg) was recorded at site 1 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. All recorded values of Fe in sediment were above the sediment quality guidelines (CCME 2012). The high Fe concentrations recorded during the study period at all sites be as a result of natural processes such as weathering of igneous and sulphate ores, metamorphic and sedimentary rocks, Furthermore, these high concentrations may be attributed to mine and sewage discharges. According to Dallas and Day (2004), high Fe concentrations may inhibit a wide range of enzyme pathways in aquatic organisms.

Lead

Lead (Pb) is a toxic metal which readily accumulate in living tissues and has a potential of causing death of an organism (Dallas and Day 2004). According to DWAF (1996b, c), Pb exists in different oxidation states of which all are important in the environment. The water quality has a significant influence on the toxicity of Pb to aquatic organisms. Solubility of Pb compounds and the concentration of Ca+ and Mg2+ in water affect the toxicity of Pb (DWAF 1996a). The sources of Pb in aquatic environment include industrial and urban waste discharge, burning of fossil fuel and mining. Lead was undetected in the water column. In sediment, the lowest Pb value (3.20 mg/kg) was recorded at site 1 during summer while the highest Pb value (12 mg/kg) was recorded at site 2 during winter (Appendix A: Table 3). The Pb mean values ranged from 4.1 mg/kg to 5.6 mg/kg with the lowest value recorded at site 1 and the highest value recorded at site 2 (Table 3.5). There was no significant difference ($p>0.05$) among the sites. All the recorded Pb values were within the acceptable range for sediment quality guidelines for aquatic life (Table 3.3; CCME 2012). The high Pb concentration at site 2 may be associated with the sedimentation and runoff entering the river during rainfall. According to DWAF (1996c), Pb can be toxic to most vertebrate species.

Magnesium

Magnesium (Mg) together with calcium forms main constituent of water hardness (DWAF 1996a). Magnesium occurs naturally in the environment and may enter aquatic environments as a result of weathering of ferromagnesium containing rocks. The concentration of Mg^{2+} in freshwaters mainly depend on the rock types of the area, however the concentration usually ranges between 1 and 100 mg/ℓ (DWAF 1996a). In water, the lowest Mg value (9 mg/ℓ) was recorded during summer at site 1, while the highest Mg value (21 mg/ℓ) was recorded during winter at site 4 (Appendix A: Table 2). There was a significant difference ($p<0.05$) among the sites. Magnesium concentrations in unimpacted freshwater ecosystems are usually between 4 and 10 mg/ℓ (DWAF 1996a; d). In sediment, the lowest Mg value (13002 mg/kg) was recorded at site 1 during winter while the highest value (36688.80 mg/kg) was recorded at during spring (Appendix A: Table 3). The lowest mean value (16550.70 mg/kg) was recorded at site 1 while the highest mean value (28969.30 mg/kg) at site 4 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. However, there are no available sediment guidelines for magnesium, thus no comparison was made. Site 1

Potassium

Potassium (K) is an alkali metal, normally found in freshwater bodies in concentrations lower than 20 mg/ℓ (DWAF 1996c). Low concentrations of K in natural freshwaters are related to the resistance of potassium containing rock to weathering. However, other sources of K such as industrial discharges and agricultural runoff may increase concentration of K in aquatic ecosystems. In water, the lowest value of K (1.10 mg/ℓ) was recorded at site 1 during autumn and the highest value of K (1.80 mg/ℓ) was recorded at site 2 and site 4 during summer and at site 2 during winter Appendix A: Table 2). The lowest mean value (1.21 mg/ℓ) was recorded at site 1 while the highest mean value (1.73 mg/ℓ) was recorded at 4 (Table 3.4). There was a significant difference ($p<0.05$) among the sites. In sediment, the lowest K value (2882.6 mg/kg) was recorded at site 1 during summer while the highest K value (10803.59 mg/kg) was recorded at site 4 during autumn (Appendix A: Table 3). The lowest mean value (2957.80 mg/kg) for K was recorded at site 1 while the highest value (8088.10 mg/kg) was recorded at site 4 (Table 3.5). There was a significant difference ($p<0.05$) among the sites. The toxic effects of K to aquatic ecosystems are not known and there are no SAWQG available. However, potassium is known to occur in freshwater in concentrations ranging between 2 to 5 mg/ℓ (DWAF 1996c).

Nickel

Nickel (Ni) is an essential trace element in nutrition of many organisms. Nickel is naturally insoluble in many solvents including water thus it is mainly tied up to the soil in large quantities (Hansen et al. 2005). In water Ni was detected only in winter with the lowest value of 0.001 mg/ℓ and highest value of 0.002 mg/ℓ (Appendix A: Table 2). Both the two recorded values fell within the TWQR for aquatic life (Table 3.3; USEPA 2012). In the sediment the lowest Ni value (34.4 mg/kg) and the highest Ni value (1392 mg/kg) were recorded at site 1 during and winter, respectively (Appendix A: Table 3). The lowest mean average value (243.1 mg/kg) was recorded at site 3 while the highest mean value (374.1 mg/kg) was recorded at site 1 (Table 3.5). There was no significant difference ($p>0.05$) among the sites and no sediment guidelines are available for Ni.

Selenium

Selenium (Se) is a non-metallic element which naturally occurs as calcium selenite, ferric selenite and elemental Se that occurs in five oxidation states, namely, -II, 0, II, IV and VI (DWAF 1996a; Dallas and Day 2004). The state of Se is dependent on the pH and redox potential of an aquatic ecosystem (DWAF 1996a). The solubility and toxicity of Se increases with the increase in pH (Dallas and Day 2004). Selenium was undetected in the water column, however it was detected in sediment. The lowest Se value (0.06 mg/kg) was recorded at site 4 during winter while the highest Se value (4.4 mg/kg) was recorded at site 3 during summer (Appendix A: Table 3). The lowest mean value (0.1 mg/kg) for Se was recorded at site 1 while the highest mean value of 1.6 mg/kg was recorded at site 3 (Table 3.5). There was no significant difference ($p>0.05$) among the site and there are no sediment guidelines available for Se.

Zinc

Zinc (Zn) is also one of the essential nutritional trace elements in organisms (DWAF 1996c). Zinc naturally occurs in the environment in rocks and ores and it enters the aquatic environment due to erosion and weathering. Anthropogenic activities such as mining and industrial activities also plays a role in releasing Zn into the aquatic environment (DWAF 1996b). Two oxidation states of zinc tend to be found in aquatic ecosystem are Zinc as a metal and Zn (I) with Zn (II) being more toxic to fish even at relatively lower concentrations (DWAF 1996a). In water, the lowest Zn value (0.014 mg/ℓ) was recorded at site 3 and site 4 during winter and the highest Zn value (0.06 mg/ℓ) was recorded at site 1 during summer (Appendix A: Table 2). The highest mean average value (0.04 mg/ℓ) was recorded at site 2 while all the other three sites had an average mean value of 0.03 mg/ℓ (Table 3.4). The concentrations of Zn recorded in water column were above the TWQR for aquatic ecosystems (DWAF 1996c). In sediment, the lowest Zn value (16 mg/kg) was recorded at site 4 during winter while the highest Zn value (214.89 mg/kg) was recorded at site 2 during spring (Appendix A: Table 3). The mean average values ranged from 59.8 mg/kg at site 4 to 149.1 mg/kg at site 1 (Table 3.5). The highest Zn value was above the accepted concentrations for sediment quality for aquatic life (CCME 2012). There was no significant difference ($p>0.05$) among the sites for both Zn concentrations in water column and in sediment.

3.4 CONCLUSION

The water temperature varied during the four sampling seasons, however all values recorded were within the expected surface water temperature range (5 to 30°C). DO levels were within the TWQR for aquatic ecosystems suggested by DWAF (1996c). However, during autumn the DO percentage values recorded were all below the TWQR for aquatic ecosystems (DWAF 1996c). All pH values recorded throughout the study were slightly alkaline (ranged from 7.56 to 8.13), this pH values may be influenced by TDS concentrations. Although the lowest water temperature value was recorded during autumn, the highest DO value was recorded during winter. Winter water temperature values were relatively lower, thus DO levels can be expected to be higher. Total nitrogen concentrations were relatively low throughout the study although a higher value of 2.13 mg/ℓ was recorded in winter. The river could be classified as being mesotrophic since mean nitrogen concentration fell between 0.5 and 2.5 mg/ℓ (DWAF 1996c). Concentrations of phosphorus that are less than 5 mg/ℓ indicate eutrophic conditions in the river.

The concentrations of metals such as Al, Cr and Zn were above the TWQR for aquatic ecosystems (DWAF 1996c). As, B, Ba, Cd, Fe, Cu, Mg and Ni concentrations were within their respective water and sediment quality guidelines used (DWAF 1996c; CCME 2012; BCEPD 2006; USPA 2012). Some metals are insoluble in water if the pH is alkaline, thus they may precipitate and sink to the sediment at the river bed where they accumulate. It is for this reason that metal accumulation in sediment was also determined, however determining metal accumulation in either water column or in sediment is a complex study as the solubility of one metal may be dependent on the other. According to Oberholster (2009), pollutants that enter the rivers in Steelpoort catchment area are mainly from acid mine drainage, discharge of treated, partially treated and untreated domestic and industrial sewage from municipal sewage treatment works. However, the physico-chemical parameters, macro-nutrients, and metal concentrations results show no indication of severe impact of these anthropogenic activities in Steelpoort River. The water quality of the Steelpoort River was acceptable during the study period although some constituents were above the TWQR for aquatic ecosystems. It is important to continuously monitor the water quality of this river since the anthropogenic activities may continue to pose a threat to the river.

CHAPTER 4

MACROINVERTEBTARES AS BIOINDICATORS

4.1 INTRODUCTION

Biological monitoring or biomonitoring refers to “the systematic use of living organisms (bioindicators) or their responses to determine the condition or changes of the environment” (Everall et al. 2017). According to Fierro et al. (2017), a bioindicator is “an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment)”. One of the biological monitoring tools mainly used in southern Africa is the South African Scoring System version 5 (SASS5). The presence, abundance and/or the behaviour of biological indicators reflect the effect of stressor on the biota (Ojija and Laizer 2016). The most commonly used bioindicators include, plankton, insects, molluscs, fish, plants and birds. Each of these bioindicators shows important information for the biomonitoring of wide variety of pollutants in aquatic ecosystem (Zhou et al. 2008; Souto et al. 2019). According to Bere et al. (2014), many developing countries have been biased towards the analysis of physical and chemical properties in the assessment of stream ecological health and water quality with biomonitoring being largely neglected. However, the physical and chemical analysis only gives fragmented overview of the state of aquatic systems. SASS5 is used to get a more time-integrated indication of the water quality reflecting conditions that are not present at sampling and analysis period (Gordon et al. 2015).

SASS was originally modified from the British Monitoring Working Party system (BMWP) and it uses aquatic macro-invertebrates as bioindicators (Chutter 1998). Aquatic macro-invertebrates, like any other ideal bio-indicator have at least some of the following characteristics; wide distribution, low mobility (for local indication), taxonomic soundness (can be easily identified by nonspecialist), high sensitivity to environmental stressors, numerical abundance, well-known ecological characteristic, suitability for use in the laboratory and ability of quantification and standardisation (Souto et al. 2019). These organisms inhabit all types of lotic water bodies, from slow flowing muddy rivers to fast flowing mountain rivers (Ferronato et al. 2019). Aquatic macro-invertebrates are key components of aquatic food webs, they link organic matter and macro-nutrient resources with higher trophic levels (Guo et al. 2016). Most of these organisms have sedentary habits which make them good representatives of site-specific ecological conditions (Li et al. 2010). Macro-invertebrate assemblages tend to be made of many orders, families and species among which there is a variety of trophic levels and pollution tolerances, thus providing essential information for interpreting cumulative effects (Boyle and Strand 2003; Mouillot et al. 2013). Karaouzas et al. (2019) reported that macro-invertebrate community structure frequently changes in response to change in environmental conditions. Such community structure change in predictable ways enabling the use of macro-invertebrates in evaluating anthropogenic influences (Everall et al. 2017).

4.2 METHODS AND MATERIALS

Macro-invertebrates were sampled seasonally at four selected sites following the SASS version 5 procedure recommended by Dickens and Graham (2002). A standard SASS net (30 mm by 30 mm) was used to collect benthic macro-invertebrates in three different biotopes at each site. The biotopes sampled were stone and rock biotopes (S), vegetation biotopes (VEG) and gravel, sand and mud biotopes (GSM). These biotopes were sampled using different methods described by Davis and Christids (1997).

Figure 4.1: (A+B) Sampling of macro-invertebrates.

In stone biotopes, stone in current (SIC) and stone out of current (SOOC) were sampled. SIC is characterised by movable stones of relatively cobble size (≥3 cm to approximately

25 cm in diameter) in areas with relatively fast-moving water. Stones out of current is characterised by stones of the same size as for SIC, however these cobbles are located in areas with relatively slow-moving water. The kick sampling method was used to sample both SIC and SOOC, a net was placed on the bottom of the river, just downstream of the stones to be kicked, in a position where the current would carry the disturbed invertebrates into the net. Two minutes was spent sampling SIC and 1 minute was spent sampling SOOC. Both SIC and SOOC macro-invertebrate samples were combined into a single sample. This sample was then identified in the field according to the SASS procedure. All the individuals of the families identified were counted and recorded.

Vegetation biotopes were subdivided into marginal vegetation (MV) and submerged vegetation (SV). Marginal vegetation includes overhanging reeds, grasses and twigs growing on the edge of stream both in current (MVegIC) and out of current (MVegOOC), often emerged. The net was scraped back and forth through vegetation in a distance of approximately 2 meters of vegetation ranging along the river. Submerged vegetation includes filamentous algae and roots of floating aquatic vegetation that are totally submerged into the water. Sampling was achieved by pushing the net under water against and through vegetation in an area of approximately one square meter. Samples collected from MV and SV were combined into a single vegetation biotope sample, and identified and counted.

Gravel, sand and mud biotopes are characterised by small stones of less than 2 cm, sand grains of less than 2 mm and silt and clay particles which are less than 0.06 mm in diameter. Gravel was stirred by shuffling it with feet whilst continuously sweeping the net over the disturbed area to catch the disturbed biota. This was done ranging along the river covering areas with sand, gravel and mud. One minute was spent sampling GSM. Samples collected were then identified and counted.

All macro-invertebrate samples collected were identified in the field and released back into the stream. The samples which could not be identified in the field were then preserved in 70% ethanol and stored in 1liter polypropylene buckets for later identification. The unidentified samples were labelled according to the site and biotope they were collected from and transported to the laboratory. In the laboratory, each biotope sample was carefully studied under the microscope and was identified using

Aquatic Invertebrates of South African Rivers illustrations guide and guide to the Freshwater Invertebrates of South Africa volume 1 to 10 (Dickens and Graham 2002).

Shannon Weiner Diversity Index was performed to determine the fish species diversity variation among the four selected sites. All measured system variables were correlated with the distribution of both macro-invertebrate taxa and fish species through performing the Canonical Correspondence Analysis (CCA) using Canoco version 4.5.

4.3 RESULTS AND DISSCUSION

The use of aquatic macro-invertebrates in biomonitoring is not restricted to SASS5 index, there are several analyses which can be done with the aid of aquatic macro-invertebrate data, thus there is a wide range of biomonitoring tools in different regions around the globe based on aquatic macro-invertebrates. Therefore, the analysis included not only the SASS5 index but also macro-invertebrate distribution, abundance and analysis of Ephemeroptera, Plecoptera and Trichoptera (EPT) richness and diversity.

4.3.1 MACRO-INVERTEBRATE DISTRIBUTION

A total of 8,556 individual aquatic macro-invertebrates were collected from all sites during the period of the study. Fifty families were recorded from 12 orders (Table 4.1). The number of families recorded per order ranged from 1 family to 10 families.

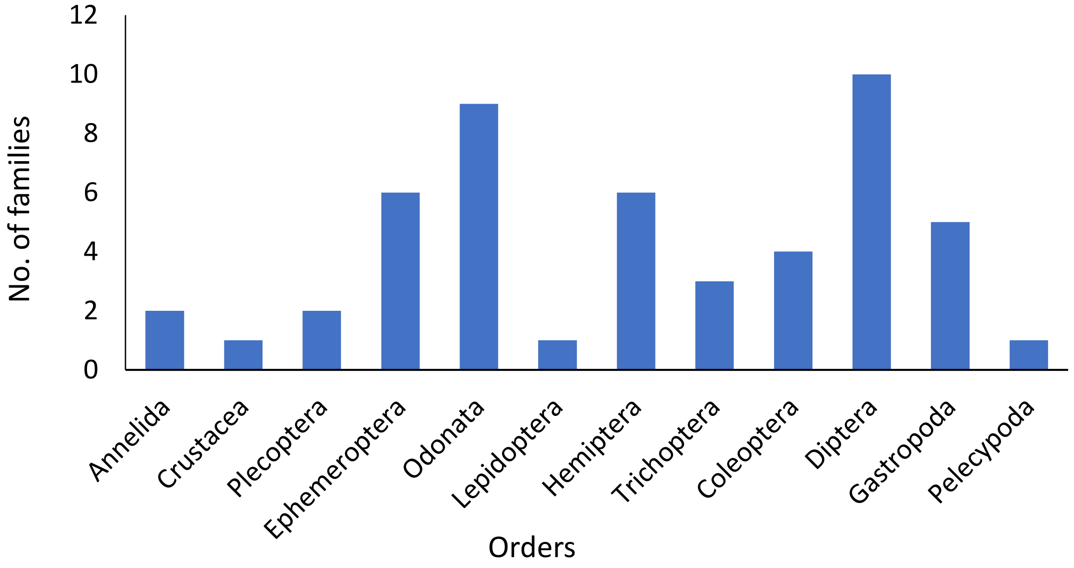

Figure 4.2: Total number of families per order recorded in Steelpoort River.

The lowest number of families (1) was recorded under order Crustacea, Lepidoptera and Pelecypoda while the highest number of families (10) was recorded under the order Diptera (Figure 4.2). The lowest number of macro-invertebrate individuals (1) was recorded under two orders, namely Crustacea and Lepidoptera while the highest number of macro-invertebrates (4603) was recorded under order Ephemeroptera (Table 4.1).

4.3.2 MACRO-INVERTEBRATE ORDERS AND FAMILY DISTRIBUTION AND RICHNESS ACROSS ALL SELECTED SITES

The distribution of macro-invertebrates is indicated in Table 4.1. Site 1 had the highest number of macro-invertebrate individuals (2978) with 10 orders recorded. Thirty-five families were identified within the 10 orders recorded at site 1 (Table 4.1; Figure 4.3). This site was mostly dominated by highly and moderately sensitive families namely; Baetidae (1022 individuals) followed by Caenidae (578 individuals) and Hydropsychidae (435 individuals) (Table 4.1). Other highly sensitive families recorded include Perlidae (33 individuals), Prosopistomatidae (25 individuals) and Notonemouridae (1 individual) (Table 4.1).

Site 2 had the second largest number of macro-invertebrates (2893). The 2893 individual macro-invertebrates were shared among 11 orders and 34 families (Table 4.1; Figure 4.3). Site 2 was also dominated by highly and moderately sensitive taxa, the most dominant family being Baetidae with 1743 individuals followed by Caenidae with 206 individuals and then by Hydropsychidae with 167 individuals (Table 4.1).

Site 3 had the least number of macro-invertebrates (1330) as compared to all sites. However, 32 families were observed from 10 orders at site 3. The highly and moderately sensitive families recorded from sites 1 and 2 were also present at site 3, however the abundance was greatly reduced with Baetidae family having 338 individuals and Caenidae having 63 individuals. Family Hydropsychidae had 242 individuals. Most of the families recorded at sites 1 and site 2 were not recorded at site 3, among these families are Lymnaeidae, Planorbinae, Physidae, Perlidae and Notonemouridae (Table 4.1).

Site 4 had 1355 number of macro-invertebrates consisting of 8 orders and 27 families (Table 4.1; Figure 4.2). The most dominant family recorded at site 4 was Baetidae (409 individuals) followed by Hydropsychidae (196 individuals) and Gomphidae (100 individuals) (Table 4.1).

The highest number of macro-invertebrates recorded at site 1 may be attributed to relatively low level of pollution since there are less anthropogenic activities around this site while the lowest number of macro-invertebrates at site 3 may be attributed to the relatively high pollution level as this site is located downstream of industrial and mining sites.

Table 4.1: Aquatic macro-invertebrates collected at four sites in the Steelpoort River (August 2017 – May 2018).

Order Group	Family Group	Site 1	Site 2	Site 3	Site 4	Total number	% per family
Annelida	Oligochaeta	87	25	22	13	147	1.718
	Hirudinea	-	1	-	-	1	0.012
Crustacea	Potamonautidae	-	1	-	-	1	0.012
Plecoptera	Notonemouridae	1	1	-	2	4	0.047
	Perlidae	33	14	-	-	47	0.549
Ephemeroptera	Baetidae	1022	1743	338	409	3512	41.047
	Caenidae	578	206	63	57	904	10.566
	Heptageniidae	20	28	4	27	79	0.923
	Leptophlebiidae	33	-	5	4	42	0.491
	Prosopistomatidae	25	-	-	-	25	0.292
	Tricorythidae	17	12	12	-	41	0.479
Odonata	Calopterygidae	1	-	1	6	8	0.094
	Chlorocyphidae	1	2	2	-	5	0.058
	Synlestidae (Chlorolestidae)	-	8	-	-	8	0.094
	Coenagrionidae	42	111	10	40	203	2.372
	Lestidae	-	-	2	-	2	0.023
	Aeshnidae	3	-	-	-	3	0.035
	Corduliidae	1	-	-	-	1	0.012
	Gomphidae	121	117	136	100	474	5.54
	Libellulidae	12	50	79	92	233	2.723
Lepidoptera	Crambidae (Pyralidae)	-	-	1	-	1	0.012
Hemiptera	Belostomatidae	4	24	17	11	56	0.655
	Gerridae	3	-	-	12	15	0.175
	Naucoridae	30	9	9	44	92	1.075
	Nepidae	7	1	3	-	11	0.129
	Pleidae	-	-	-	1	1	0.012
	Veliidae	-	-	-	2	2	0.023
Trichoptera	Ecnomidae	-	-	-	1	1	0.012
	Hydropsychidae	435	167	242	196	1040	12.155
	Leptoceridae	27	62	16	23	128	1.496
Coleoptera	Elmidae	144	87	47	55	333	3.892
	Gyrinidae	46	6	3	21	76	0.888
	Hydrophilidae	-	-	2	-	2	0.023
	Psephenidae	7	-	-	-	7	0.082
Diptera	Athericidae	-	-	-	5	5	0.058

Table 4.1 continued

	Ceratopogonidae	60	13	26	6	105	1.227
	Chironomidae	149	76	94	72	391	4.57
	Culicidae	6	5	2	1	14	0.164
	Muscidae	-	2	2	40	44	0.514
	Psychodidae	9	-	10	-	19	0.222
	Simuliidae	11	43	44	78	176	2.057
	Syrphidae	-	-	2	-	2	0.023
	Tabanidae	27	31	114	37	209	2.443
	Tipulidae	13	3	18	-	34	0.397
	Ancylidae	-	2	-	-	2	0.023
	Lymnaeidae	1	11	-	-	12	0.14
	Physidae	-	21	-	-	21	0.245
	Planorbinae	1	1	-	-	2	0.023
Gastropoda	Thiaridae	-	7	2	-	9	0.105
Pelecypoda	Corbiculidae	1	3	2	-	6	0.07
Total number per site		2978	2893	1330	1355		
H' (Shannon Weiner diversity index)		2.20	1.75	2.45	2.48		
Total No. of all macro-invertebrates						8556	

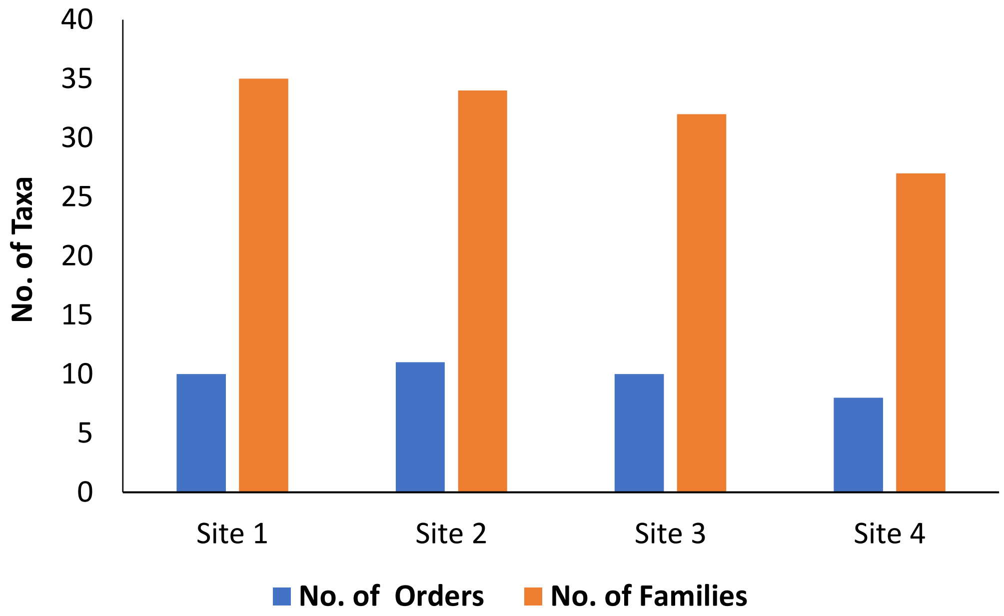

Figure 4.3: The number of orders and families at four sites in the Steelpoort River.

4.3.3 MACRO-INVERTEBRATE ABUNDANCE PER SYSTEMATIC ORDER

The macro-invertebrate abundance varied among the different orders and is illustrated in Figure 4.3. Ephemeroptera had the most abundant number of macro-invertebrates recorded during the study constituting 53.79% of all the macro-invertebrates collected. Within this order, 6 families were recorded among which Baetidae was the most dominant constituting 41.05% followed by Caenidae constituting 10.57% of the total macro-invertebrates sampled (Table 4.1; Figure 4.3). Trichoptera had the second most abundant number of macro-invertebrates constituting 33.66% of all recorded individuals (Figure 4.3). Three families were recorded in order Trichoptera. Family Hydropsychidae being the most dominant and constituted 12.16% of all macro-invertebrates sampled with the remaining two families constituting less than 2% all together (Table 4.1).

The third most abundant order was Diptera which constituted 11.68% (Figure 4.3). Within this order, 10 families were recorded among which Chironomidae had the most abundant number of individuals (391) which constituted 4.57% followed by Tabanidae (209 individuals) constituting 2.44% and Simuliidae with 176 individuals constituting 2.06% of all the macro-invertebrate individuals recorded (Table 4.1).

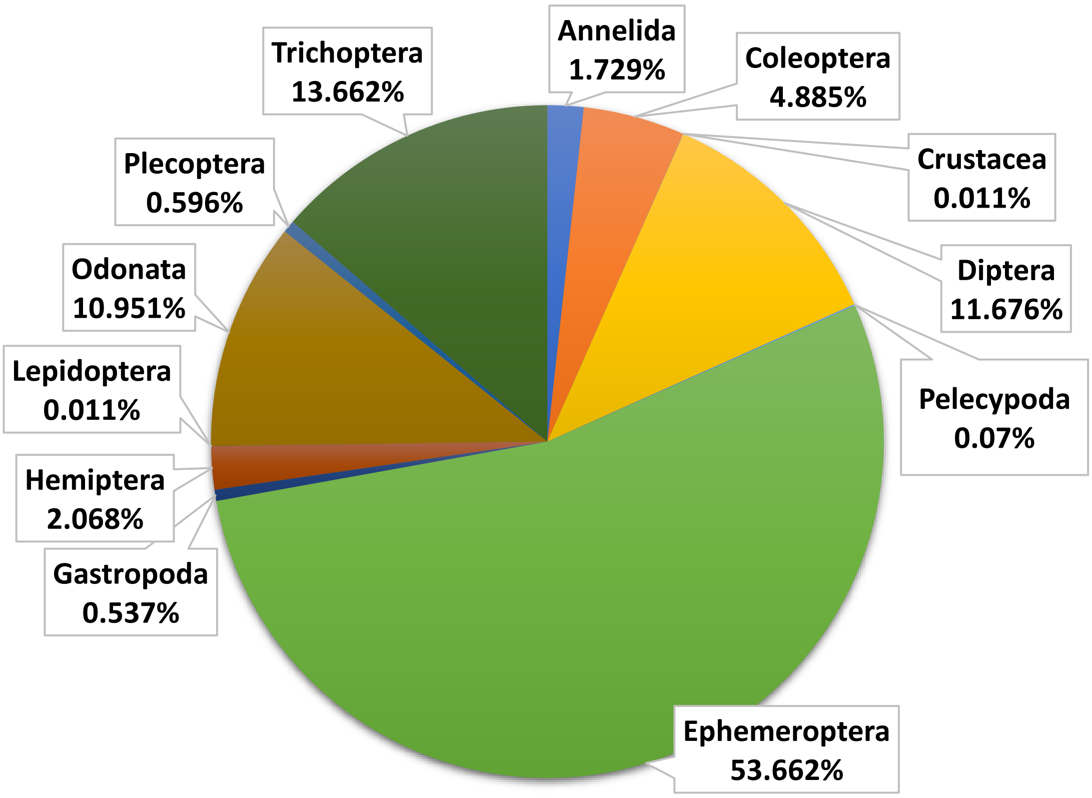

Figure 4.4: Macro-invertebrate abundance per systemic order throughout the study at all sites.

Five orders had very low macro-invertebrate abundance, all together constituted less than 5% of the total number. These orders include Crustacea, Gastropoda, Lepidoptera Pelecypoda and Plecoptera where each of them constituted less than 1% (Figure 4.4).

One family (Potamonautidae) was recorded under Crustacea and one family (Crambidae) was recorded under Lepidoptera with only one individual recorded in each family. One family (Corbiculidae) was recorded under order Pelecypoda where six individuals were recorded. The family Corbiculidae was recorded at sites 1, 2 and 3 (Table 4.1). Families of the order Ephemeroptera are known to have different sensitivity scores ranging from below 5 to above 10. Thus, the domination of the order Ephemeroptera in the period of study at this selected study area could be an indication that the quality of water in the Steelpoort River is slightly polluted as there is macro-invertebrate diversity that include both sensitive family (Prosopistomatidae) and tolerant family (Caenidae). Trichoptera, Diptera and Odonata were also among the abundant orders, all these three orders are generally characterised by families with moderate pollution sensitivity, the presence of these orders in high abundant maybe evident that the river is in good condition (Tonkin et al. 2016).

4.3.4 SPATIAL MACROI-NVERTEBRATE ABUNDANCE AND DIVERSITY

The abundance of macro-invertebrates varied with respect to sampling site during each survey. The sites with high invertebrate abundance tend to have relatively high family richness as well.

Table 4.2: Macro-invertebrate abundance at four sites in the Steelpoort River (August 2017 – May 2018).

Orders	Site 1	%	Site 2	%	Site 3	%	Site 4	%	Total order abundance
Annelida	87	58.78	26	13.51	22	14.86	13	8.784	148
Coleoptera	197	47.13	93	22.25	52	12.44	76	18.182	418
Crustacea	-	0.00	1	100.00	-	0.00	-	0.000	1
Diptera	275	27.53	173	17.32	312	31.23	239	23.924	999
Pelecypoda	1	16.67	3	50.00	2	33.33	-	0.000	6
Ephemeroptera	1695	36.82	1989	43.21	422	9.17	497	10.797	4603
Gastropoda	2	4.35	42	91.30	2	4.35	-	0.000	46
Hemiptera	44	24.86	34	19.21	29	16.38	70	39.548	177
Lepidoptera	-	0.00	-	0.00	1	100.00	-	0.000	1
Odonata	181	19.32	288	30.74	230	24.55	238	25.400	937
Plecoptera	34	66.67	15	29.41	-	0.00	2	3.922	51
Trichoptera	462	39.52	229	19.59	258	22.07	220	18.820	1169
Total site abundance	2978		2893		1330		1355		

Site 1 had the highest abundance of macro-invertebrates as compared to all the sites, with the most abundant group Ephemeroptera which constituted 36.82% of all members of Ephemeroptera recorded during the study. The second most abundant taxa at site 1 was Trichoptera constituting 39.5% of all individuals belonging to the order Trichoptera followed by Diptera with 27.5% being presented at this site (Table 4.2). The lowest abundant taxa recorded at site 1 were Gastropoda and Pelecypoda (Table 4.2). Forty-three percent of most abundant order (Ephemeroptera) was recorded at site 2. The second most abundant order at site 2 was Odonata followed by Trichoptera and Diptera with 30.7%, 19.6% and 17.3%, respectively. The order Crustacea was only recorded at site 2. Site 3 has the lowest number of macro-invertebrates. However, the taxa Ephemeroptera, Diptera and Trichoptera were the most abundant (Table 4.2). Like all other sites, at site 4, Ephemeroptera was the most abundant taxon at site 4. Only 10.8% of all recorded Ephemeroptera was recorded at site 4. The second most abundant taxon was Diptera followed by Odonata and Trichoptera (Table 4.2).

The distribution and abundance of macro-invertebrate at different site may be attributed to the difference in land use between the selected sites as these difference in land uses are reflected in macro-nutrients and metal concentrations. This is supported by several other studies (e.g. Chakona et al. 2009; Mwedzi et al. 2016) that have shown low macro-invertebrate diversity and abundances in areas where there is mining and agricultural practices taking place. According to Kaaya et al. (2015), macro-invertebrates are related or affected by local factors, thus macro-invertebrate community structure is controlled mainly by microhabitat characteristics. In this study, sites 3 and 4 was found to be highly modified as compared to sites 1 and 2 as a result sites 3 and 4 has relatively low macro-invertebrate abundance.

4.3.5 ANALYSIS OF EPHEMEROPTERA, PLECOPTERA AND TRICHOPTERA (EPT) RICHNESS AND DIVERSITY

EPT index refers to the three groups of aquatic macro-invertebrates which are sensitive to pollution. The EPT index is the sum of the total number of taxa (families) recorded in each of the three sensitive orders (Ephemeroptera/mayflies; Plecoptera/stoneflies; Trichoptera/caddisflies). Higher values of this index are indication of less stressful conditions whereas lower values are indication of potentially stressful conditions in the section of the stream. To determine the measure of community balance, the ratio of EPT to Chironomidae (EPT/C ratio) abundance was also calculated. A relatively high ratio and even distribution among all four major groups reflect a good biotic condition and good water quality while a relatively low ratio and skewed population with disproportionate number of Chironomidae relative to the more sensitive groups indicate environmental stress and poor water quality (USEPA 2007).

Table 4.3: The EPT family richness (A) and EPT/C (B) comparisons among four sites.

A

	Family Richness			
	Site 1	**Site 2**	**Site 3**	**Site 4**
Ephemeroptera	6	4	4	4
Plecoptera	2	2	0	1
Trichoptera	2	2	2	3
EPT	**10**	**8**	**6**	**8**

B

	Group Abundance			
	Site 1	**Site 2**	**Site 3**	**Site 4**
Ephemeroptera	1695	1989	422	497
Plecoptera	34	15	0	2
Trichoptera	462	229	258	220
Chironomidae	149	76	94	72
EPT/C	**14.70**	**29.38**	**7.23**	**9.99**

Patterns of abundance, richness and diversity vary under different spatial and temporal scales. Comparisons of EPT family richness and abundance showed variation among the four selected sites. These variations are an indicative of different water quality at different site. A high EPT value is an indicative of good water quality while a low EPT value is an indicative of poor water quality. The highest EPT value of 10 was recorded at site 1. Sites 2 and 4 had EPT value of 8 and site 3 had the lowest EPT value of 6 (Table 4.3A). This indicated that the water quality at site 3 was poor as compared to the other three sites. The ETP/C ratio supported the analysis of EPT. The highest EPT/C ratio (29.38) was observed at site 2 followed by EPT/C ratio of 14.70 at site 1 and then site 4 with an EPT/C ratio of 9.99 (Table 4.3B). The lowest EPT/C ratio of 7.23 was recorded at site 3 (Table 4.3B). These ratios revealed that site 2 has relatively good biotic condition as compared to other sites, while at site 3 there is potential environmental stress. The EPT index and EPT/C indicated that sites 1 and 2 had relatively good water quality while site 3 had poor water quality.

4.3.6 SOUTH AFRICAN SCORING SYSTEM

Table 4.4: SASS score, number of taxa and average score per taxon obtained at each site at Steelpoort River.

	Site 1				Site 2				Site 3				Site 4			
	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
SASS score	107	175	176	117	68	162	157	140	57	94	115	141	60	99	131	133
No of Taxa	16	26	25	18	12	26	25	24	12	16	20	24	11	17	20	22
ASPT	6.69	6.73	7.04	6.50	5.67	6.23	6.28	5.83	4.75	5.88	5.75	5.88	5.45	5.82	6.55	6.05

The SASS scores obtained throughout the study ranged from 57 to 176 with the lowest SASS score recorded at site 3 during winter while the highest SASS score was recorded at site 1 during summer (Table 4.4). The SASS score means ranged from 102 to 144, the lowest mean SASS score was recorded at site 3, while the highest mean SASS score was recorded at site 1 (Figure 4.5). Site 1 had a SASS score range of 107-176 with the lowest SASS score recorded during winter and the highest SASS score recorded during summer. Site 3 had a SASS score range of 57-141. Like in site 1 the lowest SASS score was recorded during winter. However, highest SASS score recorded during autumn (Table 4.4).

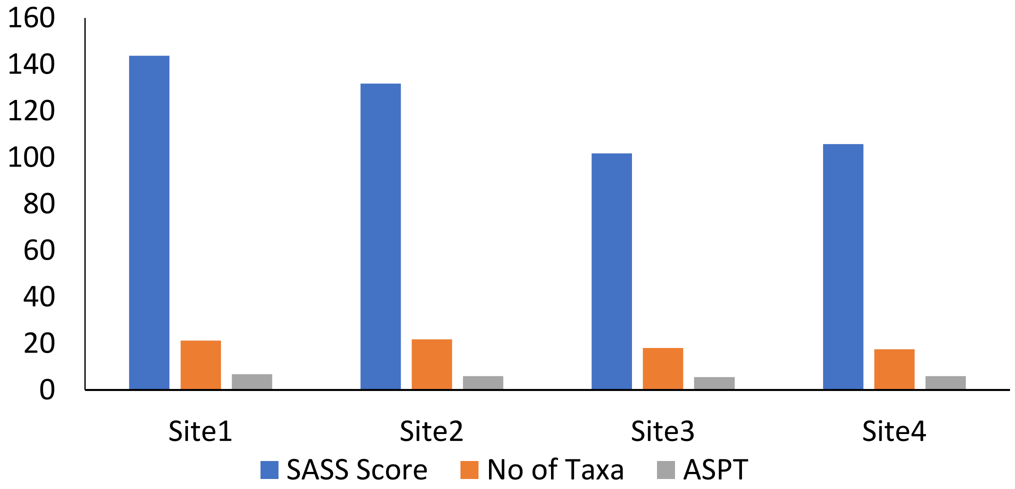

Figure 4.5: The mean SASS scores, No. of taxa and ASPT for macro-invertebrate collected at each site in Steelpoort River.

The sequence of mean SASS scores from high to low was; Site 1 > Site 2 > Site 4 > Site 3 (Table 4. 4, Figure 4.5). Based on the SASS scores obtained, site 1 is the least polluted site with the best water quality as compared to the other sites. Site 2 is the second least polluted site followed by site 4, whereas site 3 is the most impacted site and has poor water quality. This might be as a result of the anthropogenic activities along the river from upstream to the downstream thus high abundance and high taxa richness is associated with good water quality (Jackson et al. 2016). The intensity of anthropogenic activities increased from upstream to the downstream. However, from site 3 to site 4 there is a decrease in anthropogenic activities such as industries and mining, and may have contributed to the higher SASS score at site 4 than at site 3 although site 4 is downstream of site 3.

The number of taxa recorded ranged from 11 to 26 with the lowest number of taxa recorded at site 4 during winter and the highest number of taxa recorded at two sites, site 1 during summer and at site 2 during spring (Table 4.4). The lowest mean number of taxa (17.5) was recorded at site 4 followed by the second lowest mean number of taxa of 18 at site 3. The highest mean number of taxa (21.75) was recorded at site 2, followed by site 1 (21.25) (Figure 4.5). The high taxa richness and the high SASS score at sites 1 and site 2 suggest that these sites had more pollution sensitive families as compared to site 3 and site 4 (Dickens and Graham 2002). A gradual decline of SASS score and number of taxa was observed at site 3 which implies a major deterioration in the water quality however, the SASS score increased at site 4 which indicates that the water quality was better at site 4. This pattern can be observed in Figure 4.5 and may be evidence of different levels of anthropogenic impacts.

The ASPT recorded ranged from 4.75 to 7.04 with the lowest value recorded at site 3 and the highest value recorded at site 1 (Table 4.4). The mean ASPT scores ranged from 5.57 to 6.74. The SASS score and the mean average ASPT recorded at site 1 and site 2 indicate that the water quality at these sites relatively good, as these sites were less impacted. Site 3 had a lowest mean ASPT of 5.57 which indicates that there was a slight deterioration in the water quality at site 3. Site 4 had an ASPT of 5.97 which was slightly higher than that of site 3. This indication that the water quality at site 4 is better compared to that of site 3 (Table 4.4, Figure 4.5). Lower ASPT at sites 3 and 4 may be attributed to the increase in mining activities near these two sites.

4.3.7 CANONICAL CORRESPONDENCE ANALYSIS

The relationships between the physico-chemical parameters and the distribution of macro-invertebrate were analysed using he canonical correspondence analysis.

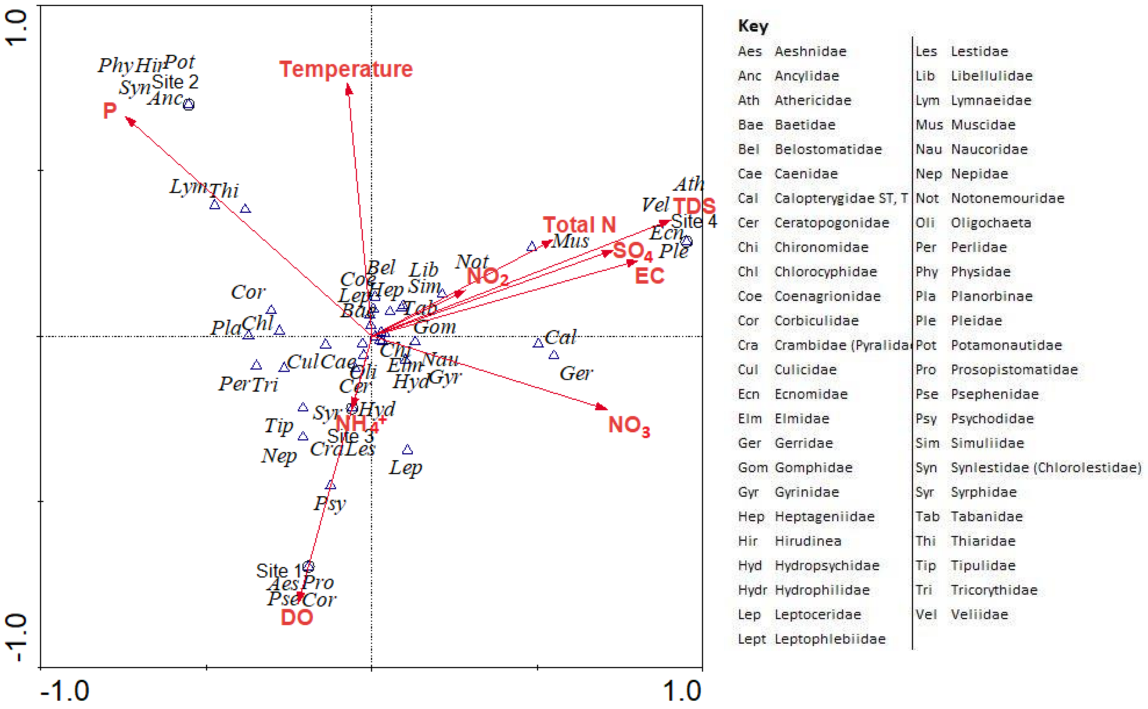

Figure 4.6: CCA plot of the relationship between macro-invertebrates and water quality parameters.

A correlation between macro-invertebrate community structure and system variables (TDS, EC, DO, NH_4^+, P, SO_4, NO_2, NO_3, total nitrogen and temperature) was observed in the study. All the ten main water quality parameters tested were important in prediction of macro-invertebrates (Figure 4.6). The distribution of family Athericidae, Ecnomidae, Pleidae and Veliidae were strongly correlated with TDS, SO_4 and EC and was strongly associated with site 4. The distribution of family Corbiculidae, Prosopistomatidae, Aeshnidae and Psephenidae at site 1 were strongly correlated with DO (Figure 4.6). The distribution of Potamonautidae, Synlestidae (Chlorolestidae), Ancylidae and Physidae at site 2 were strongly correlated to Phosphate and temperature (Figure 4.6). The distribution of macro-invertebrate at site 3 was strongly influenced by NH_4^+. However, the distribution of more than 50% of all families recorded were strongly correlated with lower concentrations of the water quality parameters evaluated as these families are distributed at the zero axis. Total nitrogen was strongly correlated with the distribution of Muscidae while nitrite was strongly correlated with Notonemouridae, Libellulidae, Tabanida and Simulidae. It is important to note that most of the system variables were within the target water quality range (DWAF 1996a; b; c).

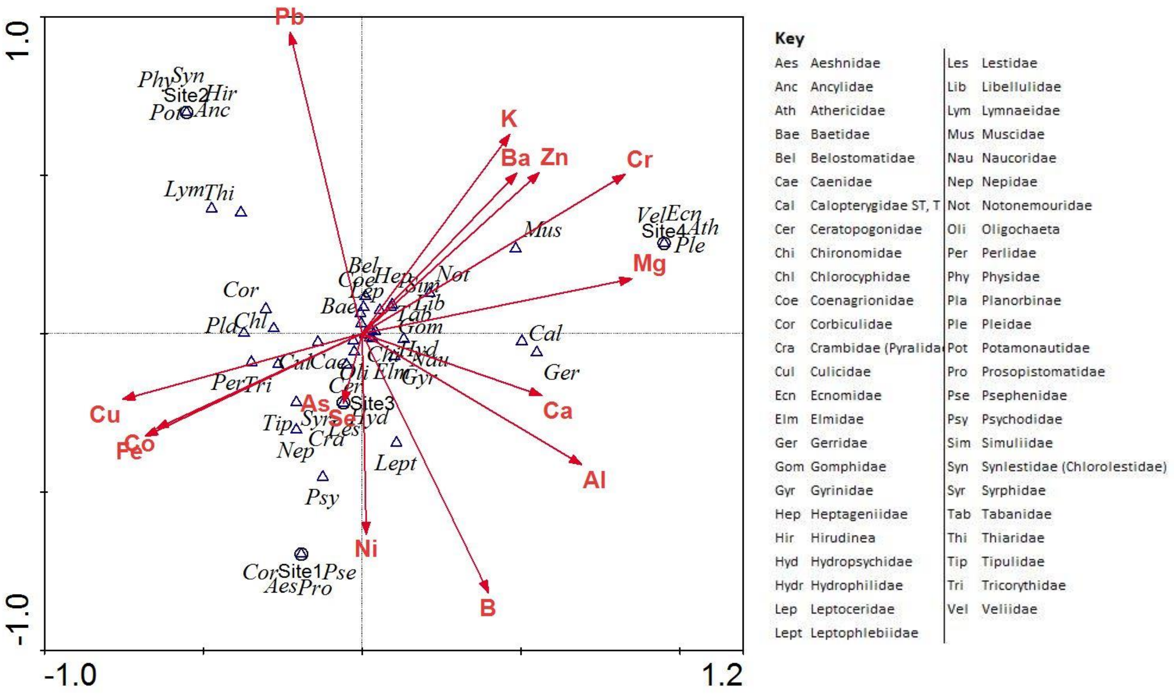

Figure 4.7: CCA plot of the relationship between macro-invertebrates and metal concentrations.

The community structure of macro-invertebrates was well correlated with metal concentrations. Higher concentrations of Pb were mostly correlated with the distribution of families such as Ancylidae, Hirudinea, Physidae, Potamonautidae and Synlestidae (Chlorolestidae) at site 2. Higher concentrations of Mg and Cr were correlated with Athericidae, Veliidae, Ecnomidae and Pleidae (Figure 4.7). Mg and Cr had stronger influence in the distribution of macro-invertebrates at site 4. Higher concentration of metals such as B, Ba, Zn, K, Al, Cu, Fe and Co did not have a strong correlation or strong influence on the distribution of macro-invertebrates, however most families were present where these metals were found to be in mid/average concentrations (Figure 4.7). As and Se showed a strong correlation to the distribution of Lestidae, Crambidae (Pyralidae), Hydrophilidae, Ceratopogonidae and Syrphidae at site 3 (Figure 4.7). At site 1, Ni had a strong influence in the distribution of Aeshnidae, Corbiculidae, Prosopistomatidae and Psephenidae (Figure 4.7).

Table 4.5: Summary of CCA results for environmental factors (system variables and metals) and macro-invertebrate taxa.

Axes	1	2	3	4	Total Inertia
Eigenvalues	0.139	0.121	0.088	0	0.348
Species-environment correlations	1.00	1.00	1.00	0	
Cumulative percentage variance					
* of taxa data	39.90	74.70	100.00	0	
* of taxa-environment relation	39.90	74.70	100.00	0	
Sum of all eigenvalues					0.348
Sum of all canonical eigenvalues					0.348

The taxa-environment factor correlation (r) for factor 1, 2 and 3 were 1.00 (Table 4.4). The cumulative variation explained by first axis was 39.9% while for the second axis was 74.7% and for the third axis was 100% (Table 4.4). This implies that the measured environmental variables were sufficient in explaining much of variance of the benthic macro-invertebrate's assemblages at sites 1, 2 and 3.

4.4 CONCLUSION

The results indicated that the water quality of the Steelpoort River is generally good. However, there is degradation of water quality from upstream to downstream. As expected, the pattern of change in macro-invertebrate community structure and water quality showed evidence of increasing intensity of anthropogenic activity occurring from upstream to downstream. Higher macro-invertebrate abundances, diversity and richness were observed at sites located upstream of an area of more intensive mining (sites 1 and 2), while sites downstream of more intensive mining area had relatively low macro-invertebrate abundance, diversity and richness. Macro-invertebrate assemblages successfully indicated variation in the level of pollutants and water quality at different sites with site 1 being the least impacted site and site 3 being the most impacted. Canonical correspondence analysis successfully showed strong correlation between most environmental variables and distribution of macro-invertebrates.

CHAPTER 5

FISH AS BIOINDICATORS

5.1 INTRODUCTION

Biological assessment is fundamental for the determination of the ecological status and conservation value of water bodies. Like macro-invertebrates, fish has successfully been used as bioindicators of changes in the hydromorphological status since Karr (1989) developed the index of biotic integrity (IBI). This is mainly because fish have a broad ecological tolerance and are highly responsive to changes in the trophic status of the water body they live in (Garcia et al. 2006). According to Birk et al. (2012) and Hering et al. (2013), fish are excellent bioindicators both at local and basin-scale. They have been successfully used in assessing different disturbances such as organic and macro-nutrient pollution, hydromorphological alterations and changes in land use (Hering et al. 2013). Fish in their natural environments are typically subjected to numerous stressors which include natural fluctuations in the system variables and anthropogenic stressors such as contaminant loading. Thus, the composition and structure of fish community have the potential to integrate information from the lower trophic levels (Brand and Fischer 2016).

Various fish indices have been developed all over the world, including South Africa. Kleynhans (1999) developed the Fish Assemblage Integrity Index (FAII), Kotzé (2001) developed the weighted Sensitivity Index of Biotic Integrity (SIBI) and then the Fish Response Assessment Index (FRAI) was developed by Kleynhans (2007). The FRAI was developed as part of the tools in the EcoClassification process to determine the reserve “the amount of water available within the water resource that are available for the future supply of water for human use as well as ecological requirements of the water resource” (NWA 1998). The FRAI index is exclusively for the determination of Present Ecological Status (PES) for riverine systems. This index is a habitat-based cause and effect-based model which takes into account the attributes of each fish species which include environmental preferences and intolerances. To determine PES, various environmental requirements and habitat drivers incorporated in the FRAI metrics are used (Malherbe et al. 2016)

5.2 METHODS AND MATERIALS

5.2.1 FISH RESPONSE ASSESSMENT INDEX

Fish were sampled according to the FRAI index protocol, however, few alterations from the protocol were made such as time spent sampling at a site. Three sections at each sampling site were chosen for sampling of fish, these sections included different velocity-depth classes. The sampled sections were fast-deep, fast-shallow and slow-shallow. A standard electro-shocking effort was used to sample each velocity-depth class at each sampling site. A standard electro-shocking effort generator (Honda mgs2500 2kva) was used. Thirty minutes were spent sampling a site ranging across all chosen sections. However, the sampling sections were selected to avoid the influence of one section on the other as suggested by Kleynhans (2008). Different sites were dominated by different velocity-depth classes. As such, all sampled individuals at each section of a sampling site were combined into a single sample and they were identified to species level using a reference guide (Skelton 2001). The number of individuals of each species collected was recorded. After identification and counting, the individuals were released back to the stream, however at least one individual of each recorded species was euthanised and preserved in a polypropylene bucket with 70% ethanol and kept in the laboratory as reference samples.

The fish data collected was incorporated into the FRAI metrics which was weighted and the percentage change from the reference condition was calculated for each site. These percentage change values are expressed as a percentage score and an ecological category of either A (100%: Unmodified), B (80–99%: Largely natural), C (60–79%: Moderately modified), D (40–59%: Largely modified), E (20–39%: Seriously modified) or F (0–19%: Critically modified) was assigned to each site (Kleynhans 2008). The reference list for the fish community and the Frequency of Occurrence (FROC) database was derived from previous surveys on the Steelpoort River in the Eastern Bankenveld Ecoregion (Kleynhans et al. 2007). The list of all reference species and FROC data is provided in Table 5.1. The reference fish species or fish community refers to the fish species that has been recorded previously in an ecoregion. Site 1 was located in Ecoregion Eastern Bankenveld 9.03 (B4STEE-TIGER) while sites 2, 3 and 4 were located in Ecoregion Eastern Bankenveld 9.03 (B4STEE-IF R09) (Kleynhans et al. 2007). The reference fish community were used to complete the FRAI index and to compare the observed fish community to the expected fish community with respect to each sampling site. According to Kleynhans (2007), the FRAI index is generally a robust index, however some uncertainty in the FRAI index results may be present because most of the intolerance and preference used within the database are expert derived information rather than empirical data.

Table 5.1: Reference species list for the different reaches within the Steelpoort River during the study (Kleynhans et al. 2007).

References Species (Site 1 - Site 4)	Species Code	Steelpoort River Site 1 Reference Frequency of Occurrence (FROC)	Steelpoort River Site 2 - Site 4 Reference Frequency of Occurrence (FROC)
Anguilla mossambica Peters, 1852	AMOS	-	3
Amphilius uranoscopus Pfeffer, 1889	AURA	1	3
Enteromius anoplus (Weber, 1897)	BANO	1	-
Labeobarbus marequensis Smith, 1841	BMAR	1	1
Enteromius neefi (Greenwood, 1962)	BNEE	-	1
Enteromius paludinosus (Peters 1852)	BPAU	-	3
Enteromius trimaculatus (Peters, 1852)	BTRI	1	1
Enteromius unitaeniatus (Günther, 1866)	BUNI	-	1
Clarias gariepinus Burchell, 1822	CGAR	-	1
Chiloglanis paratus Crass, 1960	CPAR	-	1
Chiloglanis pretoriae Van Der Horst, 1931	CPRE	1	1
Chiloglanis swierstrai Van Der Horst, 1931	CSWI	-	1
Labeo molybdinus Du Plessis, 1963	LMOL	-	1
Oreochromis mossambicus Peters, 1852	OMOS	-	1
Opsaridium peringueyi Gilcharist and Thompson, 1913	OPER	-	1
Pseudocrenilabrus philander weber, 1897	PPHI	1	-
Tilapia sparrmanii Smith, 1840	TSPA	1	1

FROC: 1 = Present at very few sites (<10% of sites); 2 = Present at few sites (>10–25%); 3 = Present at about >25–50% of sites; 4 = Present at most sites (> 50–75%); 5 = Present

5.2.2 STATISTICAL ANALYSIS

Shannon Weiner Diversity Index was performed to determine the fish species diversity variation among the four selected sites. Multivariate statistical analyses were used in the evaluation of variations between the various sites based upon their fish community structure. The multivariate technique used was gradient analysis as it explores data and determine differences in composition of various samples or sites (Van den Brink et al. 2003; Paliy and Shankar 2016). All the data were log transformed prior to analysis, assumption was made that one of the sets of environmental variables can be considered as independent and the other set is considered as dependant. The Monte Carlo permutation tests (499 unrestricted permutations) was used to test for significance of distributions. Canonical correspondence analysis (CCA) was used to determine which environmental variables were responsible for the distribution of different fish species. The CCA was done using Canoco Version 4.5.

5.3 RESULTS AND DISSCUSSION

5.3.1 FISH ABUNDANCE DISTRIBUTION AND RICHNES

A total of 623 individuals of fish and a total number of 19 different species were collected from all sites collectively throughout the study period. The temporal distribution of fish species showed that highest number of fish species (15) was recorded during autumn followed by 13 species recorded during summer (Table 5.2). The highest fish abundance (255) was recorded during summer while the second highest fish abundance (169) was recorded during autumn (Table 5.2). The lowest number of species (8) and fish abundance (75) were recorded during winter. A total number of 10 species and 124 individuals were recorded during spring. The low fish species richness and abundance recorded during winter may be attributed to low water level, thus less habitat availability. The higher fish species richness and abundance during summer may be attributed to higher water level and more habitat availability required by most fish species (Meye and Ikomi 2012; Chalifour et al. 2019).

The spatial distribution of fish across all sites revealed that site 1 has the highest fish abundance with a total of 211 fish individuals recorded. However, site 1 has the lowest number of species (5), hence this is an indication of unevenness of species diversity at site 1 (Table 5.2). Species recorded at site 1 included *Amphilius uranoscopus* Pfeffer, 1889, *Labeobarbus marequensis* Smith, 1841, *Enteromius trimaculatus* (Peters, 1852), *Chiloglanis pretoriae* Van Der Horst, 1931 and *Tilapia sparrmanii* Smith, 1840. Among the species recorded in site 1, *C. pretoriae* was found to be dominant with 139 individuals recorded. This species dominated all sites throughout the period of the study (Table 5.2). Only 1 individual of *A. uranoscopus* and *T. sparrmanii* were recorded. Site 2 has the second highest number of fish individuals (169) and has 12 fish species, among which two of them *Enteromius viviparus* (Weber, 1897) and *Oreochromis mossambicus* (Peters, 1852) were sampled once (Table 5.2). *Oreochromis mossambicus* and

Pseudocrenilabrus philander Weber, 1897 were only sampled in this site. There is no clear indication why these two species were sampled only at site 2.

Table 5.2: Fish species sampled at four sites in the Steelpoort River, August 2017– May 2018.

	Sampling sites															
	Site 1				Site 2				Site 3				Site 4			
Sampling survey (seasonal)	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut
Amphilius uranocopus (AURA)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enteromius eutaenia (BEUT)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Enteromius lineomaculatus (BLIN)	-	-	-	-	-	1	7	-	-	-	-	-	-	-	4	2
Labeobarbus marequensis (BMAR)	11	15	14	14	-	8	5	4	1	3	29	11	-	3	14	5
Enteromius neefi (BNEE)	-	-	-	-	-	-	5	4	-	-	-	-	-	-	2	2
Enteromius trimaculatus (BTRI)	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enteromius viviparus (BVIV)	-	-	-	-	1	-	-	-	-	-	-	-	-	1	12	-
Clarias gariepinus (CGAR)	-	-	-	-	1	-	2	-	-	-	-	-	-	1	3	2
Chiloglanis paratus (CPAR)	-	-	-	-	-	-	-	-	-	-	8	4	-	4	5	2
Chiloglanis pretoriae (CPRE)	5	59	37	38	28	7	18	11	1	12	20	25	2	1	17	8
Chiloglanis swiestrai (CSWI)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Glossogobius giuris (GGIU)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Labeo cylindricus (LCYL)	-	-	-	-	1	2	-	-	6	3	-	1	-	-	-	-
Labeo molybdinus (LMOL)	-	-	-	-	-	-	-	-	3	-	4	3	-	-	-	-
Mesobola brevianalis (MBRE)	-	-	-	-	-	-	-	5	-	-	-	-	-	1	-	-
Oreochromis mossambicus (OMOS)	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Opsaridium peringueyi (OPER)	-	-	-	-	-	-	15	7	-	-	3	1	-	-	7	-
Pseudocrenilabrus philander (PPHI)	-	-	-	-	-	2	14	16	-	-	-	-	-	-	-	-
Tilapia sparramanii (TSPA)	1	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-
Total Abundance per Season	Win= 75				Spr= 124				Sum= 255				Aut= 169			
Total Richness per Season	Win= 8				Spr= 10				Sum= 13				Aut= 15			
Total Abundance per Site	Site 1= 211				Site 2= 169				Site 3= 138				Site 4= 105			
Total Richness per Site	Site 1= 5				Site 2= 12				Site 3= 6				Site 4= 13			
H’	0.87				1.88				1.42				2.12			

Notes: Win= Winter; Spr= Spring; Sum= Summer and Aut= Autumn

H’ = Shannon Weiner Diversity Index

One hundred and thirty-eight individuals of fish were collected during the study period. Six species (*L. marequensis*, *C. pretoriae*, *Labeo molybdinus* Du Plessis, 1963, *Opsaridium peringueyi* Gilcharist and Thompson, 1913, *B. viviparus*, *Labeo cylindricus* Peters, 1852 were recorded at site 3 (Table 5.2). Thirteen species and 105 fish individuals were recorded at site 4, with three species *Glossogobius giuris* Hamilton-Buchanan, 1822, *Enteromius eutaenia* (Boulenger, 1904), *Chiloglanis swiestrai* Van Der Horst, 1931 being recorded only from this site (Table 5.2). The fish distribution in the river was also influenced by the velocity-depth classes present at the sampling site as the velocity-depth classes has an influence in the availability and diversity of the micro-habitat (Wyżga et al. 2009).

The Shannon Weiner Diversity Index showed variation in species diversity among the different sites. Site 1 has the lowest species diversity (H'= 0.87), however this is not an indication of a significant loss of fish species diversity at this site since most of the reference fish species were recorded. This site is subjected to relatively low anthropogenic activities. However, slight modification of velocity-depth and flow due to the bridge and tree branches blocked by the bridge just upstream of the site may have negative impact on habitat availability at site 1. Cover modification as a result of rapid growing riparian vegetation may create new habitat for fish species not previously recorded at a site (Wepener et al. 2011). The second lowest species diversity (H'= 1.42) was recorded at site 3. Relatively high modification and loss of habitat has occurred at this site mainly as a result of sedimentation into the stream from the riparian area. The flow conditions at this site has also been greatly modified as a result of debris material of an old bridge which blocks tree branches and alter flow of water.

Site 4 has the highest species diversity (H'= 2.12). The fish community structure recorded during the study period at site 4 was different from the reference fish community structure (Kleynhans et al. 2007). These differences may be as a result of the combined effects of unfavourable habitat (as reflected in the predominance of the fast-deep and slow-deep habitat) and altered water quality (Wepener et al. 2011). Site 2 has the second highest species diversity, supported by a H' value of 1.88 (Table 5.2). Nevertheless, the change of the fish community structure has occurred and may be associated with sand mining rapidly carried out in the river by the nearby villagers at this site which in turn changes the flow type and the substrate of the river bed. Although habitat changes have occurred in each of the selected sites, the changes in the water and sediment quality remain the most important potential drivers of this change in fish community structure (Wyżga et al. 2009).

5.3.2 FISH RESPONSE ASSESSMENT INDEX

The FRAI results indicate that the present ecological status of the fish community decreases from moderately modified C category at site 1 to a transition of largely modified to seriously modified category D/E at site 3. However, the ecological state of the river recovers to D category downstream at site 4 (Table 5.3). The PES (category C) at site 1 indicates that a loss and change of natural habitat and biota have occurred, this change could be attributed to a loss of habitat and biota (Kleynhans et al. 2007). The ecological category C/D found at site 2 indicates that loss and changes of natural habitat has occurred. However, the FRAI scores of at sites 1 2 were relatively similar to each other (Table 5.3).

Table 5.3: FRAI assessment for the sampling surveys from August 2017 – May 2018 for the Steelpoort River.

Sampling Sites	FRAI Score (%)	Ecological Category	Ecological Category Description
Site 1	62.5	C	Moderately modified. A loss and change of natural habitat and biota have occurred but the basic ecosystem functions are still predominantly unchanged.
Site 2	61.4	C/D	Transition between moderately and largely modified. A large loss of natural habitat, biota and basic ecosystem functions is occurring.
Site 3	41.7	D/E	Transition between largely modified and seriously modified. The loss of natural biota and basic ecosystem function is intensifying.
Site 4	57.2	D	A large loss of natural habitat, biota and basic ecosystem functions has occurred.

FRAI scores showed evidence that site 3 is the most affected among the four sites. This is revealed by the PSE category D/E which indicates that the fish community structure has been largely modified and has the potential of moving to a state of being seriously modified. Site 4 PES category (D) indicates that the river is recovering from an impact that occurred upstream (Kleynhans et al. 2007; Table 5.3).

The fish reference community in Table 5.1 provides a comparison for the fish that were collected during the surveys in Table 5.2. It is evident that most of the reference fish community were collected during the surveys (Kleynhans et al. 2007). However, some species which are not in the fish reference community for these ecoregions were collected. These species include *E. eutaenia*, *Enteromius lineomaculatus* (Boulenger, 1903), *Enteromius neefi* (Greenwood, 1962), *E. viviparus*, *C. swiestrai*, *G. giuris*, *L. cylindricus* and *Mesobola brevianalis* Boulenger, 1908. The results of the fish community clearly showed temporal variation with each survey resulting in different species and abundance of species present at each of the chosen sites. The reference fish community data for site 1 has seven species (Kleynhans et al. 2007). Five of these species were observed and had higher FROC as compared to the reference FROC (Table 5.1 and Table 5.2). The flow at site 1 was generally lower than normal and may have influence in the abundance and availability of different fish species (King 1998).

The reference fish community for sites 2, 3 and 4 showed at Table 5.1 indicated that 15 species were expected to be present at each one of these sites. At site 2, only 7 species of the reference fish community were observed, while at site 3 only 5 species were observed throughout the study period. However, the number of species observed increased at site 4 with eight of the reference species collected. Five fish species which were never recorded before at site 3 and 4 were recorded during the study period. At site 3 only one species that was never recorded before was recorded during the study period (Table 5.1 and Table 5.2). It is evident that there is a noticeable change in the fish community structures within the study area and that this change is in the form of an increase in FROC of those species that has been observed and in the form of introduction of other species to ecoregions they were never recorded from before with the disappearance of the reference species.

5.3.3 CANONICAL CORRESPONDENCE ANALYSIS

To integrate the responses of the different fish attributes to environmental parameters a CCA was completed.

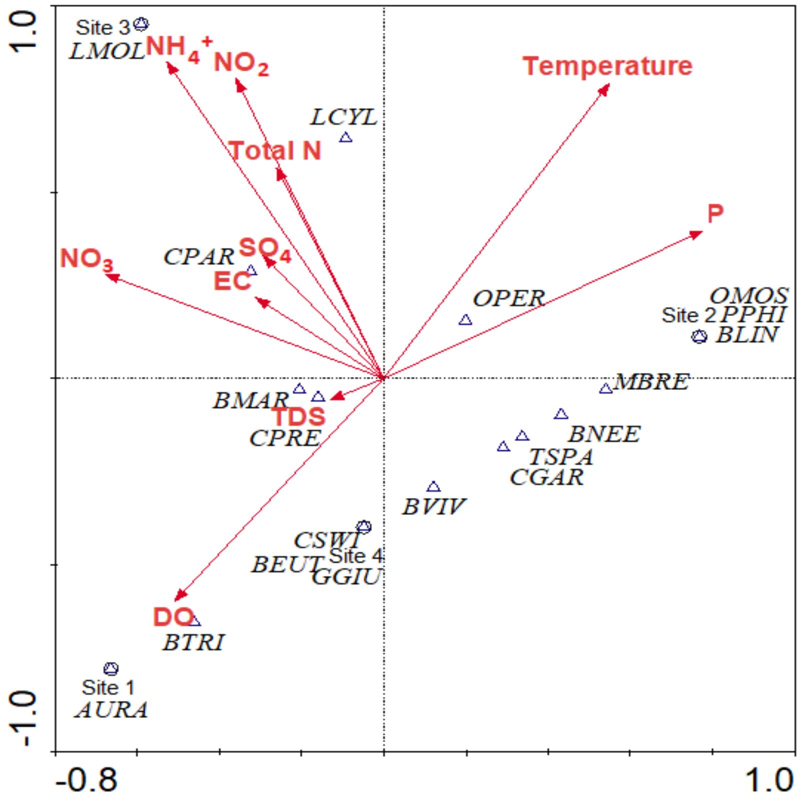

Figure 5.1: CCA plot of the relationship between fish species sampled and system variables.

The distribution of most fish species showed weak correlation to the environmental variables (Figure 5.1). However, some species such as *A. uranoscopus* and *E. trimaculatus* showed a strong correlation with high concentrations of Dissolved Oxygen (DO) at site 1 (Figure 5.1). *Chiloglanis paratus* Crass, 1960 showed a strong correlation with higher concentrations of Electrical Conductivity (EC), SO_4 and NO_3. Ammonium and NO_2 had a strongly influence in the distribution of *L. molybdinus* and *L. cylindricus* at site 3 (Figure 5.1). The distribution of *M. brevianalis* and *C. pretoriae* were strongly influenced by TDS, DO and EC (Figure 5.1). Higher levels of temperature did not have a strong influence on the distribution of any fish species. The distribution of *C. swierstrai*, *G. giuris* and *E. eutaenia* at site 4 were strongly correlated to moderate to higher concentrations of DO (Figure 5.1).

The distribution of *O. mossambicus*, *P. philander* and *B. lineomaculatus* were strongly correlated to phosphorus at site 2.

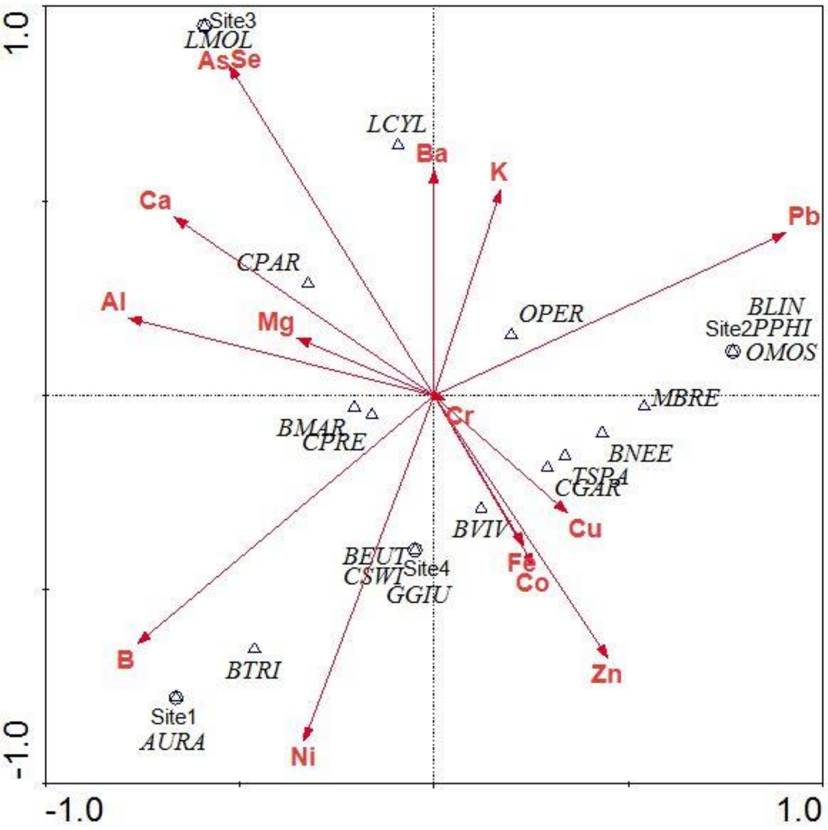

Figure 5.2: CCA plot of the relationship between fish species sampled and sediment metal concentrations.

A strong correlation between most metal concentrations and distribution of fish species were observed (Figure 5.2). Higher concentrations of arsenic (As) and selenium (Se) were strongly correlated to the distribution of *L. molybdinus* at site 3. *Chiloglanis paratus* distribution was strongly correlated by higher concentrations of Barium (Ba), Potassium (K) and moderate concentrations of arsenic (As), selenium (Se) and lead (Pb) (Figure 5.2). The distribution of *C. swierstrai*, *B. eutaenia* and *G. giuris* at site 4 was strongly influenced by moderate to higher level of Nickel (Ni). Higher level of Ni and Boron (B) has a strong correlation with *A. uranoscopus* at site 1 (Figure 5.2). Chromium (Cr) and Aluminium (Al) did not show any strong correlation or influence in the distribution of any species although Cr is being mined at the Steelpoort Catchment area. Higher concentrations of Copper (Cu) and moderate concentrations of Zinc (Zn) had a strong influence in the distribution of *B. neefi*, *C. gariepinus* and *T. sparrmanii*. At site 2, Pb had a strong correlation with the distribution of *B. lineomaculatus O. mossambicus* and *P. philander* (Figure 5.2).

Table 5.4: The summary of CCA results for environmental factors (physico-chemical parameters and metals) and fish.

Axes	1	2	3	4	Total Inertia
Eigenvalues	0.36	0.28	0.26	0	0.90
Species-environment correlations	1.00	1.00	1.00	0	
Cumulative percentage variance					
*of species data	40.00	71.10	100	0	
*of species-environment relation	40.00	71.10	100	0	
Sum of all eigenvalues					0.90
Sum of all canonical eigenvalues					0.90

The taxa-environment factor correlation (r) for factor 1, 2 and 3 were 1.000 (Table 5.4). The cumulative variation explained by first axis was 40% while for the second axis was 71.10% and for the third axis was 100% (Table 5.4). The cumulative variation explained by the three axis implies that the measured environmental variables were sufficient in explaining much of variance of the fish community structure.

5.4 CONCLUSION

Biological monitoring of river systems using fish as bioindicators has been widely recognised as an appropriate aspect of water resource management and conservation in South Africa (Bere 2016). The FRAI results were able to indicate variation in the fish community structure among different sites. The PES showed a decline of water quality from upstream to downstream. Site 1 was the least impacted site followed by site 2 and site 4 while site 3 was the most impacted site with poor water quality compared to other three sites (sites 1, 3 and 4). The FRAI results supported the SASS results obtained in Chapter 4. The diversity, abundance and distribution of the fish were strongly influenced by system variables and the concentration of metals at each site, although some fish species were less influenced by the system variables. It can be concluded that the river is generally in transition between ecological category C and D. However, there was very little fish monitoring data available for a comparison among the sites. Only data from the national river monitoring programme were available and used for comparison within this study.

Linking fish community changes to individual environmental parameters has been nearly impossible since there is no concurrent water, sediment or habitat data (RHP 2003; Malherbe 2016). There is a need for long term monitoring data of fish communities, environmental and habitat requirements which will give a better interpretation of the various fish community structures. However, the different fish community structures observed in this study were driven by multiple factors including climate, habitat modifications, flow volumes, water quality and potential anthropogenic activities as also mentioned by Malherbe (2016). Increasing human populations and development in the catchment area may result in the degradation of water quality.

CHAPTER 6

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

In South Africa, the Olifants River System of which the Steelpoort River is a tributary, has been constantly reported as one of the polluted river systems as a result of being subjected to prolonged pollution mainly from mining and industrial activities (Morokong et al. 2016; Njiraini et al. 2016). These activities have led to the deterioration of the health status of the river system including the Steelpoort River and continues to pose a threat to the aquatic ecosystem of this river (Ashton and Dabrowski 2011). The main aim of this study was to investigate the influence of mining activities on the water quality and health status of the Steelpoort River. This was achieved through assessing the water quality of the river by determining the concentration levels of physico-chemical parameters, macro-nutrients and metals of the water and sediments, the response of invertebrate assemblages to water quality using SASS 5 and also the diversity of fish using FRAI.

Water quality

The water quality of the Steelpoort River was generally at accepted condition for aquatic ecosystems with respect to the South African Water Quality Guidelines (SAWQG), this is because most of the system variables measured (temperature, Dissolved Oxygen (DO), pH, Total Dissolved Solids (TDS), total nitrogen, ammonium (NH_4), phosphorus (P) and sulphate (SO_4) were within the Target Water Quality Range (TWQR) (DWAF 1996a; b; c). However, some values above the TWQR were recorded. These include total nitrogen at sites 2, 3 and 4 and NH_4 at site 3. These concentrations indicate mesotrophic conditions. Higher levels of ammonium are known to have negative effects on the respiratory system of mayfly larvae and pose risk of growth rate reduction and cause changes in the tissue of gills, liver and kidneys of many fish species (DWAF 1996c). The pH was slightly alkaline throughout the study period while the mean water temperature ranged from 18.73°C to 22.08°C. There was no significant different ($p>0.05$) among the sites for all the system variables except for Electrical Conductivity (EC) which was high at site 4.

Higher metal concentrations were recorded in sediment compared to water. However, both metal concentrations in water and in sediment indicated that there is an increase of inflow of metals from upstream to downstream. Most metals were within the recommended water and sediment quality range (DWAF 1996c; CCME 2012). These metals include; As, B, Ba, Cd, Cu, Pb, Ni and Cr. However, in water Cu was higher at site 2 compared to the other sites. All recorded concentrations of Cr in the sediment were high at all sites. Iron and Zn were generally the most concentrated metals in all samples and were above the recommended quality ranges throughout the study period. The high concentrations of Cr, Fe and Zn might be strongly associated with the mining activities. The other source of these metals in the water column might be sediments on the river bed as sediments releases suspended substances back into the water column (Liu et al. 2019). However, some of the metals recorded in sediment were not detected in the water. Highly intense mining area upstream of sites 3 and 4 may be contributing to the higher concentrations of metals at these sites. The inflow from Dwars River might be contributing to the higher concentrations of macro-nutrients and metals recorded at sites 3 and 4.

Macro-invertebrate distribution, richness and abundances

The macro-invertebrate assemblages observed supported the hypothesis that areas subjected to less pollutants generally tend to support higher diversity of life than areas subjected to high level of pollutants (Wright and Ryan 2016, Fierro at al. 2017). In this study, site 1 was located in area with less anthropogenic activities (Chapter 2), thus it is expected to be the least affected site. This site had both the highest diversity of macro-invertebrate families and the highest macro-invertebrate abundance. The diversity of macro-invertebrate families and their abundance showed a decline from upstream to downstream, thus site 4 has the lowest macro-invertebrate diversity and site 3 has the lowest macro-invertebrate abundance. Although pollution played a major role in shaping the macro-invertebrate community structure at all four sites, the availability of the microhabitats at these sites may also impact the presence of some macro-invertebrate families as also reported by (Ferronato et al. 2019). Although site 3 was found to be the most polluted site, a higher number of macro-invertebrate families (32) was recorded at this site compared to 27 families recorded at site 4 (Chapter 4). However, it was evident that site 3 had poor water quality condition. The order Ephemeroptera was dominated at all sites. This order is known for its moderate to higher sensitivity to pollution (Kaaya et al. 2015). Larval stages of families within this order, such as Baetidae and Leptophlebiidae, are known to be sensitive to oxygen depletion (Menetrey et al. 2008; Herman and Nejadhashemi 2015). Sites 1 and 2, with higher abundance of Ephemeroptera, may be associated with low levels of pollution or good water quality as compared to sites 3 and 4 with low abundance of Ephemeroptera. Ephemeroptera together with other two sensitive orders, Plecoptera and Trichoptera,

were used in the analysis of EPT richness and diversity and EPT to Chironomidae ratio (EPT/C ratio). Chironomidae is known to be less sensitive and more tolerant to pollution (Dickens and Graham 2002; Rak et al. 2017). Site 1 had the highest EPT while site 3 had the lowest EPT. Higher EPT scores indicate domination of higher pollution sensitive families at a site, thus good water quality (De Walt et al. 2018). However, the highest EPT/C ratio was recorded at site 2, while the lowest ETP/C ratio was recorded at site 3. Site 3 has been shown to have poor water quality throughout the study period. This site is subjected to higher level of habitat modification as well as industrial and mining effluents.

South Africa Scoring System Version 5

The SASS scores obtained also showed a trend of deterioration in water quality from upstream to downstream, with site 3 having the lowest SASS score and site 1 having the highest SASS score. The ASPT trend also supported the SASS scores, with site 1 having the highest ASPT score while site 3 has the lowest ASPT score. High SASS score and ASPT score at site 1 may be due to the presence of highly sensitive families and their high abundance (De Walt et al. 2018). The presence of these families is associated with higher diversity of micro-habitat, good water quality and less inflow of pollutants (Gordon et al. 2015; Bawa et al. 2018). At site 3, the low SASS score and low ASPT score may be associated with low number of sensitive families, thus major deterioration of water quality has been observed (Bawa et al. 2018; De Walt et al. 2018). There is a higher level of pollutants entering the stream upstream of site 3 which may be negatively affecting the water quality at this site. SASS score and ASPT at site 4 are higher than at site 3, this is an indication that the river health status is being restored (Bassey 2019). The Canonical Correspondence Analysis (CCA) indicated a strong correlation between some system variables, metal concentrations and the presence of macro-invertebrate. Although some system variables strongly correlated with some macro-invertebrate families, all the variables were within the accepted water quality ranges. However, sensitive macro-invertebrate families such as Pleidae, Ecnomidae, Athericidae and Synlestidae had strong correlation with higher concentrations of Pb, Cr and Mg. Lestidae and Pyralidae were strongly correlated with As and Se (see Chapter 4). These strong correlations indicate the influence of pollutants on the distribution and composition of macro-invertebrate structures in the river system (Haggag et al. 2018; Liu et al. 2019). Site 1 was located in an area with low anthropogenic influence and was expected to have good water quality compared to sites 3 and 4 located in area with intense mining activities. The micro-invertebrate results found supported the theory of finding good water quality in a less impacted site as also mentioned by (Bassey 2019).

Fish abundance, distribution and richness

The fish abundance decreases from upstream to downstream, with site 1 having the highest fish abundance followed by site 2 and site 3. Site 4 had the lowest fish abundance. Although site 4 has the lowest abundance, it has the highest species richness while site 1 had the lowest species richness. The Shannon Weiner Diversity Index (H') supported that site 1 has the lowest species richness while site 4. However, this comparison does not indicate higher loss of species from the reference species at site 1 as the species richness at site 4 is higher than that of site 1 as indicated on the reference species list (Kleynhans et al. 2007). Fish community structure recorded at site 1 shows slight modification from the reference condition as compared to sites 2, 3 and 4 while site 3 has undergone relatively high modification. Thus, site 1 was considered the least modified site compared to sites 2, 3 and 4.

Fish Response Assessment Index

The FRAI index also showed a trend of degradation of water quality from upstream to downstream. Site 1 has an Ecological Category (EC) of C (moderately modified) while site 2 is in a transition stage between EC of C and D (moderately modified and largely modified). Site 3 was found to be in a transition stage between EC of D and E (largely modified and seriously modified). This trend was influenced by an increase of influx of pollutants from agricultural land, industrial and mining area and increase in the extent in which habitats are being altered from upstream to downstream. According to Zhao at al.

(2018), the increase of pollution into streams generally result in decrease in the fish diversity. Site 4 has an EC of D, this shows that the quality of the river at this site is being restored from the major degradation that occurred at site 3. The Canonical Correspondence Analysis showed a weak correlation between the distribution of fish species and system variables, however, a strong correlation between the metals and the distribution of fish species was observed (see Chapter 5). The majority of fish species were strongly correlated to a higher concentration of at least one metal except for *Mesobola brevianalis* Boulenger, 1908, *Chiloglanis pretoriae* Van Der Horst, 1931, *Labeobarbus marequensis* Smith, 1841 and *Enteromius neefi* (Greenwood, 1962). This could be evidence for the hypothesis that system variables and metals can have diverse effects on the health and distribution of aquatic biota including fish species (Liao et al. 2018).

Generally, the water quality of the Steelpoort River was found to be relatively good, with moderate modifications in the status of the river. This can be concluded taking into consideration all the results obtained from the analysis of water quality parameters, indices based on macro-invertebrates and fishes. However, it is important to note that the Steelpoort River is constantly exposed to the risk of inflow of pollutants. Thus, for future studies, it is important to assess metal accumulation in fish and macro-invertebrates and the risk associated with consumption of fish from the Steelpoort River System.

Biomonitoring of river systems

A wide range of natural and synthetic chemical substances produced over the past decade continue to affect the water quality of many river systems up to date. Although these substances did not evoke an immediate effect in ecosystems, they have accumulated in the water bodies and have certainly contributed to the structural and functional changes of the aquatic ecosystems (Keck et al. 2017). These substances have been monitored over the years in many countries worldwide, nevertheless the emphasis of such monitoring was on the chemical and physical properties of water bodies and lacked essential biological information. The findings of this study showed that the river health status and quality of the water of the Steelpoort River is deteriorating from upstream to downstream. It is for this reason that it is important to conduct further research and biomonitoring should be conducted in the Olifants River system which the Steelpoort River is a tributary.

For future studies, monitoring river systems and other water bodies should include evaluation of metal bioaccumulation, toxicity tests in both fish and macro-invertebrates. Human health risk assessment should be considered as well since communities near the Steelpoort River consume fish from the river.

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APPENDIX A: WATER QUALITY VARIABLES

Table 1: Seasonal water quality variables recorded at Steelpoort River (August 2017 – May 2018).

	Summer				Autumn				Winter				Spring			
Water Quality Parameters	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Water temperature (°C)	22.7	25	24.3	21.6	11.3	19.2	17.9	16.5	16.7	19.5	19.7	17.3	24.2	23.9	26.4	27.4
Dissolved Oxygen (mg/ℓ)	8.78	7.35	7.53	8.06	9.91	6.19	6.71	5.23	9.06	8.97	10.09	10.97	8.18	8.69	9.08	7.58
Dissolved Oxygen (%)	102	89.7	90	91.4	73.1	63.7	69.7	51.7	95.1	98.9	111	115.7	97.2	104	113.5	95.9
pH	8.65	8.74	8.7	8.65	8.13	7.56	7.7	8.13	8.92	8.89	8.5	8.92	8.57	8.71	8.65	8.71
Conductivity (mS/m)	178	223	267	276	188.9	242.5	255.3	288.2	246	214	362	396	259	250	289	301
TDS (mg/ℓ)	89	110	133	138	146.9	176.8	119	196.6	123	107	181	198	130	125	144	150
Nitrate (mg/ℓ NO_3^-N)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.5	0.7	0.83	0.67	1.97	1.63	-	-	-	-
Nitrite (mg/ℓ NO_2^-N)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.09	<0.05	0.03	0.04	0.04	0.05	-	-	-	-
Ammonium (mg/ℓ NH_4^+)	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.05	0.07	0.12	0.07	-	-	-	-
Total Nitrogen	-	-	-	-	-	-	0.59	0.70	0.91	0.78	2.13	1.75	-	-	-	-
Phosphate (mg/ℓ PO_4^-)	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.07	0.05	0.13	0.04	-	-	-	-
Sulphates (SO_4^-)	8	12	9	13	-	-	-	-	20.97	19.33	35.87	32.43	-	-	-	-

Table 2: The seasonal metal concentrations of the water at four sites in the Steelpoort River (August 2017 – May 2018).

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Ag (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Al (mg/ℓ)	< 0.100	0.764	< 0.100	1.126	< 0.100	< 0.100	< 0.100	0.213	< 0.100	< 0.100	0.319	< 0.100	< 0.100	< 0.100	0.183	< 0.100
As (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Au (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
B (mg/ℓ)	< 0.010	0.010	< 0.010	0.011	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	0.004	0.006	< 0.010	< 0.010	0.013	0.015
Ba (mg/ℓ)	0.035	0.035	0.031	0.055	0.035	0.033	0.034	0.031	0.024	0.022	0.023	0.020	0.042	0.030	0.042	0.028
Be (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Bi (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ca (mg/ℓ)	17.66	26.8	24.65	29.77	20.07	22.69	24.88	25.39	24.15	25.55	29.65	28.44	23.22	25.08	26.45	26.15
Cd (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ce (mg/ℓ))	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Co (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Cr (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.013	0.004	0.009	0.004	< 0.010	< 0.010	< 0.010	< 0.010
Cs (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Cu (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Dy (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010

Table 2 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Er (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Eu (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Fe (mg/ℓ)	0.151	0.993	0.137	1.25	0.131	0.035	0.111	0.334	0.272	0.08	0.4	0.046	0.088	0.033	0.211	0.06
Ga (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.001	0.001	0.001	0.001	< 0.010	< 0.010	< 0.010	< 0.010
Gd (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ge (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Hf (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Hg (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.005	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ho (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
In (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ir (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
K (mg/L)	1.153	1.788	1.618	1.829	1.136	1.615	1.726	1.716	1.207	1.813	1.741	1.703	1.362	1.642	1.75	1.663
La (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
La (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Li (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Lu (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010

Table 2 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Mg (mg/ℓ)	9.177	14	11.23	17.8	10.69	11.7	14.33	15.53	11.24	12.61	16.87	21.22	11.94	11	12.93	15.17
Mn (mg/ℓ)	< 0.025	0.106	0.056	0.116	< 0.025	0.04	< 0.025	0.029	< 0.025	< 0.025	0.026	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025
Mo (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.001	0.001	0.001	0.001	< 0.010	< 0.010	< 0.010	< 0.010
Na (mg/ℓ)	8.206	14.66	11.45	15.53	9.713	11.96	14.86	16.26	10.52	15.47	20.99	22.08	11	12.33	20.57	15.59
Nb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Nd (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ni (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.001	< 0.001	0.002	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Os (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.002	0.002	0.002	0.002	< 0.010	< 0.010	< 0.010	< 0.010
P (mg/ℓ)	0.023	0.059	0.026	0.053	0.051	0.045	0.040	0.042	0.007	< 0.001	0.004	0.005	0.058	0.027	3.391	0.056
Pb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Pd (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.001	0.001	0.001	0.001	< 0.010	< 0.010	< 0.010	< 0.010
Pr (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Pt (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Rb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.002	0.002	0.001	0.001	< 0.010	< 0.010	< 0.010	< 0.010
Rh (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ru (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010

Table 2 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Sb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Sc (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	0.003	0.002	0.003	0.003	< 0.010	< 0.010	< 0.010	< 0.010
Se (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Si (mg/ℓ)	7.405	9.824	6.464	9.979	8.428	7.431	7.511	8.316	9.9	6.981	8.445	8.521	9.416	6.8	7.859	7.418
Sm (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Sn (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Sr (mg/ℓ)	0.074	0.111	0.103	0.115	0.083	0.098	0.104	0.104	0.096	0.108	0.123	0.122	0.113	0.114	0.113	0.118
Ta (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Tb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Te (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Th (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Ti (mg/ℓ)	0.017	0.024	0.022	0.030	0.019	0.021	0.023	0.025	0.009	0.009	0.013	0.011	0.022	0.022	0.025	0.020
Tl (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Tm (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
U (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010

Table 2 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
V (mg/ℓ)	< 0.010	0.017	< 0.010	0.019	< 0.010	< 0.010	0.013	0.015	0.003	0.003	0.037	0.033	< 0.010	< 0.010	0.020	0.023
W (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Y (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Yb (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010
Zn (mg/ℓ)	0.064	0.054	0.054	0.050	0.024	0.029	0.024	0.030	0.015	0.021	0.014	0.014	0.015	< 0.010	< 0.010	< 0.010
Zr (mg/ℓ)	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.010	< 0.001	< 0.001	< 0.001	< 0.001	< 0.010	< 0.010	< 0.010	< 0.010

Table 3: The seasonal metal concentrations of the sediment at for sites in the Steelpoort River (August 2017 – May 2018).

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Ag (mg/kg)	0.00	0.00	8.40	2.00	0.00	0.00	0.00	0.00	0.16	0.17	2.21	0.60	4.11	0.53	0.00	0.00
Al (mg/kg)	42482.63	32742.50	36643.60	43675.19	53091.82	42918.00	66971.21	59399.20	29380.00	48617.00	53535.00	53471.00	54277.78	28148.90	52916.53	45503.80
As (mg/kg)	0.80	1.20	2.40	0.80	0.99	0.88	0.78	0.90	1.42	1.58	2.12	1.11	2.35	1.38	1.25	1.03
Au (mg/kg)	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.45	0.15	0.27	0.00	0.00	0.00
B (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	72.00	35.00	47.00	59.00	0.00	0.00	0.00	0.00
Ba (mg/kg)	123.35	206.32	178.40	204.55	183.45	312.84	460.07	543.39	168.00	385.00	369.00	257.00	171.54	152.72	376.11	337.28
Be (mg/kg)	0.40	0.40	1.20	0.40	0.39	0.38	0.73	0.52	0.48	0.90	0.81	0.54	0.60	0.60	0.00	0.00
Bi (mg/kg)	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	1.38	0.17	1.00	0.00	0.00	0.00
Ca (mg/kg)	29336.13	29135.15	34482.80	34761.09	39631.54	30458.80	50158.74	39843.11	22593.00	45194.00	43266.00	44972.00	40541.57	18740.52	44502.80	36885.34
Cd (mg/kg)	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.05	0.06	0.16	0.03	0.25	0.00	0.00	0.00
Ce (mg/kg)	7.19	9.20	8.40	5.99	12.15	13.51	11.83	13.51	17.00	20.00	15.00	10.00	15.45	12.09	14.57	14.36
Co (mg/kg)	99.80	95.56	88.40	69.92	90.07	90.83	37.94	40.08	105.00	51.00	50.00	54.00	82.65	94.18	60.52	72.92
Cr (mg/kg)	72.26	165.13	486.80	601.28	69.59	522.69	612.67	650.91	102.00	77.00	341.00	995.00	60.40	609.56	476.84	1460.68
Cs (mg/kg)	0.00	0.00	1.20	0.40	0.35	0.37	0.42	0.59	0.36	0.57	1.67	0.55	0.73	0.46	0.54	0.54
Cu (mg/kg)	47.50	47.18	36.80	29.17	46.60	40.78	19.44	21.00	55.00	38.00	23.00	21.00	50.01	57.19	32.23	29.20

Table 3 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Dy (mg/kg)	0.80	1.20	1.20	0.80	1.60	1.65	1.27	1.70	1.68	2.44	1.85	1.29	1.74	1.17	1.55	1.62
Er (mg/kg)	0.40	0.80	0.00	0.40	0.90	0.96	0.78	0.93	1.01	1.52	1.16	0.81	1.07	0.70	1.00	0.95
Eu (mg/kg)	0.40	0.40	0.80	0.40	0.82	0.82	0.96	1.08	0.64	1.19	1.21	0.79	1.01	0.52	0.98	0.84
Fe (mg/kg)	236696.61	206607.36	161490.00	118347.98	158940.12	174818.00	51117.55	49199.60	237942.00	88476.00	59098.00	79842.00	227826.54	240527.74	137828.27	124917.30
Ga (mg/kg)	28.74	35.59	30.40	30.36	34.70	48.62	63.42	69.28	27.00	31.00	28.00	25.00	51.42	36.80	89.92	66.77
Gd (mg/kg)	0.80	1.20	1.20	0.80	1.75	1.74	1.38	1.68	1.99	2.79	2.11	1.40	2.14	1.55	1.91	1.73
Ge (mg/kg)	1.20	1.20	1.60	1.20	1.27	1.34	0.95	1.04	1.53	1.51	1.50	1.38	0.45	0.00	0.97	1.55
Hf (mg/kg)	1.20	1.20	7.60	1.60	0.00	0.00	0.00	0.00	2.37	2.00	19.00	2.06	5.79	1.66	1.85	1.73
Hg (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ho (mg/kg)	0.00	0.40	0.40	0.00	0.32	0.34	0.27	0.34	0.34	0.51	0.43	0.29	0.36	0.25	0.30	0.29
In (mg/kg)	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.05	0.04	0.18	0.03	0.27	0.00	0.00	0.00
Ir (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.10	0.00	0.00	0.00	0.00
K (mg/kg)	2882.63	5548.98	4607.20	7801.84	2081.04	5913.20	9561.38	10803.59	3488.00	10283.00	8752.00	7079.00	3379.70	3849.50	8382.59	6668.00
La (mg/kg)	2.79	4.00	0.00	2.40	5.73	6.86	6.50	7.43	8.14	11.00	8.04	5.09	7.36	5.82	7.50	6.89
Li (mg/kg)	3.19	5.20	0.00	4.00	6.76	5.41	7.45	8.80	3.26	10.00	7.51	6.51	6.19	3.51	5.67	7.70
Lu (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.24	0.23	0.14	0.00	0.00	0.00	0.00

Table 3 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Mg (mg/kg)	14930.14	17185.13	29224.00	29668.40	18569.66	18838.80	19203.12	23954.89	13002.00	14884.00	26604.00	25565.00	19701.04	18403.99	26550.32	36688.77
Mn (mg/kg)	1906.59	1854.86	1659.60	1469.04	1673.05	1717.60	830.87	869.06	2013.00	1216.00	1089.00	1143.00	2236.21	2312.18	1619.41	1716.74
Mo (mg/kg)	0.80	0.80	0.80	0.40	1.62	2.74	3.98	5.63	0.81	0.40	0.67	0.45	0.43	0.51	0.48	0.82
Na (mg/kg)	11130.54	8293.48	7728.80	9805.03	14297.41	10142.00	14520.19	13626.75	7904.00	13190.00	12188.00	11502.00	13331.73	5982.44	11068.69	8751.90
Nb (mg/kg)	5.19	4.00	5.60	3.60	0.00	1.10	0.00	0.00	15.00	4.40	11.00	4.19	5.63	6.82	4.69	6.65
Nd (mg/kg)	3.59	0.00	0.00	0.00	6.78	7.76	6.42	7.79	8.60	12.00	8.48	5.50	8.29	6.90	8.34	7.82
Ni (mg/kg)	37.92	84.77	167.20	163.40	31.87	96.75	101.45	115.76	1392.00	758.00	583.00	725.00	34.43	142.54	120.69	185.05
Os (mg/kg)	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.11	0.05	1.37	0.62	0.22	0.09	0.00	0.00
P (mg/kg)	1342.91	1309.48	1255.60	1277.67	1446.25	1255.42	1125.30	1254.50	687.00	767.00	324.00	285.00	2396.18	1627.98	1271.79	1270.67
Pb (mg/kg)	3.19	4.00	3.60	4.39	3.34	3.87	4.60	5.14	6.51	12.00	6.28	5.67	3.28	2.66	3.66	3.55
Pd (mg/kg)	0.00	0.00	2.40	0.40	0.00	0.00	0.00	0.00	0.30	0.38	1.43	0.66	0.49	0.00	0.00	0.00
Pr (mg/kg)	0.80	1.20	1.20	0.80	1.57	1.84	1.58	1.87	2.18	2.93	2.24	1.40	1.99	1.58	1.98	1.93
Pt (mg/kg)	0.00	0.00	1.20	0.40	0.00	0.00	0.00	0.00	0.02	0.00	0.29	0.13	0.37	0.00	0.00	0.00
Rb (mg/kg)	3.19	8.40	12.80	8.79	10.22	19.33	29.02	35.47	12.00	35.00	31.00	14.00	13.82	14.58	25.34	21.22
Rh (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ru (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Sb (mg/kg)	0.00	0.00	0.80	0.00	0.00	0.00	0.00	0.00	0.10	0.16	0.69	0.13	0.95	0.23	0.00	0.46
Sc (mg/kg)	22.75	21.59	26.40	13.18	32.12	25.20	16.24	18.27	47.00	71.00	71.00	65.00	26.75	22.97	20.81	22.81
Se (mg/kg)	0.00	0.40	4.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08	0.06	0.00	0.00	0.00	0.00
Si (mg/kg)	143117.76	162738.90	189724.00	225773.07	160195.60	176916.00	232543.00	251213.60	107725.00	226018.00	236500.00	218198.00	164720.20	133025.90	216585.50	211909.70
Sm (mg/kg)	0.80	1.20	1.20	0.80	1.61	1.71	1.43	1.69	1.83	2.65	2.05	1.35	2.05	1.48	1.75	1.77
Sn (mg/kg)	0.80	0.40	1.20	0.00	0.00	0.00	0.00	0.00	1.06	1.42	0.90	0.63	1.13	1.05	0.32	0.59
Sr (mg/kg)	133.73	103.56	102.00	75.91	176.22	118.48	197.85	174.40	92.00	162.00	171.00	163.00	181.23	74.69	152.95	148.22
Ta (mg/kg)	0.00	0.00	2.40	0.00	0.00	0.00	0.00	0.00	2.97	0.15	4.71	0.37	2.52	0.00	0.00	0.00
Tb (mg/kg)	0.00	0.00	0.40	0.00	0.25	0.27	0.00	0.26	0.29	0.42	0.38	0.23	0.35	0.22	0.31	0.27
Te (mg/kg)	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.11	0.35	0.00	0.00	0.00
Th (mg/kg)	0.80	0.80	7.60	1.20	0.00	0.00	0.00	0.00	2.98	3.57	14.00	2.76	3.45	0.95	1.10	1.39
Ti (mg/kg)	47624.35	35434.63	25988.00	18009.19	23876.44	28246.09	4741.30	3592.28	48404.00	10603.00	5114.00	12254.00	30652.35	40799.68	19744.58	19659.67
Tl (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.25	0.50	0.16	0.33	0.00	0.00	0.00
Tm (mg/kg)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.22	0.22	0.13	0.00	0.00	0.00	0.00
U (mg/kg)	0.00	0.00	0.00	0.40	0.34	0.40	0.32	0.44	0.84	1.04	0.80	0.57	0.48	0.35	0.41	0.48

Table 3 continued…

Metals and Metalloids	Summer				Autumn				Winter				Spring			
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
V (mg/kg)	1260.68	1434.63	0.00	654.02	739.33	1241.30	176.43	155.45	1302.00	424.00	212.00	384.00	887.47	1904.95	848.52	669.44
W (mg/kg)	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.75	0.24	1.48	0.39	0.91	0.31	0.00	0.14
Y (mg/kg)	4.39	6.40	6.40	3.60	7.75	8.68	7.01	8.23	7.41	12.00	8.61	6.12	8.34	6.02	8.89	9.76
Yb (mg/kg)	0.40	0.80	0.80	0.40	0.85	0.90	0.74	1.04	1.05	1.52	1.19	0.85	0.83	0.61	0.89	0.88
Zn (mg/kg)	197.60	190.32	120.80	73.91	0.00	0.00	0.00	0.00	261.00	23.00	0.00	16.00	137.88	214.89	122.44	149.19
Zr (mg/kg)	42.71	33.99	38.40	35.56	31.69	44.59	32.47	45.16	63.00	60.00	35.00	48.00	35.21	41.88	50.29	66.15

APPENDIX B: MACRO-INVERTEBRATES AS A BIOINDICATOR

Table 1: The seasonal macro-invertebrates count in the Steelpoort River (August 2017 – May 2018).

		Seasons															
		Summer				Autumn				Winter				Spring			
Orders	Taxa	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
	Oligochaeta	22	6	14	9	1	1	2	2	29	9	3	0	35	9	3	2
Annelida	Hirudinea	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Crustacea	Potamonautidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Notonemouridae	0	0	0	2	0	0	0	0	0	0	0	0	1	1	0	0
Plecoptera	Perlidae	13	2	0	0	16	3	0	0	0	0	0	0	4	9	0	0
	Baetidae 1sp																
	Baetidae 2sp																
	Baetidae >2sp	420	321	68	55	280	550	142	35	151	459	15	80	171	413	113	239
	Caenidae	185	44	1	9	13	8	39	14	56	35	6	2	324	119	17	32
	Heptageniidae	8	8	0	13	0	12	4	14	6	6	0	0	6	2	0	0
	Leptophlebiidae	0	0	3	0	0	0	2	1	0	0	0	0	33	0	0	3
	Prosopistomatidae	5	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0
Ephemeroptera	Tricorythidae	3	0	0	0	0	1	0	0	0	0	0	0	14	11	12	0
	Calopterygidae ST, T	0	0	0	0	0	0	1	5	1	0	0	0	0	0	0	1
	Chlorocyphidae	1	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0
	Synlestidae (Chlorolestidae)	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
	Coenagrionidae	9	41	0	6	8	37	5	25	11	7	5	0	14	26	0	9
	Lestidae	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Aeshnidae	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Odonata	Corduliidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 1 continued…

		Seasons															
		Summer				Autumn				Winter				Spring			
Orders	Taxa	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Odonata	Gomphidae	53	24	45	21	36	18	19	33	17	42	37	9	15	33	35	37
	Libellulidae	1	20	2	11	0	23	46	44	5	0	10	11	6	7	21	26
Lepidoptera	Crambidae (Pyralidae)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	Belostomatidae	0	18	13	4	0	4	4	2	0	0	0	0	4	2	0	5
	Gerridae	0	0	0	12	3	0	0	0	0	0	0	0	0	0	0	0
	Naucoridae	12	4	4	6	3	1	4	6	0	0	0	0	15	4	1	32
	Nepidae	0	0	0	0	7	1	3	0	0	0	0	0	0	0	0	0
	Pleidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Veliidae	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Trichoptera	Ecnomidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Hydropsychidae 1sp Hydropsychidae 2sp Hydropsychidae >2sp	97	87	11	40	156	42	75	50	20	7	18	1	162	31	138	105
	Leptoceridae	27	51	8	6	0	2	2	3	0	0	0	0	0	9	6	14
Coleoptera	Elmidae	37	49	3	18	43	28	12	15	3	0	0	0	61	10	32	22
	Gyrinidae	1	6	0	0	0	0	0	16	0	0	0	0	45	0	3	5
	Hydrophilidae	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Psephenidae	3	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0
Diptera	Athericidae	0	0	0	1	0	0	0	3	0	0	0	1	0	0	0	0

Table 1 continued…

		Seasons															
Orders	Taxa	Summer				Autumn				Winter				Spring			
		Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Diptera	Ceratopogonidae	9	5	3	0	0	3	7	5	45	2	0	1	6	3	16	0
	Chironomidae	43	15	0	0	8	22	39	50	10	3	7	4	88	36	48	18
	Culicidae	3	3	0	0	1	2	1	1	0	0	0	0	2	0	1	0
	Muscidae	0	0	0	0	0	0	1	0	0	0	1	40	0	2	0	0
	Psychodidae	0	0	10	0	0	0	0	0	3	0	0	0	6	0	0	0
	Simuliidae	2	30	5	3	9	1	15	1	0	0	0	1	0	12	24	73
	Syrphidae	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Tabanidae	3	14	21	19	11	13	70	10	4	1	15	4	9	3	8	4
	Tipulidae	3	3	16	0	7	0	0	0	0	0	2	0	3	0	0	0
Gastropoda	Ancylidae	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Lymnaeidae	0	1	0	0	0	0	0	0	0	0	0	0	1	10	0	0
	Physidae	0	18	0	0	0	1	0	0	0	0	0	0	0	2	0	0
	Planorbinae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Thiaridae	0	4	0	0	0	1	2	0	0	2	0	0	0	0	0	0
Pelecypoda	Corbiculidae	1	1	0	0	0	0	1	0	0	2	1	0	0	0	0	0

APPENDIX C: FISH AS A BIOINDICATOR

Table 1: The seasonal fish count in the Steelpoort River (August 2017 – May 2018).

Species scientific name	Species Code	Winter				Spring				Summer				Autumn			
		Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
Amphilius uranocopus	AURA	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Enteromius eutaenia	BEUT	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
Enteromius lineomaculatus	BLIN	0	0	0	0	0	1	0	0	0	7	0	4	0	0	0	2
Labeobarbus marequensis	BMAR	11	0	1	0	15	8	3	3	14	5	29	14	14	4	11	5
Enteromius neefi	BNEE	0	0	0	0	0	0	0	0	0	5	0	2	0	4	0	2
Enteromius trimaculatus	BTRI	14	0	0	0	0	0	0	0	1	0	0	3	1	0	0	0
Enteromius viviparus	BVIV	0	1	0	0	0	0	0	1	0	0	0	12	0	0	0	0
Clarias gariepinus	CGAR	0	1	0	0	0	0	0	1	0	2	0	3	0	0	0	2
Chiloglanis paratus	CPAR	0	0	0	0	0	0	0	4	0	0	8	5	0	0	4	2
Chiloglanis pretoriae	CPRE	5	28	1	2	59	7	12	1	37	18	20	17	38	11	25	8
Chiloglanis swiestrai	CSWI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Glossogobius giuris	GGIU	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Labeo cylindricus	LCYL	0	1	6	0	0	2	3	0	0	0	0	0	0	0	1	0
Labeo molybdinus	LMOL	0	0	3	0	0	0	0	0	0	0	4	0	0	0	3	0

Table 1 Continued…

Species scientific name	Species Code	Winter				Spring				Summer				Autumn			
		Site 1	Site 2	Site3	Site4	Site 1	Site 2	Site3	Site4	Site 1	Site 2	Site3	Site 4	Site 1	Site 2	Site3	Site4
Mesobola brevianalis	MBRE	0	0	0	0	0	0	0	1	0	0	0	0	0	5	0	0
Oreochromis mossambicus	OMOS	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Opsaridium peringueyi	OPER	0	0	0	0	0	0	0	0	0	15	3	7	0	7	1	0
Pseudocrenilabrus philander	PPHI	0	0	0	0	0	2	0	0	0	14	0	0	0	16	0	0
Tilapia sparramanii	TSPA	1	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0