ASSESSMENT OF THE IMPACT OF WATER AND SEDIMENT QUALITY ON THE DIVERSITY OF AQUATIC MACRO-INVERTEBRATE COMMUNITIES IN THE DWARS RIVER OF THE OLIFANTS RIVER SYSTEM, LIMPOPO PROVINCE

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 execution, and that all material contained herein has been duly acknowledged.”

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TV MMAKO   **DATE**

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ABSTRACT

Freshwater ecosystems in South Africa are losing their quality and quantity over time due to pollution mainly from mining, agriculture, industries, deforestation, sewage systems, construction of dams, channel modification and over extraction of water. The Dwars River, a tributary of the Olifants River, is of no exception, as recent studies indicated an increase in nutrient input possibly from agriculture. The Dwars River is an important source of water for nearby communities (Ga-Mampuru). The aim of the study was to assess water and sediment quality of the Dwars River using macroinvertebrates as bioindicators of pollution. Water and macroinvertebrates sampling were done seasonally from July 2017 to May 2018. The water quality results indicated that non-toxic constituents such as salinity and EC (Electrical Conductivity) were above permissible limits stipulated by the DWAF (1996) guidelines.

More sensitive taxa were found upstream, despite high concentrations of some nutrients and metals in the water column. The high abundance and distribution of macroinvertebrates observed upstream was confirmed by the Canonical Correspondence Analysis (CCA), South African Scoring System (SASS5) and Average Score Per Taxon (ASPT) results during the study. Site 1 was dominated by the most sensitive taxa and this could be due to high dissolved oxygen content and increased heterogeneity of the area. Site 4 was dominated by the most tolerant taxa, according to the CCA, SASS score and ASPT results. This could possibly be due to the nutrients and heavy metals washed from upstream, which get adsorbed by the sediment. The results for species abundance, diversity and richness indicated that Ephemeroptera was the most abundant, while Diptera was the most diverse. Ephemeropterans are known to be indicators of good water quality. Site 1 had the highest number of families and orders while site 4 had the least families and orders. Ephemeroptera, Plecoptera and Tricoptera (EPT) taxa richness and Shannon diversity (H') index values are high upstream and decrease downstream. Overall, the SASS5 indices, CCA and physicochemical results indicated that the water quality in the Dwars River is deteriorating in most impacted sites.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

1.1 GENERAL INTRODUCTION

Water is the most vital resource for survival of all plants and animals. Freshwater encompasses a tiny fraction of the earth surface (about 0.8%), but amazingly, these systems are a home to many varied species (Dalu et al. 2017; Edokpayi et al. 2017). Freshwater ecosystems provide an array of services which include drinking, transportation, recreation, flood control and purification of industrial and human wastes (Kumar 2015; Mangadze et al. 2019). The biodiversity in freshwater ecosystems is declining due to the continued deterioration of the quantity and quality of water resulting from the pressure of human activities (Matlou et al. 2017; Addo-Bediako et al. 2018). These activities include mining, agriculture, industries, deforestation, sewage systems, channel modification and over extraction of water (Dalu et al. 2017; Matlou et al. 2017; Addo-Bediako et al. 2018).

South Africa is a semi-arid country with inadequate freshwater ecosystems and has been rated as the 30th driest country in the world (Owusu-Sekyere et al. 2016; Zengeni et al. 2016; Du-Plessis & Schloms 2017). Several rivers in this country get dried most of the year due to low seasonal rainfall (Zengeni et al. 2016; Mosase & Ahiablame 2018). This situation is made worse by increased pollution from human activities (Dalu et al. 2017; Addo-Bediako et al. 2018). When water is polluted its features or properties are compromised and this may result in deleterious effects on the aquatic biota (Edokpayi et al. 2017). Effluents released from anthropogenic activities change the structure and distribution of aquatic communities including native species (Matlou et al. 2017).

Water quality of most South African rivers has deteriorated drastically due to industrialisation and urbanisation (Koff et al. 2016; Addo-Bediako et al. 2018). This encompasses the Olifants River System which is currently under stress due to effluents released from mining, agriculture, and industrial activities (Wolmarans et al. 2014; Matlou et al. 2017). The Dwars River which forms an important part of the Olifants River System is of no exception. Along the Dwars River, there are continuous agriculture, mining and industrial activities with informal settlements (Magala 2015). The impact of the continuous anthropogenic activities in the Dwars River on the aquatic biota and water quality is not currently known.

1.2 PROBLEM STATEMENT

Fresh water ecosystems are losing their integrity over time due to anthropogenic activities (Mangadze et al. 2019). There have been major changes during the past five years in the Dwars River such as, road construction, pipelines crossing or watercourse diversion and an increase in soil erosion which can lead to loss of species' habitat (Magala 2015). This situation is worsened by continuous agriculture, mining, industries and informal settlements present in the catchment of the river (Magala 2015). It is currently unclear how these anthropogenic activities are affecting the quality and quantity of the water. The chemicals released from mining and industrial sectors are of great concern. This is because of their ability to accumulate and persist in an aquatic environment, thereby affecting the aquatic biota and the health of nearby residents depending on the river for water and food (Oelofse 2008). A recent study in water quality of the Dwars River has reported an increase in nutrient content, possibly from agriculture (Magala 2015). The level of other pollutants, such as metals in the river and the impact of pollution on the aquatic biota have not been assessed. In this study, the current ecological state of the Dwars River was assessed and the impact of mining and agricultural activities on water quality and aquatic macroinvertebrates was ascertained. Also measures to protect the health of the ecosystem were proposed.

1.3 HYPOTHESIS:

There is an increase in pollution of water and sediment in the Dwars River which is affecting the aquatic macroinvertebrate assemblages.

1.3.1 AIMS AND OBJECTIVES

The aim of the study was to assess water and sediment quality of Dwars River using macroinvertebrates as bio-indicators of pollution

The objectives of the study were to:

i. Determine the quality of water with respect to physicochemical parameters, nutrients and metals and compare them with the water quality guidelines

ii. Determine the level of heavy metals in the sediment, and to compare them with the guideline values.
iii. Investigate the effect of water and sediment quality on aquatic macro-invertebrates.

1.4 LITERATURE REVIEW

1.4.1 POLLUTION OF FRESHWATER ECOSYSTEMS

Globally, freshwater ecosystems are recognised as the most threatened systems due to pollution from natural and artificial sources (Munir et al. 2016; Marr et al. 2017). Anthropogenic activities such as mining, agriculture and industries are the leading cause of degradation worldwide (Marr et al. 2017; Rasifudi et al. 2018). Partly treated or untreated effluents usually end up in freshwater bodies though runoff (Okeyo et al. 2018). Contaminants degrade water quality and can interfere with the capacity of the river to cleanse itself which can result in poor health of the overall ecosystem (Rashid & Romshoo 2012). When the quality of water is compromised, this implies that it will no longer satisfy any of its intended use (Alavaisha et al. 2019). Most rivers in South Africa are affected by an increase in urbanization and industrialization (Kumar 2015; Addo-Bediako et al. 2018). One of such rivers is the Olifants River System which is recognised as one of the hardest working rivers (Wolmarans et al. 2014) and has been rated as the third most polluted river in South Africa (Matlou et al. 2017). Several tributaries and impoundments of the Olifants River System are also subjected to similar pressures (Jooste et al. 2015).

The Dwars River is a tributary of the Steelpoort River and forms part of the Olifants River System. The main anthropogenic activities in the Dwars River catchment are mining, industrial, agricultural and informal settlements (Magala 2015). The effluents from mining, agriculture and industries contain, amongst others, chemicals such as, metals and pesticides. Metals and pesticides are persistent and their presence in water and sediment may lead to bioaccumulation in living organisms which may affect their overall health (Edokpayi et al. 2017; Brink & Kamish 2017). Heavy metals which enter the river settle down in the river sediment by the process of adsorption; therefore, sediments serve as sink that retain heavy and trace metals (Naggar et al. 2018). A recent study on the physicochemical properties of water in Dwars River has reported an increase in nutrient content, possibly from agriculture (Magala 2015). When nutrients are present in excess they may lead to eutrophication (Naidoo 2005; Struijs et al. 2010). There are few studies on water quality in the Dwars River and recently the catchment has experienced major changes such as road construction, watercourse diversion, and increased informal settlements, mining and agricultural activities. The Dwars River needs to be continuously studied because the above-mentioned anthropogenic activities might have deleterious effect on the aquatic biota. Previous studies have concentrated on the nutrient loading and the physicochemical properties of water, so this study aims to investigate the impact of anthropogenic activities on both the nutrient level and on the aquatic macroinvertebrate assemblages. Therefore, the research is urgently required to clarify how the present anthropogenic activities are affecting the water quality and the aquatic biota in the river.

Different land-use activities happening in the catchment of rivers have been linked with water quality deterioration, which can affect the composition and diversity of the biota (Addo-Bediako et al. 2018; Rasifudi et al. 2018). In the past few years, an interest for rapid assessment in biomonitoring of water quality across the world has increased (Matlou et al. 2017; Mangadze et al. 2019). To understand the function and structure of any aquatic system, it is critical to obtain both biological and physicochemical data because they complement each other (Mangadze et al. 2019). Biomonitoring employ a wide range of bioindicators and biomarkers from subcellular level to population level (Hamza-Chaffai 2014). These include the communities of diatoms, bacteria, protozoans, algae, macroinvertebrates and fish (Szczerbinska & Galczynska 2015). Aquatic macroinvertebrates are one of the most popular bioindicators used in distinct parts of the world (Matlou et al. 2017). This is because macroinvertebrates continuously dwell in water and they respond to every perturbation they encounter in their environment, such as pollution (Rasifudi et al. 2018). Most of macroinvertebrates are bottom dwellers which occupy the sediment which acts as a sink for pollutants (Oberholster et al. 2013; Dalu et al. 2017). Aquatic macroinvertebrates are clearly visible to the naked eye, they occupy sedentary habitats and have rapid life cycles (Matlou et al. 2017; Dalu et al. 2017). They have various species with varied sensitivity to stressors and they are ubiquitous, thus, they are considered as good bioindicators (Dalu et al. 2017; Rasifudi et al. 2018).

1.5 SIGNIFICANCE OF THE PROPOSED RESEARCH

This study provides information on the recent health status of the river and its biota with the continuous mining, industrial, agricultural activities and informal settlements. It would further help to evaluate the effect of anthropogenic activities on the water quality and aquatic macro-invertebrates. The information collected would assist the authorities to make informed decisions to conserve the river.

1.6 THE STUDY AREA

The Dwars River is a tributary of the Steelpoort River, and Steelpoort River joins the Olifants River. The Dwars River is of great ecological importance, due to the great geology which changes from mountainous to bushveld coupled with the Veloren valley nature reserve (Magala 2015). This river is an important source of water, with two impoundments responsible for water supply to most small towns and nearby settlements (Magala 2015). In the Dwars River catchment, there is continuous mining and agricultural activities with informal settlements which may impact water quality and the aquatic biota (Magala 2015).

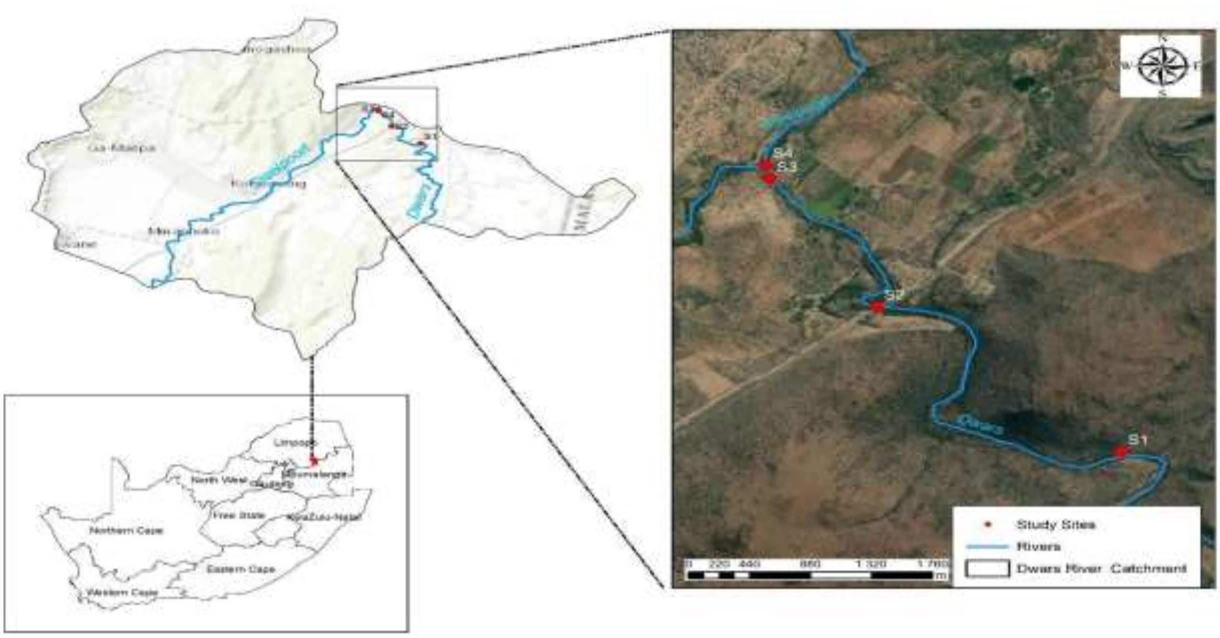

Figure 1.1 Dwars River map showing the four sampling sites (Google Earth 2017).

1.6.1 DESCRIPTION OF THE STUDY SITES

Site 1

This site is secluded (24º51’19” S 30º6’9” E) and is about 4.23 km from Tweefontein mine water return dam. The river substrate is composed of clay and silt with small to medium sized boulders (Figure 1.2). Some parts of this area are shaded by a canopy of trees. The vegetation along the river bank consists of reeds and grass with few acacia trees.

Site 2

The site is situated underneath a concrete bridge (24º50’34” S 30º5’12” E), a walking distance from the R555 road. This site is underneath the bridge and near mining and industrial areas (the ferrochrome mine and Lion smelter) (about 16 km). The river substrate is made up of clay and silt with large boulders. The riparian vegetation has been moderately modified, and this might be due to grazing, because during the survey herds of cattle were observed drinking water from the river (Figure 1.3).

Site 3

The site is about 1.42 km away from the R555 road (24º49’54” S 30º4’47” E) and is near agricultural areas. The river bed is made up of sand and silt with medium to large boulders. The vegetation consists of mainly reeds and grass. Many people from the area come to the river to collect water and several livestock come to drink water. The marginal vegetation has been highly modified which exposes the river bank to erosion (Figure 1.4).

Site 4

This site is the confluence of the Dwars River and Steelpoort River (24º49’50” S 30º4’46” E) and is about a kilometre from Ga-Mampuru village. There is agricultural activities and informal settlements near the site. The river consists of sand and silt with fast running deep water with riffles. The riparian vegetation consists of trees, shrubs and grass. During the field campaigns, there were cattle carts fetching water and a herd of cattle drinking from this site (Figure 1.5)

1.6.2 Visual representation of study sites

Figure 1.2: Representation of the reference site which is site 1 of the Dwars River

Figure 1.3: Representation of site 2 which is underneath the bridge

Figure 1.4: Representation of site 3 before the confluence

Figure 1.5: Representation of site 4 which is the confluence of Dwars and Steelpoort Rivers

1.7 LAYOUT OF THE DISSERTATION

Chapter 1: General introduction and purpose of the study - Introduces the title of the study and further covers literature review relating to all aspects covered in the dissertation including the aim and objectives, problem statement, significance of the study and study area.

Chapter 2: Water and sediment quality - Provide the detailed description of water quality constituents and sediment metals present in the Dwars River.

Chapter 3: Aquatic macroinvertebrates - Covers the results on macroinvertebrate community structure, species abundance, richness, distribution and how these results reflect water quality of the Dwars River.

Chapter 4: Concludes the overall findings of the study.

CHAPTER 2

2.1 INTRODUCTION

2.1.1 Water and Sediment quality

Universally, water is one of the resources which are natural on earth yet limited (Munir et al. 2016). This important resource is a basic need for all forms of life including human beings (Kale 2016). Therefore, its quantity and quality should be protected always to satisfy any of its intended uses. Currently, the South African freshwater ecosystems are deteriorating due to anthropogenic activities (Matlou et al. 2017; Rasifudi et al. 2018). Increased human population coupled with improved standard of living has accelerated demand of water, which has resulted in water stress (Edokpayi et al. 2017). Water quality assessment provides a snapshot of water composition when affected by human induced modifications and nature (Mangadze et al. 2019). Poor water quality does not only affect the aquatic biota, but also affects the health of human beings (Jia et al. 2017). In any activity which compromises water quality, it makes it unfit to serve any of its intended purposes.

The chemical, biological, physical and aesthetic properties of water which determine its fitness for a range of uses and for the protection of the integrity and the health of the aquatic ecosystems is referred to as water quality (Liu et al. 2009). Water quality constituents which are either dissolved or suspended in water have a greater influence on the properties of water (Alavaisha et al. 2019). In South Africa, the Department of Water Affairs and Forestry (DWAF 1996a) initiated a seven-volume Water Quality Guideline which involves different uses, such as industrial, domestic, livestock watering and agriculture (irrigation and aquaculture), as well as for aquatic ecosystems. Briefly, the water quality guideline assists as the main source of information for defining the water quality requirements of various water uses and for the safeguard and maintenance of the health of the aquatic ecosystems. This helps to make judgements about the fitness of water to satisfy its intended use or for the protection of aquatic ecosystems.

The water quality criteria involve the Chronic Effect Values (CEV), the Acute Effect Values (AEV) and the Target Water Quality Range (TWQR) for assessing water quality constituents. The Target Water Quality Range (TWQR) is referred to as a management objective which has been derived from qualitative and quantitative criteria and is not a water quality criterion. This management objective (TWQR) has a range of concentrations whereby there are no measurable adverse health effects expected on the health of aquatic ecosystems, thereby ensures their protection (DWAF 1996a). The concentration of a constituent at which there is expected a measurable probability of chronic effects of up to 5% of species in the aquatic community is referred to as the Chronic Effect Value (CEV). Consequently, if such chronic effects persist for a while they can lead to eventual death and disappearance of vulnerable species in an aquatic system (DWAF 1996a). The Acute Effect Value (AEV) is described as the level or concentration of a constituent above which there is expected to be a greater probability of acute effects of up to 5% of species in an aquatic system community. If such acute effects prevail, even for a short period, there can be immediate death or disappearance of prone species from the aquatic community (DWAF 1996a).

Water quality problems have been largely associated with the presence of a contaminant and how these constituents interact among one another (Liu et al. 2009). The constituent-specific criteria have been divided into four categories, which are system variables, non-toxic inorganic constituents, nutrients, and toxic constituents (DWAF 1996a). System variables encompasses temperature, pH, dissolved oxygen (DO) which are referred to as constituents which regulate essential ecosystem processes such as, migration and spawning.

The Non-toxic constituents can be toxic whenever they are present in high concentrations and are regarded as system characteristics (Leske & Buckley 2003). These include electrical conductivity (EC), Total Dissolved Solids (TDS), salinity and turbidity.

Nutrients naturally occur at very low levels in both water and soil; however, their concentration may be increased by human activities (Griffin 2017). Anthropogenic activities such as runoff from agriculture, untreated sewage, and organic industrial wastes, for example, pesticides and others can accelerate the concentration of nutrients (Griffin 2017).

Toxic constituents are detrimental in an aquatic system even when they are found in low concentrations (Naggar et al. 2018). However, in an unimpacted water body these constituents rarely occur in elevated concentration levels. Metals and metalloids such as, lead (Pb), cadmium (Cd) and mercury (Hg) are mostly present in wastewater released from mining, agricultural, industrial and domestic activities (Edokpayi et al. 2017).

When defining the aquatic ecosystems, three primary abiotic and biotic components are often incorporated, which are the riparian zone, water and sediment (Santoyo et al. 2017). When constituents are present in higher concentration in an aquatic environment, they can be easily exchanged between the sediment and the water column. Sediment contaminants can be recovered again in the water column through bioavailability (Naggar et al. 2018). Bioavailability is referred to as the proportion of a substance which is available to be absorbed by living organisms and may cause adverse health effects or toxicological responses (Wepener & Vermeulen 2005). The distribution of constituents depends mainly on chemical, biological and physical factors (Naggar et al. 2018).

Sediments are regarded as the secondary source of pollution because when properties such as salinity, pH, temperature and ionic strength change, it will cause the bound contaminants to be released back into the aquatic environment (Marchand et al. 2006; Naggar et al. 2018). The danger of toxic metals present in freshwater bodies is that they have the potential to accumulate in sediments and aquatic biota and they are eventually transferred to human beings through the food chain (Younus et al. 2016; Brink & Kamish 2017). In overall, sediment quality forms an important indicator of contamination in the aquatic environment (Naggar et al. 2018).

2.2 METHODS AND MATERIALS

2.2.1 Water and sediment sampling

Seasonal water samples were collected at the four selected sites from July 2017 to May 2018. Water quality samples were collected at a depth of up to 50cm using 1000 mℓ acid pre-treated polypropylene bottles. Collected water samples were kept in a container with ice and transported to the laboratory. In the laboratory, the water samples were kept at $4^{o}C$ prior to chemical analysis at an accredited laboratory (ISO/IEC 17025: 2005) in Pretoria. A YSI Model 554 Datalogger with a 4 m multiprobe instrument was used to measure in situ, parameters such as, water pH, temperature, salinity, EC, DO and TDS. The 500 mℓ pre-treated polypropylene bottles were used to collect water for nutrient analyses within two days at the University of Limpopo Biodiversity Laboratory.

Sediment samples were collected seasonally, at the same location and time with the water samples. Where necessary, the large stones were shifted to collect beneath sediment because macroinvertebrates often attach to rocks, logs, sticks, vegetation and even burrow into the bottom sediment and sand. Samples were stored in 500 mℓ acid pre-washed polyethylene bottles and frozen in the laboratory at $-25^{o}C$ prior to analysis of heavy and trace metals at an accredited laboratory (ISO/IEC 17025: 2005) in Pretoria.

2.2.2 Laboratory analysis

The collected water samples with 1000 mℓ acid pre-treated polypropylene bottles were kept in a container with ice and transported to the laboratory. Water samples were fixed to prevent contamination of the samples. Nutrients: ammonia (NH4), nitrite (NO2), nitrate (NO3) and phosphate (PO4), turbidity, alkalinity and water hardness analyses were done within two days at the University of Limpopo Biodiversity Laboratory using a spectrophotometer (Merk Pharo 100 Spectroquant™) with Merck cell test kits. In the water laboratory, the water samples were analysed in batches with blanks using Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES: Perkin Elmer, Optima 2100 DV) and reported as mg/ℓ.

The sediment samples were wet digested using the microwave digestion system as described by Mustafa et al. (2005). For digestion, 0.5 g of air-dried sediments was accurately weighed and digested in 6 mℓ of nitric acid, HNO3 (Suprapure, Merck), 2 mℓ of perchloric acid HClO4 (Suprapure, Merck), 3 mℓ of hydrochloric acid (HCl Merck) and 2 mℓ of hydrofluoric acid (HF Merck) in a microwave digestion system. The solution was made up by addition of the deionized water and analysed for metals using an inductively coupled plasma-optical emission spectrophotometry (ICP-OES). Recoveries were within 10% of certified values and analytical accuracy was determined using certified standards (De Bruyn Spectroscopic Solutions 500MUL20-50 STD2). All sediment and water samples were subjected to the similar QC/QA. The sediment results were reported as mg/kg and mg/g where the values are high.

2.2.3 Statistical analysis

Excel was used to calculate the mean and standard deviation of water quality parameters and metals. One-Way ANOVA using R 3.1.0 statistical software (R Development Core Team. 2014) was performed, to determine whether water chemistry parameters varied among sites/seasons. Then the significance of the results was ascertained at $p<0.05$. Where there was a significant variation, the Turkey's Post-Hoc test was performed to determine where the difference occurred. Graphical parameters were drawn using Excel. The water quality results were analysed by comparing with the TWQR, AEV and CEV for aquatic ecosystems suggested by DWAF (1996a), however; where DWAF guideline is not available other guidelines were used (CCME 2012).

2.3. RESULTS

2.3.1 Physicochemical variables

The water quality results obtained during the study at different sites are represented in Table 2.1, and Figures 2.1, 2.2 and 2.3. The measured depth ranged from 0.13 m at Site 3 to 0.68 m at Site 4. The river width ranged from 4.9 m at Site 1 to 11 m at Site 4.

Table 2.1 The water quality results recorded at different sites in the Dwars River

Water parameters	Site 1 Mean	SD	Site 2 Mean	SD	Site 3 Mean	SD	Site 4 Mean	SD	Water Quality Guidelines
Temperature (^{0}C)	21.3	6.7	17.6	6.1	21.4	6.3	20.8	4.0	Should not vary more than 10% from normal value[1]
DO (mg/ℓ)	9.13	1.7	7.5	1.2	6.8	1.4	7.9	1.2	-
DO (%)	73.9	20.7	66.7	20.1	66.1	21.2	75.8	23.7	80-120% of saturation[1]
pH	8.25	0.7	8.1	0.4	8.3	0.4	7.9	0.7	Should not vary by > 5%[1]; 6.5-9 .0[3]
Turbidity (NTU)	6.6	8.6	1	0	6.6	10.1	1.03	0	120 to 180 (mg/ℓ $CaCO_3$) hard water[2]
TDS mg/ℓ	295.7	147.6	308.2	134.9	311.3	135.2	172.8	38.3	TDS should not change by >15% from normal cycle[1]
Conductivity mS/m	539.5	102.3	502.8	80.6	549.7	71.4	294.7	48.04	No criteria available
Salinity (‰)	0.56	0.36	0.35	0.15	0.37	0.18	0.21	0.16	<0.05% or <0.5‰[1]
Calcium (mg/ℓ)	38.8	2.2	38.5	2.08	37.3	1.71	25.8	2.9	No criteria available
Magnesium (mg/ℓ)	37.5	5.5	38	4.97	38.8	1.5	14.3	4.03	<150[3]
Potassium (mg/ℓ)	1.13	0.5	1.2	0.5	1.2	0.5	1.7	0.06	No criteria available
Sodium (mg/ℓ)	20.8	4.3	21.3	2.63	22.3	1.26	13.5	2.4	No criteria available
Physical									
Velocity	21.4	33.9	15.7	19.7	24.4	21.3	20.2	20.16	
Depth	0.14	0.02	0.2	0.01	0.13	0.02	0.68	0.21	
Width	4.92	0.03	8.26	1.21	6.09	0.44	11	0	
Riparian				**Cover**					
Bank Erosion	slight		slight		more		more		
Canopy Cover	moderate		none		less		none		
Substrate				**Percentage (%)**					
Cobble	50		30		40		5		
Rock	10		60		30		0		
Sand	0		5		20		75		
Mud	40		5		10		20		

1. (DWAF 1996)-South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.
2. BC-EPD (2006)- British Columbia Environmental Protection Division: Water Quality Guidelines.
3. (CCME 2012)- Canadian Council of Ministers of the Environment: Water Quality Guidelines- Aquatic Life.

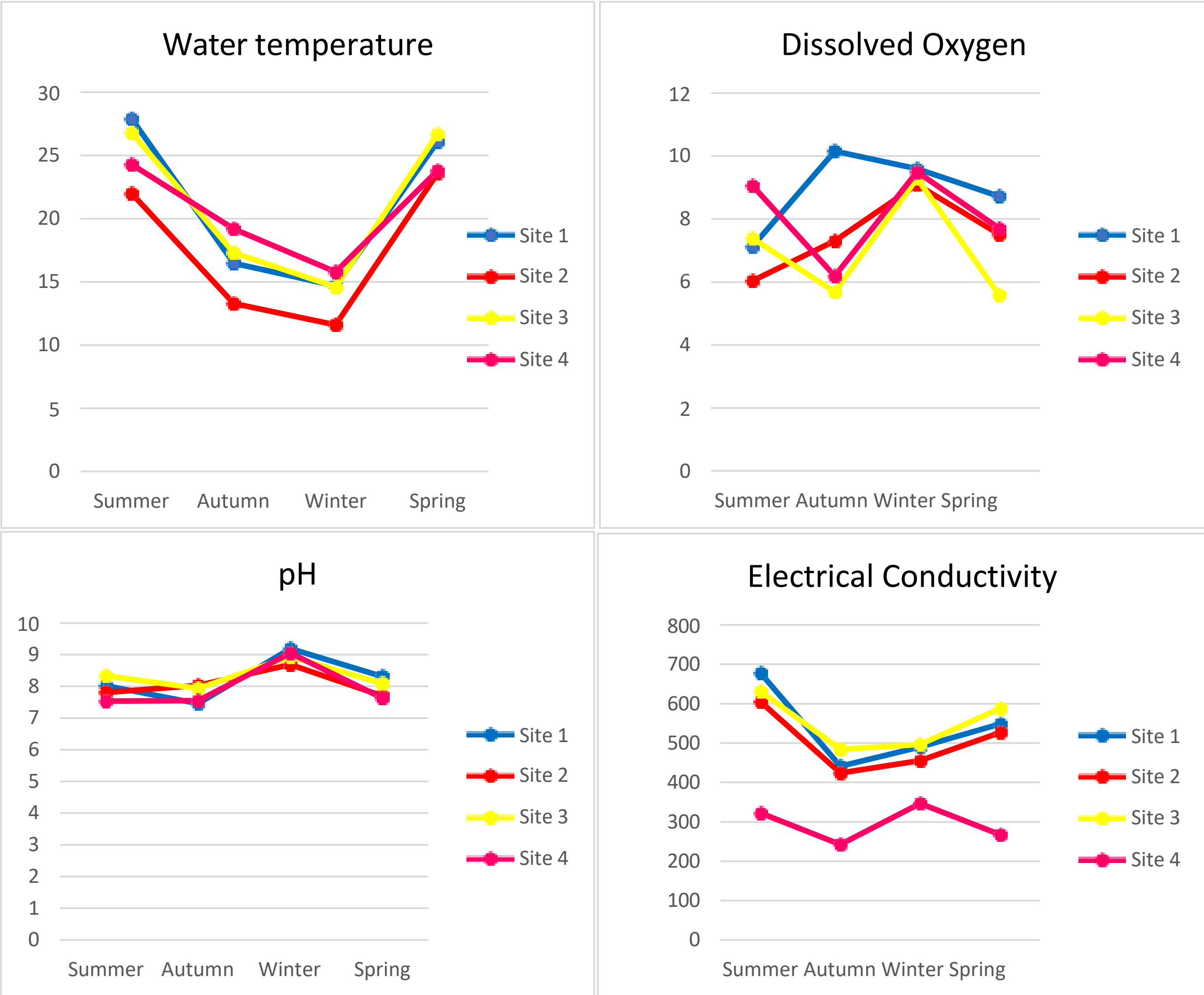

Figure 2.1 Seasonal variation of water temperature, DO, pH and EC among all four sampling sites in the Dwars River during 2017 and 2018

The mean water temperature ranged from 17.6°C to 21.4°C. The water temperature was highest in summer at Site 3 (21.4 °C) and the lowest in winter at Site 2 (17.6°C). In reference to South African inland waters, the permissible water temperature range is between 5-30°C (DWAF 1996a). Thus, during the study, the water temperature values were within the normal limits. The recorded DO concentration was highest at Site 1, with a mean value of 9.13 mg/ℓ and lowest at Site 3 with a mean value of 6.8 mg/ℓ. During the study the DO was within the permissible range limit (DWAF 1996a). The highest pH was recorded at Site 1 and Site 3, while Site 4 had the lowest pH of 7.4. Seasonally, an increase in pH was noted during winter, with a value of 8.9 and the lowest during summer, with a value of 7.8. The highest EC value was recorded at Site

3 with a mean of 549.7 mS/m and the lowest at Site 4 with a mean of 294.7 mS/m. Seasonally, summer had the highest EC value of 559.1 mS/m while autumn had the lowest value of 397.9 mS/m. All the variables were within the TWQR limit except for salinity and EC.

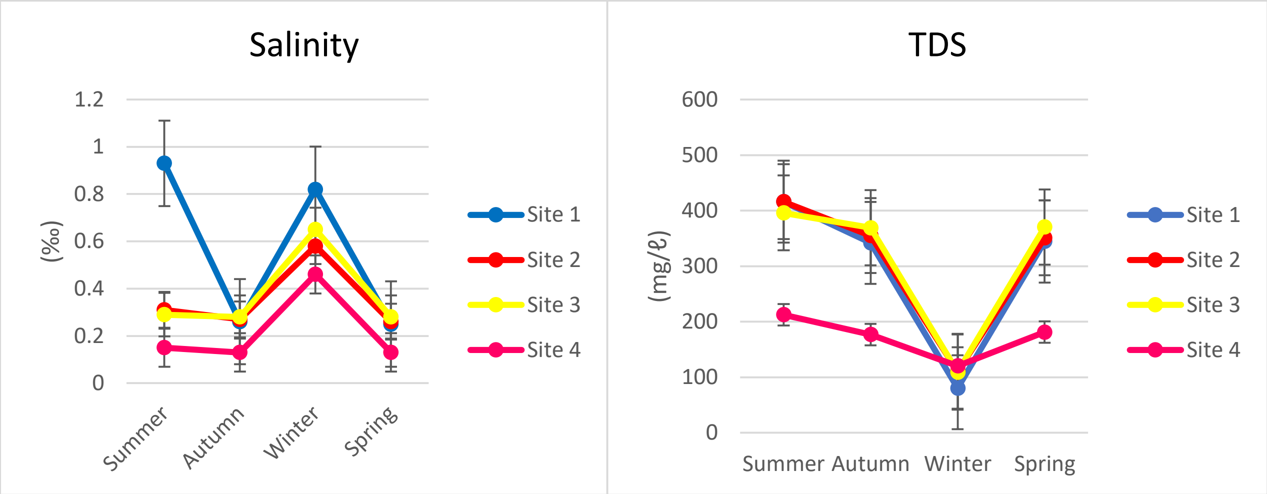

Figure 2.2 Seasonal variation of salinity and TDS among four sampling sites in the Dwars River during 2017 and 2018

The highest salinity mean concentration (0.56‰) was recorded at Site 1 and the lowest concentration (0.21‰) was at Site 4. Seasonally, winter had the highest salinity of 0.63 ‰, while spring had the lowest (0.23 ‰) salinity. The limit for salinity levels in freshwater ecosystems should be <0.5‰ or not change by 0.05% from the normal cycle (DWAF 1996a). Site 1 was above the permissible limit. Seasonally, salinity was higher during winter than the other seasons. The SAWQG limit for TDS is unavailable, however, DWAF (1996a) has proposed that the TDS concentration should not be changed by >15% from the normal cycle under un-impacted conditions at any time of the year. The TDS recorded was high at Site 3 and low at Site 4. SAWQG of TDS is site specific and all measured values were within permissible limit.

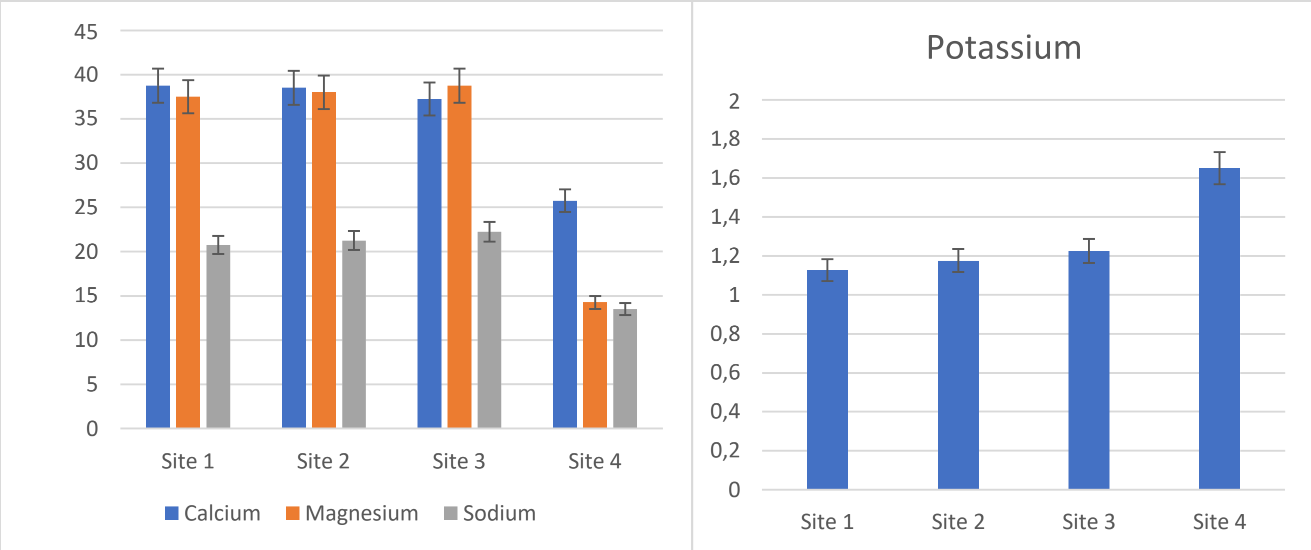

Figure 2.3 Concentration of major ions recorded at different sites in the Dwars River.

The highest calcium concentration was recorded at Site 1 with a mean of 38.75 mg/ℓ and the lowest concentration was recorded at Site 4 with a mean of 25.75 mg/ℓ. Seasonally, winter and summer had the highest calcium concentration of 36 mg/ℓ, while spring had the lowest mean concentration of 33 mg/ℓ. The highest magnesium concentration was recorded at Site 3 with a mean of 38.8 mg/ℓ and the lowest was at Site 4 with a mean of 14.2 5mg/ℓ. The permissible limit of magnesium for domestic use is between 4 and 10 mg/ℓ (DWAF 1996c). The highest potassium concentration was recorded at Site 4 with a mean of 1.65 mg/ℓ in summer and the lowest concentration was recorded at Site 1 with a mean of 1.13 mg/ℓ in autumn. The highest sodium concentration was recorded at Site 3 with a mean of 22.25 mg/ℓ and the lowest concentration was at Site 4 with a mean of 13.5 mg/ℓ. Seasonally, summer had the highest sodium concentration of 22 mg/ℓ while autumn had the lowest mean concentration of 18 mg/ℓ. The concentration of major ions recorded during the study were within the permissible limit except for magnesium.

Table 2.2 The concentrations of nutrients recorded in the Dwars River

Nutrients (mg/ℓ)	Site 1		Site 2		Site 3		Site 4		Target Water Quality Range
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Nitrate	16.5	4.92	14.8	4.30	14.4	2.43	1.9	2.36	13[2]
Nitrite	0.33	0.1	0.03	0	0.03	0	0.02	0	0.06[2]
Phosphate	0.04	0	0.05	0	0.04	0	0.04	0	< 0.005 (oligotrophic); > 0.25 (hypertrophic)[1]
Ammonia	0.04	0	0	0	0.04	0	0.04	0	<0.007[1]; <0.354[2]
Total Nitrogen	16.54	4.93	14.81	4.29	14.42	2.39	1.92	2.40	< 0.5 (oligotrophic; >10 (hypertrophic)[1]

1. (DWAF 1996a) - South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems.
2. BC-EPD (2006) - British Columbia Environmental Protection Division: Water Quality Guidelines.

The highest nitrate and nitrite concentrations were recorded during summer at Site 1, with a mean value of 16.5 mg/ℓ and 0.33 mg/ℓ respectively. Inorganic phosphorus concentration was elevated during winter at Site 2, with a mean value of 0.05 mg/ℓ. However, at all sampled sites, it ranged from 0.04 mg/ℓ to 0.05 mg/ℓ (Table 2.2) and the water condition was eutrophic in terms of inorganic phosphorus. Ammonia was only detected at Site 1, Site 3 and Site 4, with a mean value of 0.04 mg/ℓ, and was above the stipulated guideline limit (Table 2.2). The highest nitrogen concentration was during summer at Site 1, with a mean value of 16.54 mg/ℓ. Site 1, Site 2 and Site 3 experienced hypertrophic conditions, while Site 4 was mesotrophic, in terms of the nitrogen levels. One-Way ANOVA indicated significant difference in physicochemical parameters such as temperature, DO, pH and turbidity among the sitations ($p<0.05$). However, there was no significant difference in EC among the sites ($p>0.05$). The difference lies between Site 3 and Site 2 for temperature; Site 1 and Site 3 for DO; Site 1 and all the Sites (2,3 and 4) for pH and Site 3 and Site 4 for TDS.

All metals recorded in water were within the stipulated TWQR guidelines (DWAF 1996a; CCME 2012; BC-EPD 2006; USEPA 2012), except for aluminium, chromium and zinc. (Table 2.3; Figure 2.4). The highest concentration of aluminium, chromium and zinc were recorded at Site 1 during summer, with an average of 0.50 mg/ℓ, 0.02 mg/ℓ and 0.08 mg/ℓ respectively. Sediment metals and metalloids such as aluminium, barium, boron, iron, manganese, nickel, strontium, titanium, vanadium and lead were detected within the stipulated limits, however, chromium and copper were above the stipulated limits by the guideline (CCME 2012). The highest chromium concentration was recorded during summer at Site 1, with a mean value of 5.5 mg/g and the highest copper concentration was recorded during spring at Site 4, with a mean value of 48 mg/kg (Table 2.4; Figure 2.5).

Table 2.3 Mean and standard deviation of metals in water recorded at different sites in the Dwars River (mg/ℓ).

	Site 1		Site 2		Site 3		Site 4		
Metals (mg/ℓ)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Water quality guidelines
Aluminium	0.54	0.71	0.48	0	0.16	0	0.18	0.083	0.001[1]; 0.1[3]
Iron	0.43	0.66	0.24	0.25	0.103	0.04	0.244	0.178	Vary < 10% background conc[1]; 0.3[3]
Titanium	0.04	0	0.04	0.01	0.04	0.008	0.02	0.007	No criteria available
Barium	0.09	0.13	0.04	0.03	0.025	0.006	0.035	0.003	0.7[4]
Manganese	0.14	0	0.04	0	0	0	0.04	0.01	0.18[1]; < 1.3[2]
Nickel	0.02	0	0	0	0	0	0	0	<0.47[4]
Vanadium	0.01	0	0.01	0	0.013	0.002	0.005	0	No criteria available
Chromium	0.02	0.02	0.007	0.007	0.002	0	0.001	0	Cr III: 0.012[1] ;0.0089[3]
Strontium	0.17	0.02	0.17	0.02	0.16	0.011	0.107	0.006	4.0[4]
Zinc	0.08	0.12	0.04	0.05	0.05	0.051	0.043	0.04	0.002[1]; 0.04-0.115[2]; 0.03[3]; <0.12[4]
Boron	0.02	0	0.01	0	0.02	0	0.012	0	1.5[3]; 1.2[2]

1. (DWAF 1996a)-South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.
2. BC-EPD (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 2.4 Sediment metals (mg/kg & mg/g) recorded at various sites in the Dwars River

	Site 1		Site 2		Site 3		Site 4		Water Quality Guidelines
Metals	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Aluminium	32.5	16.8	35.1	11.6	35.1	17.8	35.7	21.7	No guidelines
Barium	94.5	57.9	92	56.5	122.8	75.6	243.8	199.2	No guidelines
Boron	5.5	11	17.5	35	20.8	31.7	41	79.4	No guidelines
Chromium	5.5	2.3	4.9	2.8	2.8	1.5	0.3	0.1	0.0373 mg/g
Copper	17.1	13.8	20.8	10.1	25.3	21.3	48	20.8	35.7 mg/kg
Iron	56.7	9.6	59.6	10.9	60.4	6.9	204.3	96.2	No guidelines
Manganese	1.4	0.1	1.4	0.1	1.4	0.3	1.8	0.7	No guidelines
Nickel	464.3	123.5	459.8	122.4	421.3	247.5	526.8	857.3	No guidelines
Strontium	85.3	28.5	81.8	33	99.3	52.2	111.5	81.1	No guidelines
Titanium	1780	296.7	2845	1315.1	3020.5	1517	39018.3	21393.3	No guidelines
Vanadium	155.3	17.6	206.8	86.2	126.3	92.8	1639	971.4	No guidelines
Zinc	30	34.8	40.5	48	54.3	37.3	295.3	391.3	123 mg/kg
Lead	3.1	2	3.5	1.7	2.9	1.4	4.2	1.2	35.0 mg/kg

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

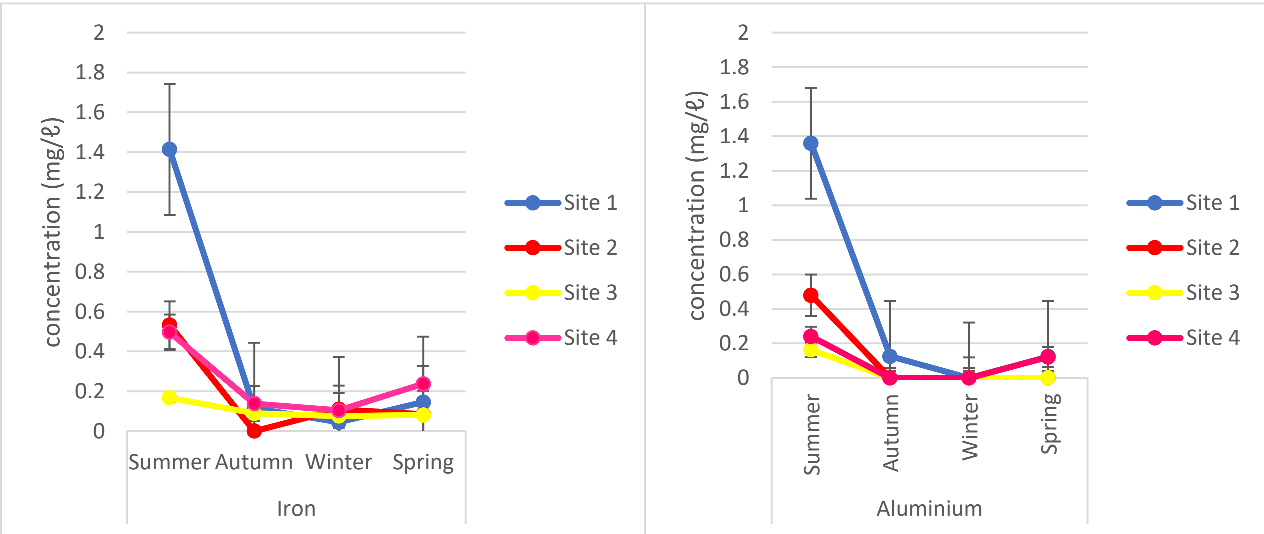

Figure 2.4 Seasonal variation of aluminium and iron recorded in water column of the Dwars River

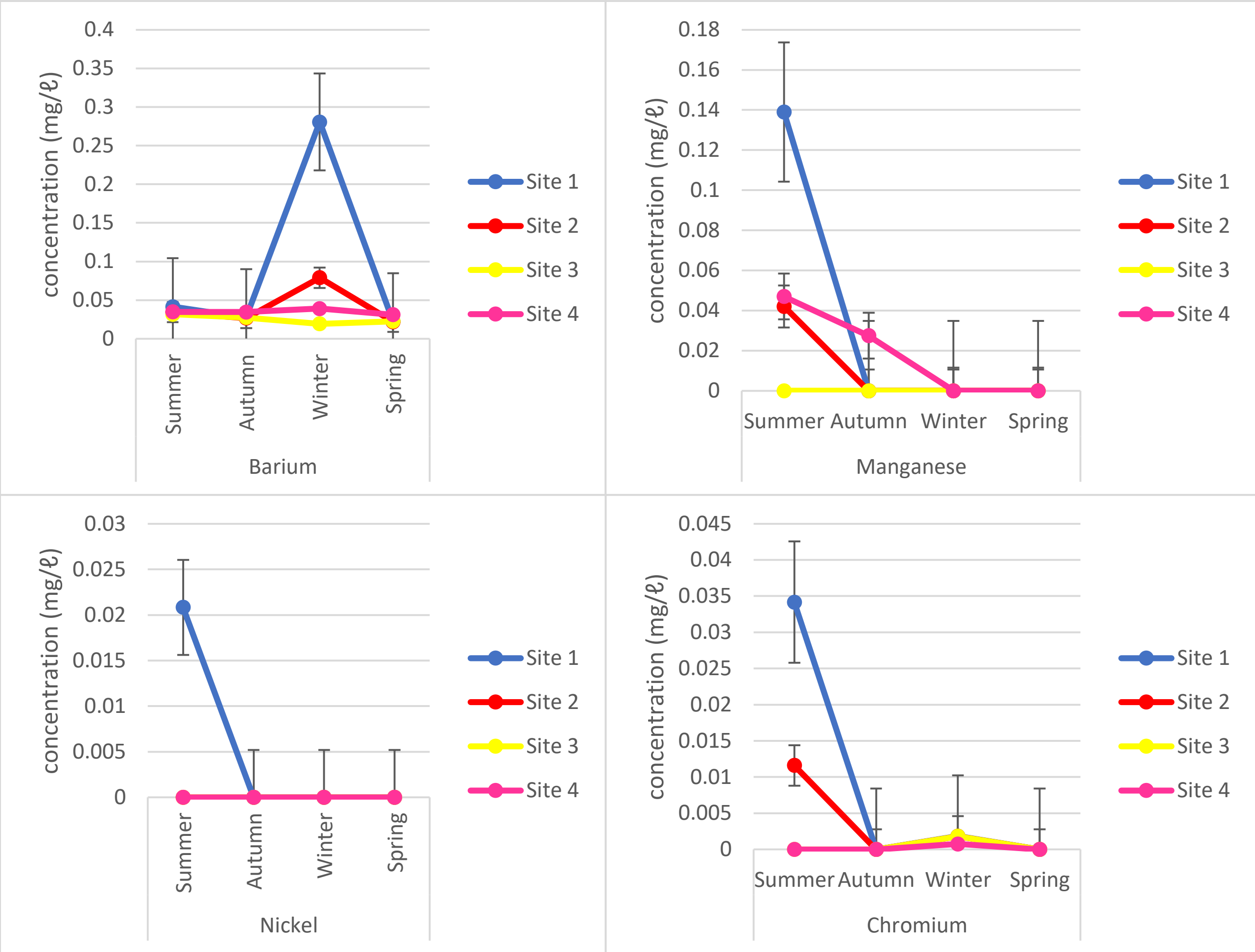

Figure 2.5 Seasonal variation of barium, manganese, nickel and chromium recorded in the water column of the Dwars River

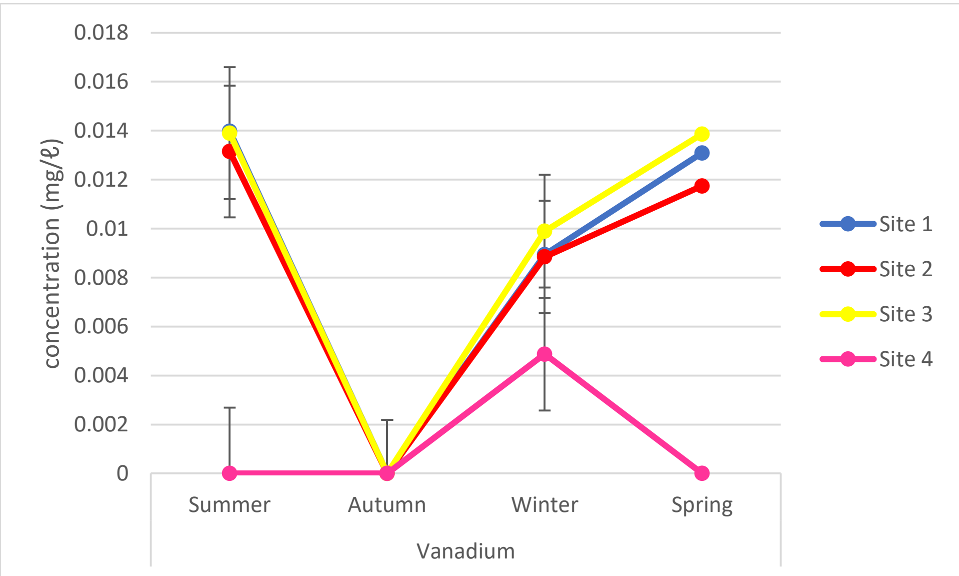

Figure 2.6 Seasonal vanadium concentration recorded in water column of the Dwars River

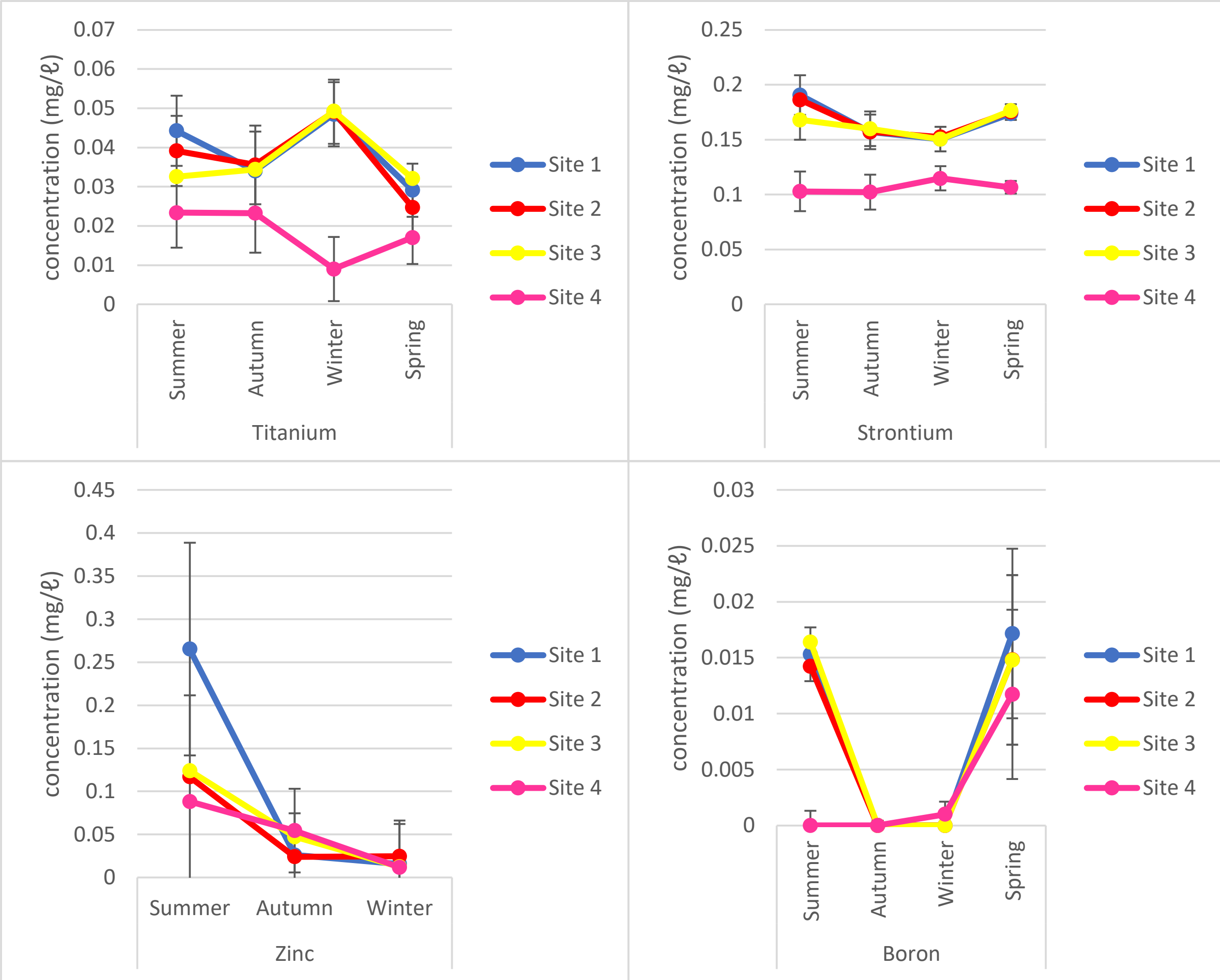

Figure 2.7 Seasonal variation of titanium, strontium, zinc and boron recorded in water column of the Dwars River

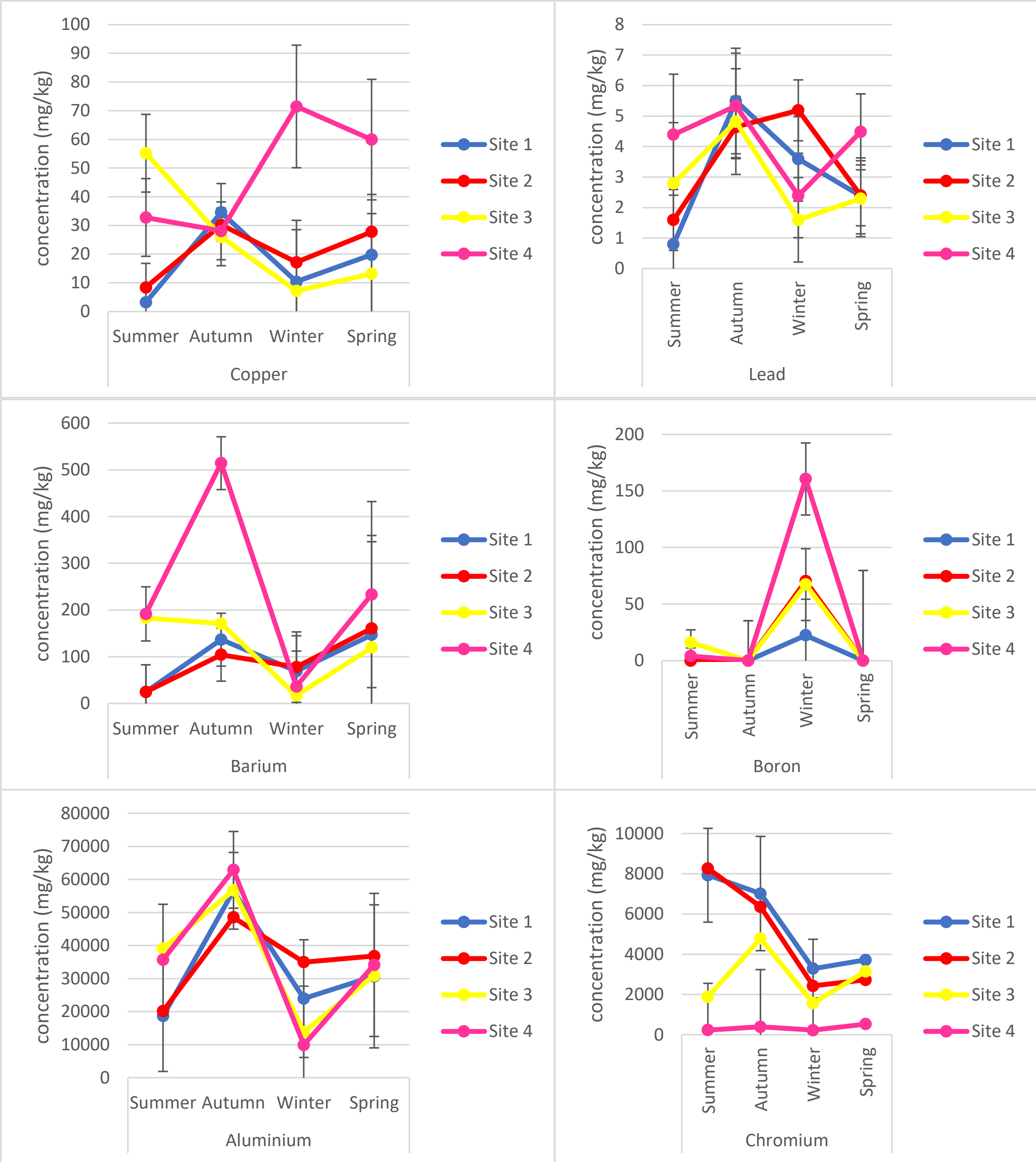

Figure 2.8 Seasonal variation of sediment metals recorded in the Dwars River

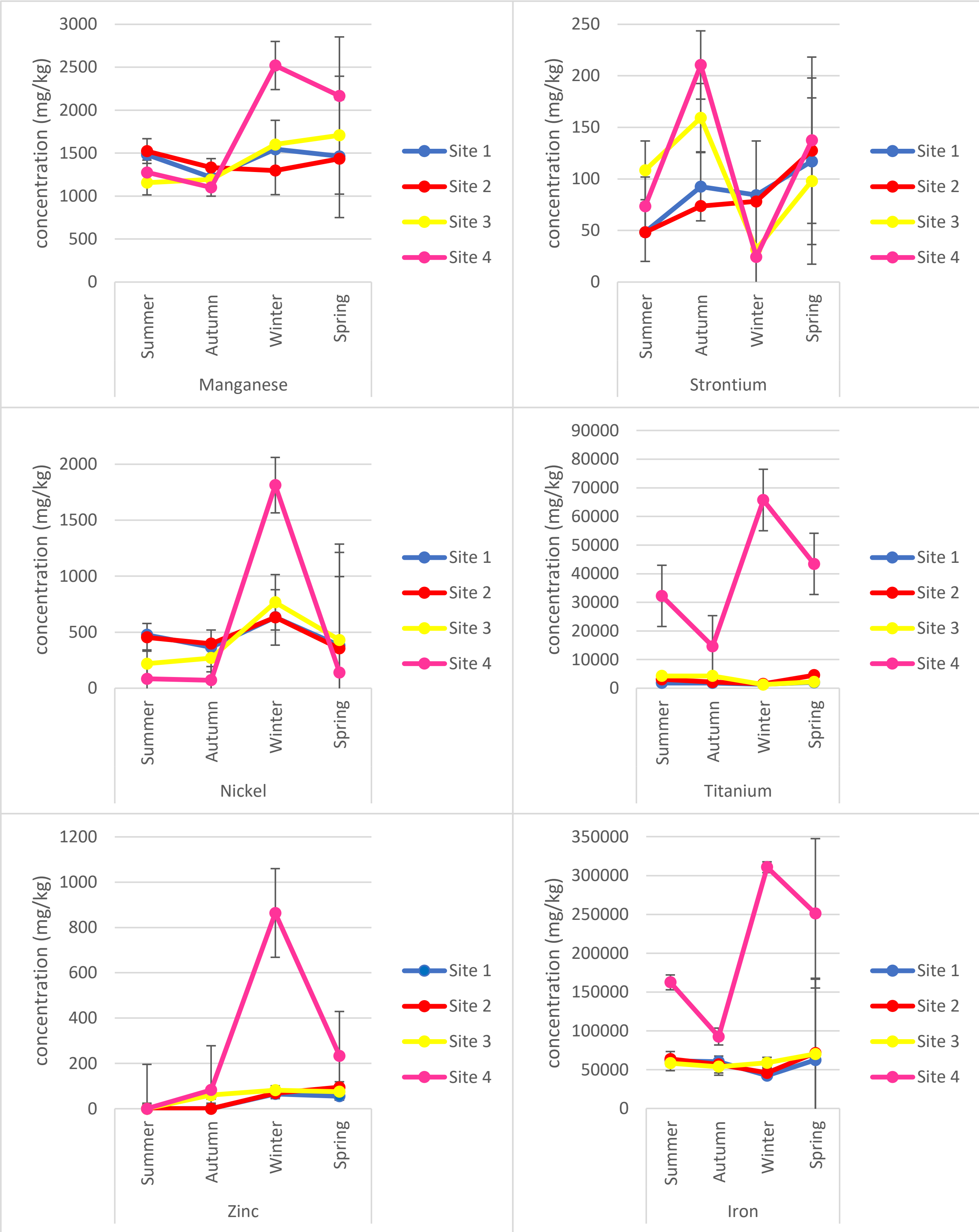

Figure 2.9 Variation of sediment metals concentrations recorded in the Dwars River

2.4 DISCUSSION

2.4.1 Water quality

2.4.1.1 Physicochemical variables

During the study, the water temperature values were within the normal limits (Table 2.1). An increase in temperature can affect substance toxicity and the rate of chemical reactions (Dallas 2008). Literature has indicated that extreme temperatures decrease oxygen solubility, its concentration and availability to aquatic biota (Dallas 2008). Normal functionality of organisms such as reproduction, growth, behaviour and metabolic rate are affected by fluctuation in temperature readings (Dallas & Ross-Gillespie 2015). The high temperature at Site 3, might be due to modified riparian vegetation, whereby most of the canopy trees has been removed which expose the river to direct sunlight. Tree harvesting in riparian zones increase the penetration of light to the water body, thus increasing the water temperature (Justice et al. 2017). The increase in temperature at Site 3 decreases the amount of DO concentration which negatively impact aerobic organisms in the river.

The recorded DO levels were high at Site 1 and low at Site 3 (Table 2.1). This range is comparable with the DO range (5.53-9.4 mg/ℓ) reported in the Swartkops River, South Africa (Odume & Muller 2011). The decrease in DO concentration at Site 3 might be due to an increase in temperature experienced at this site. During the study, the DO was within the permissible range limit (DWAF 1996a). The decline in DO concentration observed in Site 3 might be attributed to surface runoff of fertilizers or pesticides from nearby farms. Nutrient loading contains nitrogen and phosphorus which stimulate aquatic plants growth and algae, thus increasing microbial activity which depletes oxygen levels and may result in loss of sensitive species (Bouwman et al. 2012). There is a clear relationship between the decline in DO and an increase in temperature at Site 3 which might have negative impact on the aquatic biota.

The highest pH was recorded at Site 1 and Site 3, while Site 4 had the lowest pH of 7.4 (Table 2.1). The alkaline pH values recorded during the study were comparable with the results obtained in the Steelpoort River (Magala 2015). Seasonally, an increase in pH was noted during winter, with a value of 8.9 and the lowest during summer, with a value of 7.8. An increase in pH might be attributed to effluents released from industrial activities or photosynthetic plants. When aquatic plants produce their own food through photosynthesis during the day, they consume carbon dioxide which results in an increase in pH (Araoye 2009). An acidic pH condition affects the solubility of trace metals in water (Morrison et al. 2001).

The SAWQG for EC is currently unavailable, however WHO acceptable limit is 100 mS/m. During the study, measured EC values were all above the permissible limit (Table 2.1). However, Site 3 had the highest EC and Site 4 had the lowest record. Site 3 had the lowest water level of 0.13 m; this might be due to agricultural activities which uses water for irrigation purposes or an increase in evaporation during summer season. Seasonally, summer experienced the highest EC than autumn. The ions in water become more concentrated as the water level decrease which ultimately contribute to an increase in water conductivity (Mondal et al. 2016). This was evident at Site 3, whereby as the water level reduces, high electrical conductivity was experienced. The increased EC might be attributed to high dissolved salt content probably from domestic origin or agricultural activities.

The highest salinity mean concentration was recorded at Site 1 during winter and the lowest concentration was recorded at Site 4 during spring season. The limit for salinity levels in freshwater ecosystems should be <0.5‰ or not change by 0.05% from the normal cycle (DWAF 1996a). Site 1 was above the permissible limit. An increase in salinity might be due to industrial activities such as ferrochrome production happening at Site 1. According to literature, industrial activities are commonly correlated with a decline in water quality (Matlou et al. 2017). Salinity affect the physiological process of osmoregulation in aquatic organisms (Teske & Wooldridge 2004; Noyes et al. 2009).

The TDS recorded was high at Site 3 and low at Site 4. SAWQG of TDS is site specific and all measured values were within permissible limit of 1000 mg/ℓ (WHO 2006). Seasonally summer had the highest TDS and winter had the lowest. Elevated TDS decrease the clarity of water, which will affect photosynthesis and ultimately increase water temperature (Dallas & Day 2004). Industrial discharges and surface runoff from agricultural might be attributed to an increase in TDS levels (DWAF 1996a). Too high or too low TDS concentrations may limit growth and cause death to aquatic organisms (Dallas & Day 2004).

There is no target water quality range for turbidity in aquatic ecosystems, however the aquaculture guideline indicated that, <25 NTU is the permissible limit for species (DWAF 1996a). All the sites were within the permissible limit. The increase in turbidity might be due to runoff from mining and industrial activities near the area. Turbidity may alter the feeding and respiratory apparatus of some macroinvertebrates, such as Simuliids and Mayflies which may lead to change in species assemblages (Dallas & Day 2004). Seasonal variations can also influence turbidity levels in rivers, whereby during heavy rainfall runoff picks up waste and sediment particles and deposit them directly into the rivers, which increase turbidity (Zhou et al. 2015). Furthermore, during hot summer season, the water temperature increases which elevate the decomposition and growth rate of organisms such as phytoplankton and bacteria.

2.4.2 Major ions

The major ions recorded in this study include calcium, potassium, magnesium and sodium. Low levels of major ions in an aquatic environment is said to be generally non-toxic, however, when their concentrations increase, they can affect the physiology of aquatic biota (Camargo & Alonso 2006). The levels of calcium at all the sites and seasons were within the permissible limit. However, Site 1 had the highest calcium levels and Site 4 had the lowest. There is no guideline value (SAWQG) for calcium, however, DWAF (1996a) suggested a permissible limit of 250 mg/ℓ is suitable for all the users. Calcium ion naturally occurs in water; however, its presence might be enhanced by weathering of rocks such as fluorite, marble and dolomite. An increase in calcium levels might be due to effluent from industrial activities. Research indicated that calcium is vital for survival of organisms and its decrease may affect species composition through preference of species that tolerate low calcium concentration (Dalesman & Lukowiak 2010).

Potassium can be described as an alkali metal that combine chemically with water to form the positive-charged potassium ions (K^+) (DWAF 1996a). Potassium values at all the sites and seasons were within the permissible limit. Site 4 had the highest potassium levels while Site 1 had the lowest potassium concentration. An increase in potassium concentration might be attributed to the use of potassium salts and industrial effluent near this site. Potassium ions play an essential role in ionic balance of all living organisms (DWAF 1996a). There is no guideline value (SAWQG) for potassium, however, Chapman (1996) has suggested the desirable limit of <10 mg/ℓ.

All the sites and seasons experienced higher magnesium concentration above the permissible limit. An increase in magnesium concentration might be attributed to weathering of rocks containing ferro-magnesium minerals and effluents from agricultural activities happening in the catchment of the river (Ramollo 2008). An increase in magnesium and calcium concentrations is related to the type of land use activities in the catchment (Potasznik & Szymczyk 2015). The effect of magnesium on the aquatic biota is not well documented or little is known (CCME 2012).

The recorded sodium concentration was within the permissible limit at all the sites and seasons. However, the highest sodium concentration was at Site 3 and the lowest was at Site 4 (Table 2.1). The most important occurrence of sodium is its influence on TDS and is among the least toxic cation in the aquatic environment (Dallas & Day 2004). Naturally, sodium occurs in an aquatic environment and its concentration is determined by the geology of the catchment area (Dallas & Day 2004). The Industrial discharge in an area might increase sodium concentration. Sodium is important in osmotic, ionic and water balance of all living organisms (Alade & Abdulazeez 2012).

2.4.3 Nutrients

Nutrients are natural occurring in water and soil; however, their presence can be increased by effluents from human activities (Griffin 2017). Ammonia was undetected at Site 2. However, Site 1, Site 3 and Site 4 had equivalent ammonia concentration. All the recorded ammonium concentrations were within permissible limit of 0.2 mg/ℓ as stipulated by DWAF (1996a), for the aquatic ecosystem. In an alkaline environment of pH>8.5, free ammonia (NH_3) is known to be more toxic to aquatic biota (Morrison et al. 2001). Application of inorganic fertilisers may increase the concentration of ammonia in water bodies during runoff (Ahmed et al. 2017).

Nitrite (NO_2) is the intermediate product of inorganic oxidation of ammonia. The highest nitrite concentration was at Site 1 and the lowest was at Site 4. Generally, nitrites are found at very low levels in an aquatic environment because they are easily reduced to ammonia or oxidised to nitrate by both biochemical and chemical processes (DWAF 1996a). Anthropogenic activities such as industrial production of metals, dye and celluloids may increase the concentration of nitrite ions (Ahmed et al. 2017). In conversion of ammonia nitrite is known to be toxic to aquatic biota even in lower concentrations (Smith et al. 1999). However, nitrates and bicarbonates have shown to modify the toxicity of nitrite (Dallas & Day 2004; Smith et al. 1999). In this study the salinity is very high which will limit the toxicity of nitrite.

The highest nitrate concentration ranged from 1.9 mg/ℓ at Site 4 to 16.5 mg/ℓ at Site 1. There is no guideline value (SAWQG) for nitrate, however, the Canadian guidelines stipulated a limit of 13.0 mg/ℓ. Site 1 had the highest nitrate concentration above permissible limit. The high concentration of nitrate recorded might be attributed to industrial wastes, agriculture and urban runoff near the area. Over application of fertilisers in agriculture may enrich the aquatic systems which can lead to eutrophication and algal blooms (Griffin 2017).

The inorganic phosphorus concentration was between 0.025 mg/ℓ and 0.25 mg/ℓ, which was interpreted as eutrophic (DWAF 1996a). During field survey there were signs of algae growth observed at the sites. SAWQG for aquatic ecosystems classify the inorganic phosphorus into four forms, which include; the oligotrophic conditions occurring at 0.005 mg/ℓ, mesotrophic conditions occurring between 0.005 and 0.025 mg/ℓ, eutrophic conditions occurring between 0.025 and 0.25 mg/ℓ and hypertrophic conditions occurring at values >0.25 mg/ℓ (DWAF 1996a). An increase in inorganic phosphorus concentration might be due to agricultural runoff, industrial wastes and decomposition of organic matter. According to DWAF (1996a), inorganic phosphorus is the only oxy anion which originate from weathering and decomposition of organic matter and it strongly influence the rate of primary production. An excess increase in nutrients have the potential to cause eutrophication, hypoxia and ultimately death of aquatic biota (Griffin 2017).

The highest nitrogen concentration was recorded at Site 1 and the lowest was at Site 4. Site 1, Site 2 and Site 3 had higher nitrogen concentrations which were above 10 mg/ℓ and this was interpreted as hypertrophic (DWAF 1996a). Usually, hypertrophic conditions are characterised by reduced species diversity, highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae. Algal blooms may be toxic to human, livestock and wildlife (DWAF 1996a). Anthropogenic activities releasing nitrogen compounds into water ways can result in eutrophic systems which can affect the aquatic biota (Ahmed et al. 2017). An increase in nitrogen concentration might be attributed to the use of nitrogenous fertilisers in agriculture and from fossil fuel combustion (Ahmed et al. 2017).

2.4.4 Toxic constituents (metals and metalloids) in water

Toxic constituents are detrimental in aquatic systems even when they are found in low levels. However, toxic constituents are rarely elevated in unimpaired water bodies (Brink & Kamish 2017; Edokpayi et al. 2017). The highest aluminium concentration was recorded at Site 1 (0.50 mg/ℓ) and the lowest was at Site 3 (0.16 mg/ℓ). The TWQR for aluminium in freshwater is 0.001 mg/ℓ (DWAF 1996a). All the recorded aluminium concentrations were above the permissible limit. About 8% of the earth's crust is made of aluminium (Oliveira et al. 2016). Aluminium is known to be harmful because it mobilises various toxicity events and has serious ecological consequences (Correia et al. 2010). It has been documented that, certain filter-feeders and grazers can accumulate significant amount of aluminium which may affect them negatively (Oberholster et al. 2011). An increase in aluminium concentration might be attributed to mining and processing of aluminium ores coupled with industrial effluents (Oberholster et al. 2011). In sediment, the highest aluminium concentration was at Site 4 with a mean value of 35.7 mg/g and the lowest was at Site 1 with a mean of 32.5 mg/g. Seasonally, spring had the highest aluminium concentration of 33.2 mg/g while winter had the lowest mean concentration of 20.7 mg/g.

Iron is regarded as the most important micronutrient in all living organisms, however, at high concentration it can be toxic (Dallas and Day 2004). Site 1 had the highest iron concentration recorded in water column. This increase might be due to high effluent from mining and industrial activities and less rainfall. Iron has both direct and indirect effects on aquatic ecosystems and it can alter the diversity of aquatic organisms (Edokpayi et al. 2017). The recorded sediment iron concentration was very at Site 4

(241.6 mg/g). This increase in iron might be attributed to runoff from ferrochrome mines around the area.

Titanium is graded as the ninth most abundant element, which is present in igneous rocks and sediments bearing these rocks (ATSDR 1997). An increased titanium concentration might be due to surface runoff from mining or the geology of the area. Currently there are no known research which report on the ecological effects of titanium. During the study, the titanium levels were very high at Site 4 in sediment than in the water column. This is because of a constant exchange taking place between water and sediment where sediment acts as a sink for metals (Crafford & Avenant-Oldewage 2011).

A low level of barium is non-toxic to most organisms, except invertebrates (Donald 2017). In sediment, the highest barium concentration was recorded at Site 4 during spring. An increase in barium might be attributed to nearby industries which might utilize barium compounds in manufacturing paints, rubber, glass or tiles. However, barium is known to originate primarily from natural sources and is present as a trace element in igneous and sedimentary rocks (WHO 2004).

Manganese in an aquatic environment exists as compound or complexes with other organic compounds (Hashim et al. 2015). The recorded manganese concentration at all sites were within the permissible limit. An increased manganese concentration can alter metabolic pathways, specifically, the central nervous system through suppression of dopamine formation (Dallas & Day 2004). In sediment, the highest manganese concentration was recorded at Site 4 during winter season. This might be attributed to sediment, soil, metamorphic and sedimentary rocks of the area

Nickel concentration was only detected at Site 1 with a value of 0.02 mg/ℓ. Nickel has been reported to cause cancer and respiratory complications in living organisms (Oforka et al. 2012). The nickel concentration recorded in sediment was very high than in water due to the reason that sediment act as a sink which retain heavy metals (Crafford & Avenant-Oldewage 2011). An increase in nickel concentration in winter might be due to lack of rainfall and increased effluents from mining and industrial operations near the area.

In the water column the concentration of vanadium was increased at Site 1, Site 2 and Site 3. Elevated concentrations of vanadium have been reported to precipitate serum proteins and alter oxidation of tissues (Dallas & Day 2004). However, USEPA (2012), has considered the vanadium compounds to be potentially toxic. The high vanadium recorded at the sites might be due to effluent released from industrial and domestic activities near the area.

Generally, chromium exists in hexavalent (VI) and trivalent (III) oxidation forms in both aquatic and soil environments (Krishnani et al. 2004). Chromium (VI) state has been categorised as carcinogen in group A, based on its chronic effects while chromium (III) is the most insoluble and stable (Bojic et al. 2004; Krishnani et al 2004). The high concentration of chromium observed in Site 1, might be attributed to production of ferrochrome near this site. Different sources of chromium released to the environment include chromium plating, metal finishing industries, cooling towers, production of corrosion inhibitors and tanneries (Crafford & Avenant-Oldewage 2011). In literature, (chromium VI) has been reported to bioaccumulate in aquatic organisms (Krishnani et al. 2004). The stipulated sediment limit of chromium is 0.0373 mg/g (CCME 2012). The concentrations recorded at all the sites and in all the seasons were above the permissible limit and this needs to be seriously monitored as it might impact the aquatic biota. Chromium concentration in sediment is much higher than in water because of the constant exchange of heavy metals between water and sediment whereby the sediment act as a sink for heavy metals (Crafford & Avenant-Oldewage 2011).

All the recorded strontium concentrations were within the permissible limit at all sites. Strontium is regarded as a non-essential element which occasionally contributes to water hardness (Crafford & Avenant-Oldewage 2011). High concentration of strontium has been reported to accumulate in opercula and vertebrae of organisms (Crafford & Avenant-Oldewage 2011). The highest strontium concentration in sediment was recorded at Site 4 during autumn season.

Zinc is regarded as an important micronutrient often associated with cadmium in natural environments (Dallas & Day 2004). All the recorded zinc concentrations were above the permissible limits. In water column zinc exhibit two oxidation forms which are zinc (II) and as a metal (DWAF 1996a). The presence of zinc, sulphate and molybdenum in an aquatic environment decrease the toxicity of copper (Dallas & Day

2004). The high zinc concentration might be attributed to the high intensity of mining in the catchment coupled with domestic and industrial runoff.

All the recorded boron concentration at all sites were within permissible limit (CCME 2012). According to USEPA (2008), boron concentration limit recommended to protect sensitive species in an aquatic environment should be less than 1 mg/ℓ. Seasonally, boron concentration was detected during winter season. The source of boron could be from agricultural and mining seepage. The presence of boron in an aquatic system is of a major concern, due to its conservative nature, which is inability to biodegrade, undergo redox transformations, precipitation and significant sorption (Tredoux et al. 2004).

Lead is relatively accessible and potentially hazardous and carcinogenic to most forms of life including aquatic organisms (DWAF 1996a). Lead was not detected in the water column; however, it was detected in sediment. Lead can be present in several oxidation forms which are 0, I, II and IV. The Pb (II) form is known to bioaccumulate in organisms (ATSDR 2007; DWAF 1996a). Lead can alter haeme from haemoglobin molecule and its toxicity is mostly determined by water hardness, organic materials and pH (Fatoki et al. 2002). During the study, the recorded pH levels were alkaline which will reduce the toxicity of metals including lead. Different sources of lead into the aquatic systems, include industrial wastes discharge, urban storm runoff, atmospheric deposition, erosion and soil leaching (Fatoki et al. 2002).

The copper concentration was undetected at all sites in the water column; however, the highest concentration was detected in sediment at Site 4. Copper is essential in regulating both the nervous and cardiovascular systems (Rai et al. 2015). However, when copper is present in excess, it can alter both the physiological and biochemical processes through generation of free radicals (Rai et al. 2015). Toxicity of copper is mainly dependent on the water hardness (Rai et al. 2015). High copper levels observed at Site 4 might be due to industrial effluents and mine tailings runoff.

In summary, water temperature levels were normal, and the highest temperature was observed during summer. The recorded pH values were mainly alkaline during the study and this might account for the reduced concentration of most of the heavy metals in the water. Dissolved oxygen, TDS and turbidity concentrations in the water column were within permissible limit (DWAF 1996a; b). Salinity and EC concentrations in water column were above permissible limits (DWAF 1996a; WHO 2006). Nutrients such as nitrate, nitrite and total nitrogen had the highest concentrations recorded at Site 1, with values above permissible limits (CCME 2012; DWAF 1996a). The inorganic phosphorus concentration ranged from 0.025 and 0.25 mg/ℓ at all sites, indicating eutrophic condition (DWAF 1996a). The concentrations of major ions (calcium, potassium and sodium) were within the permissible limits (Chapman 1996; DWAF 1996a; DWAF 1996c). However, magnesium concentrations at all sites and seasons were above permissible limit. Metals and metalloids (iron, barium, manganese, nickel, strontium, boron and lead) concentrations were within permissible limits in the water column (CCME 2012; DWAF 1996a; USEPA 2012; WHO 2003; WHO 2004). However, aluminium, chromium and zinc concentrations were above permissible limits in water column (DWAF 1996a). The concentrations of copper and chromium in sediment were above permissible limits (CCME 2012). Overall, many metals in water column and sediment were detected above permissible limits at Site 1. The high levels of metals in the sediments indicate that metals in water from upstream are washed downstream and settle in sediment.

CHAPTER 3

3. AQUATIC MACROINVERTEBRATES BIOMONITORING

3.1 INTRODUCTION

Human-induced stressors to the aquatic environment are recognised as the main contributors to pollution world-wide (Mathers et al. 2016; Dalu et al. 2017). Reducing water contamination needs adequate, cost-effective approach and monitoring indices (Mangadze et al. 2019). The South African River Health Programme (RHP) has been recently changed to the River Ecosystem Monitoring Program (REMP) in 2016 (Mangadze et al. 2019). This program (REMP) has about seven aquatic biomonitoring indices made available to assess and monitor the health of the aquatic bodies which include the South African Scoring System version 5 (SASS5), Index of Habitat Integrity (IHI), Invertebrate Habitat Assessment System (IHAS), Riparian Vegetation Index (RVI) and Fish Assemblage Integrity Index (FAII) (Maseti 2005). Every index is established to assess and evaluate a certain health aspect of the aquatic ecosystem such as riparian vegetation, fish, habitat, invertebrates, and the geomorphological state of the river channel (Maseti 2005). The indices possess various advantages and disadvantages depending mainly on the type of the organisms used and the method of monitoring.

The River Ecosystem Monitoring Program (REMP) was initiated by the Department of Water Affairs and Forestry (DWAF), currently known as the Department of Water and Sanitation (DWS). The REMP was undertaken due to the need for monitoring the ecological state of rivers in South Africa (DWAF 2008). In the past when assessing the health status of ecosystems, the water chemistry analysis was the only indicator which was considered reliable. However, recently that method has been extended to merge both the physicochemical analysis and the biological assessments (Kalogianni et al. 2017). Biomonitoring is referred to as the use of biological responses to assess the effect of various stressors on the health and functioning of the aquatic ecosystems (Mangadze et al. 2019).

The most recognised and broadly used biomonitoring index in South Africa, is the South African Scoring System version 5 (SASS5), which was originally developed by Chutter in 1998 and later was refined from SASS version 4 to SASS version 5 (Dickens & Graham 2002). Previously SASS version 4 was used in monitoring organic pollution in aquatic systems. However, recently it included the assessment of the biological effects of other contaminants as well (Gordon et al. 2014). Beyond any doubts, SASS5 has proven to be a very sensitive tool in assessing the ecological and biological implication of pollution in an aquatic environment with the aid of bioindicators (Mangadze et al. 2019). Literature indicated that SASS5 has the potential to reflect instream sediment impacts (Gordon et al. 2014). The SASS5 sampling method has various advantages such as time saving during sampling, uncomplicated procedures and affordable to implement (Dalu et al. 2017; Mangadze et al. 2019).

The main metrics used in SASS5 are the number of taxa, SASS score and Average Score Per Taxon (ASPT) (Gordon et al. 2014). The SASS5 method involves scores which are assigned to different families/taxon classified according to their tolerance levels to pollution and the higher the score the sensitive the family (Dickens & Graham 2002). Biomonitoring programmes employ a wide array of bioindicators and biomarkers ranging from subcellular level to population level (Mangadze et al. 2019). An indicator organism which is ideal should possess various characteristics such as numerical abundance, local indication, suitability for experiments in the laboratory, high sensitivity, high ability for quantification and standardisation, easy identification, wide distribution and well-known ecological characteristics (Mangadze et al. 2019).

Fresh water organisms spent almost their entire life in water, and they respond to every change in water quality (Rasifudi et al. 2018). Macroinvertebrates have received enormous attention, as bioindicator of evaluating the health of an aquatic environment in different parts of the world (Mathers et al. 2016; Haggag et al. 2018). This is because macroinvertebrates continuously dwell in water and are affected by any disturbance encountered in the aquatic systems such as pollution (Rasifudi et al. 2018). Aquatic macroinvertebrates can provide information about the past and current health status of an aquatic environment as compared to physical and chemical assessments (Matlou et al. 2017; Rasifudi et al. 2018). Macroinvertebrates are visible to the naked eye; they occupy sedentary habitats and have rapid life cycles (Dalu et al. 2017).

Additionally, aquatic macroinvertebrates consist of distinct species with various sensitivity levels to stressors and they occupy sediment which acts as a sink for pollutants (Dalu et al. 2017; Rasifudi et al. 2018).

3.2 METHODS AND MATERIALS

3.2.1 Sampling of aquatic macroinvertebrates

Macroinvertebrates were sampled seasonally (July 2017 to May 2018) at four different sites along the Dwars River. Aquatic macroinvertebrates sampling was done using the SASS5 bio-assessment protocol (Dickens & Graham 2002). In each selected Site, 5 samples of aquatic macro-invertebrates were collected using a SASS5 net of 30 cm by 30 cm with 1 mm mesh size. The substrate was disturbed for a period of 5 min to free macroinvertebrates and biotopes sampled were stones, vegetation, and GSM (gravel, sand and mud). All macroinvertebrate samples collected were identified in the field. Macroinvertebrates were identified to family level using an invertebrate field guide, then counted and recorded in a SASS5 data sheet (Gerber & Gabriel 2002). However, macroinvertebrates families which could not be identified in the field were preserved in 70% ethanol in 1 litre polypropylene buckets and transported to the laboratory for further identification. Preservation of macroinvertebrate samples with 70% ethanol was done immediately, to prevent predacious invertebrates to prey on others.

3.2.2 LABORATORY ANALYSIS

Aquatic macroinvertebrates

After collection, samples were taken to University of Limpopo Biodiversity Laboratory to be sorted. Sorting was done by adding enough clean water into a white tray, to provide better vision and to easily pick and identify the macroinvertebrates. Leaves, debris, twigs and stones were carefully checked for clinging organisms. After, small portion of the sample was poured into a petri dish containing water with forceps, to enhance clear sorting and avoid missing other macroinvertebrates. Then observed under a stereomicroscope.

To determine the state of water quality in the Dwars River, SASS score and ASPT were calculated. The SASS5 is a scoring system whereby each taxon is assigned a score based on the degree of sensitivity/tolerance to pollution, i.e. tolerant families (1-5 scores), moderately tolerant (6-10 scores) and highly sensitive to pollution (11-15 scores) (Dickens & Graham 2002). The higher the score the more sensitive the family (Dickens & Graham 2002). Data interpretation was based on the ASPT, which is the SASS score divided by the number of taxa and the SASS score, which is the sum of all the sensitivity/tolerance scores of taxa.

3.2.3 STATISTICAL ANALYSIS

Canonical correspondence analysis (CCA) is a multivariate approach used to analyse the relationship between the biotic assemblages of taxa and their environment. In the current study, CCA was used to explore the relationship between environmental variables and macroinvertebrate assemblages. The data was log (x+1) transformed to stabilize the variance and the statistical package CANOCO was used (Ter Braak & Smilauer 2012).

3.3 RESULTS

3.3.1 Taxon diversity and richness of benthic invertebrates

A total of 7628 individual macroinvertebrates were collected. They belonged to 10 orders and 45 families. Orders included Ephemeroptera, Tricoptera, Diptera, Odonata, Coleoptera, Pelecypoda, Annelida, Hemiptera, Plecoptera and Gastropoda (Table 3.1). Site 1 had the highest abundance with 4933 individuals, while Site 4 had the lowest number (358) of individuals (Table 3.1). Seasonally, winter had the highest abundance with 3439 individuals while spring had the lowest number of individuals, 1287 (Table 3.2). Site 1 had the highest number of sensitive, moderately tolerant and tolerant individuals and Site 4 had the lowest number of sensitive, moderately tolerant and tolerant taxa (Figure 3.1). Winter had the highest number of sensitive, moderately tolerant and tolerant individuals compared to all the other seasons (Figure 3.2). The highest EPT value was obtained at Site 1, while the lowest EPT value was at Site 4. Site 1 had the highest diversity of taxa while Site 4 had the least diversity of taxa (Table 3.3).

Table 3.1 Abundance of macroinvertebrates collected at four sites in the Dwars River

Orders	Families	Site 1	%	Site 2	%	Site 3	%	Site 4	%
	Oligochaeta	13	0.26	12	1.79	6	0.36	8	2.23
Annelida	Hirudinea	0	0.00	0	0.00	0	0.00	2	0.56
Plecoptera	Perlidae	10	0.20	2	0.29	2	0.12	0	0.00
	Baetidae	468	9.49	102	15.27	191	11.44	49	13.69
	Caenidae	345	6.99	83	12.43	395	23.67	117	32.68
	Heptageniidae	16	0.32	13	1.95	13	0.78	5	1.39
	Leptophlebiidae	147	2.99	79	11.83	120	7.19	29	8.10
	Oligoneuridae	4	0.08	0	0.00	3	0.18	0	0.00
	Prosopistomatidae	3	0.06	1	0.15	0	0.00	0	0.00
Ephemeroptera	Tricorythidae	25	0.51	8	1.19	18	1.08	0	0.00
	Chlorocyphidae	10	0.20	8	1.19	19	1.14	2	0.56
	Coenagrionidae	1	0.02	0	0.00	1	0.06	0	0.00
	Lestidae	0	0.00	1	0.15	0	0.00	0	0.00
	Aeshnidae	23	0.47	0	0.00	1	0.06	0	0.00
	Corduliidae	5	0.10	0	0.00	0	0.00	0	0.00
	Gomphidae	419	8.49	28	4.19	75	4.49	41	11.45
Odonata	Libellulidae	364	7.39	7	1.05	47	2.82	16	4.47
	Belostomatidae	1	0.02	0	0.00	0	0.00	1	0.28
	Naucoridae	3	0.06	1	0.15	2	0.12	4	1.12
	Pleidae	0	0.00	0	0.00	0	0.00	3	0.84
Hemiptera	Veliidae	0	0.00	3	0.45	0	0.00	0	0.00
	Ecnomidae	1	0.02	0	0.00	0	0.00	2	0.56
	Hydropsychidae	1456	29.52	119	17.81	270	16.18	30	8.38
	Philopotamidae	0	0.00	0	0.00	1	0.06	0	0.00
	Hydroptilidae	2	0.04	0	0.00	0	0.00	0	0.00
Tricoptera	Leptoceridae	11	0.22	0	0.00	0	0.00	0	0.00
	Elmidae	470	9.53	31	4.64	204	12.22	9	2.51
	Gyrinidae	17	0.34	3	0.45	3	0.18	2	0.56
	Helodidae	1	0.02	0	0.00	0	0.00	0	0.00
	Hydraenidae	1	0.02	0	0.00	2	0.12	0	0.00
Coleoptera	Psephenidae	162	3.28	12	1.80	91	5.45	0	0.00
	Athericidae	30	0.61	6	0.90	11	0.66	6	1.68
	Ceratopogonidae	85	1.72	39	5.84	41	2.46	0	0.00
	Chironomidae	282	5.72	46	6.89	68	4.07	2	0.56
	Culicidae	0	0.00	0	0.00	2	0.12	3	0.84
	Muscidae	7	0.14	0	0.00	0	0.00	0	0.00
	Simuliidae	273	5.53	11	1.65	12	0.72	10	2.79
Diptera	Tabanidae	215	4.36	39	5.84	51	3.06	12	3.35
	Tipulidae	0	0.00	2	0.30	0	0.00	0	0.00
	Hydrobiidae	2	0.04	0	0.00	1	0.06	0	0.00
	Lymnaeidae	2	0.04	0	0.00	4	0.24	1	0.28
	Planorbinae	0	0.00	0	0.00	3	0.18	2	0.56
Gastropoda	Thiaridae	0	0.00	1	0.15	2	0.12	1	0.28
	Corbiculidae	58	1.18	11	1.65	10	0.59	1	0.28
Pelecypoda	Sphaeriidae	1	0.02	0	0.00	0	0.00	0	0.00
Total		4933		668		1669		358	

Table 3.2 Seasonal abundance of different macroinvertebrates families

	Seasons							
Families	**Summer**	**%**	**Autumn**	**%**	**Winter**	**%**	**Spring**	**%**
Oligochaeta	14	0.97	19	1.3	0	0	6	0.47
Hirudinea	1	0.07	0	0	0	0	1	0.08
Perlidae	3	0.21	1	0.07	2	0.06	8	0.62
Baetidae	109	7.56	195	13.36	347	10.09	159	12.35
Caenidae	192	13.31	115	7.88	469	13.64	164	12.74
Heptageniidae	18	1.25	4	0.27	14	0.41	11	0.85
Leptophlebiidae	107	7.42	46	3.15	120	3.49	102	7.92
Oligoneuridae	6	0.42	1	0.07	0	0	0	0
Prosopistomatidae	0	0	0	0	0	0	4	0.31
Tricorythidae	17	1.18	7	0.48	0	0	27	2.09
Chlorocyphidae	8	0.55	8	0.55	8	0.23	15	1.16
Coenagrionidae	2	0.14	0	0	0	0	0	0
Lestidae	1	0.07	0	0	0	0	0	0
Aeshnidae	0	0	0	0	24	0.69	0	0
Corduliidae	0	0	1	0.07	0	0	4	0.31
Gomphidae	48	3.33	56	3.83	393	11.43	66	5.13
Libellulidae	51	3.54	38	2.6	294	8.55	51	3.96
Belostomatidae	1	0.07	0	0	0	0	1	0.08
Naucoridae	2	0.14	7	0.48	0	0	1	0.08
Pleidae	3	0.21	0	0	0	0	0	0
Veliidae	3	0.21	0	0	0	0	0	0
Ecnomidae	0	0	1	0.07	0	0	2	0.16
Hydropsychidae	377	26.14	338	23.15	877	25.5	283	21.99
Philopotamidae	0	0	0	0	0	0	1	0.08
Hydroptilidae	0	0	0	0	0	0	2	0.16
Leptoceridae	2	0.14	1	0.07	3	0.09	5	0.39
Elmidae	185	12.83	252	17.26	134	3.9	143	11.11
Gyrinidae	2	0.14	1	0.07	16	0.47	6	0.47
Helodidae	0	0	0	0	1	0.03	0	0
Hydraenidae	0	0	3	0.21	0	0	0	0
Psephenidae	83	5.75	61	4.18	29	0.84	92	7.15
Athericidae	17	1.18	3	0.2	19	0.55	14	1.09
Ceratopogonidae	36	2.49	85	5.82	39	1.13	5	0.39
Chironomidae	26	1.8	94	6.44	218	6.34	60	4.66
Culicidae	0	0	5	0.34	0	0	0	0
Muscidae	0	0	0	0	7	0.2	0	0
Simuliidae	8	0.55	21	1.44	277	8.05	0	0
Tabanidae	83	5.76	55	3.77	140	4.07	39	3.03
Tipulidae	0	0	2	0.14	0	0	0	0
Hydrobiidae	0	0	0	0	2	0.06	1	0.08
Lymnaeidae	2	0.14	5	0.34	0	0	0	0
Planorbinae	2	0.14	3	0.2	0	0	0	0
Thiaridae	4	0.28	0	0	0	0	0	0
Corbiculidae	29	2.01	32	2.19	5	0.15	14	1.09
Sphaeriidae	0	0	0	0	1	0.03	0	0
Total	**1442**		**1460**		**3439**		**1287**	

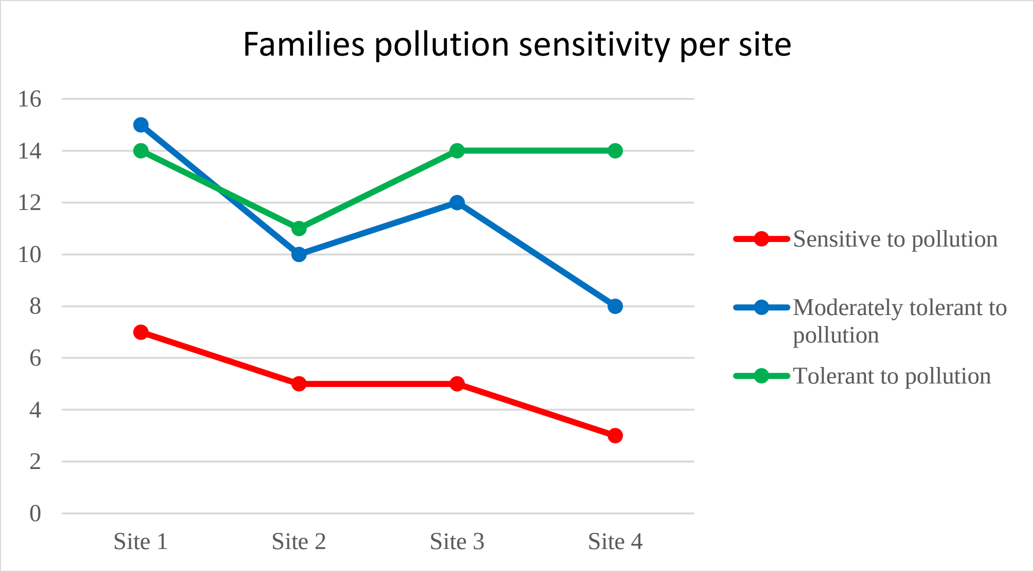

Figure 3.1 Different groups of macroinvertebrate taxa recorded at different sites of the Dwars River

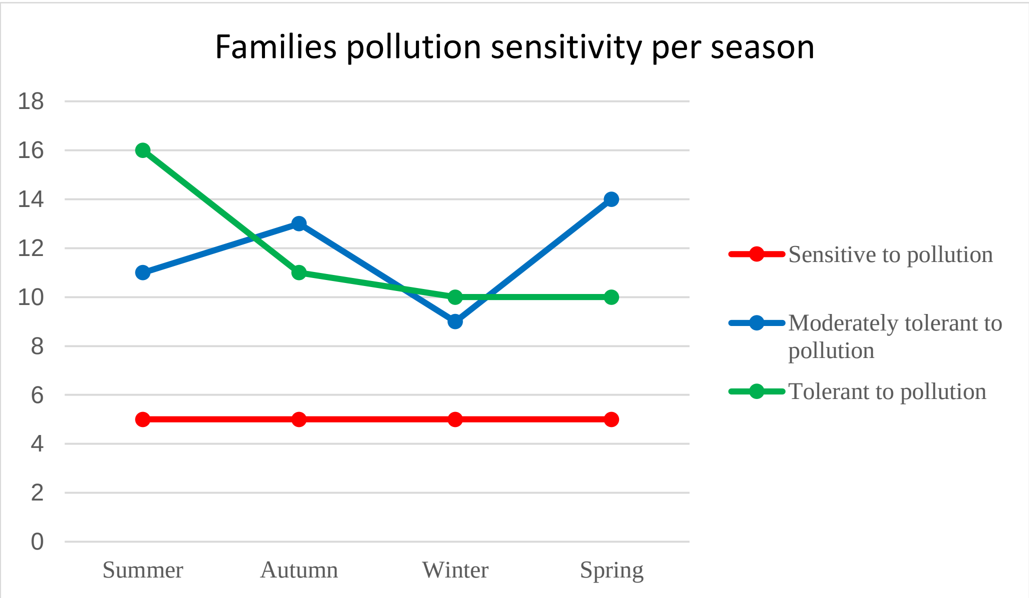

Figure 3.2 Seasonal distribution of macroinvertebrate groups recorded in the Dwars River

Table 3.3 EPT and H' values calculated from all the sites in the Dwars River.

Orders	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Ephemeroptera	7	6	6	4
Plecoptera	1	1	1	0
Tricoptera	4	1	2	2
EPT	12	8	9	6
Shannnon Weiner (H')	2.4	2.6	2.4	2.3

3.3.3 SOUTH AFRICAN SCORING SYSTEM VERSION 5 EDITION (SASS5)

Table 3.4 SASS5 indices calculated (SASS score, number of taxa and ASPT) per sites in the Dwars

	Site 1						**Site 2**						**Site 3**						**Site 4**					
	Summer	Autumn	Winter	Spring	**Total**	**Mean**	Summer	Autumn	Winter	Spring	**Total**	**Mean**	Summer	Autumn	Winter	Spring	**Total**	**Mean**	Summer	Autumn	Winter	Spring	**Total**	**Mean**
SASS Score	160	149	172	179	660	165	130	128	121	120	499	125	162	163	114	112	551	138	65	70	104	46	285	71
No. of Taxa	24	22	24	23	93	23	19	19	16	18	72	18	24	25	15	16	80	20	14	13	14	10	51	13
ASPT	6.7	6.7	7.2	7.8	28	7.1	6.8	6.7	7.6	6.7	27.8	7	6.8	6.5	7.6	7	28	7	4.6	5.4	7.4	4.6	22	5.5

The highest SASS Score, number of taxa and ASPT were obtained at Site 1, while the lowest SASS Score, number of taxa and ASPT were obtained at Site 4. Seasonally, all the four seasons had a SASS Score greater than 100 and ASPT value greater than 6, which could be interpreted as natural water quality and high habitat diversity (Chutter 1995). However, in terms of spatial distribution at Site 4, the mean SASS Score and ASPT value indicate that the area is disturbed. The EPT orders were chosen because they are known to have very sensitive families to pollution, and they reside in rivers with enough dissolved oxygen (Dalu et al. 2017).

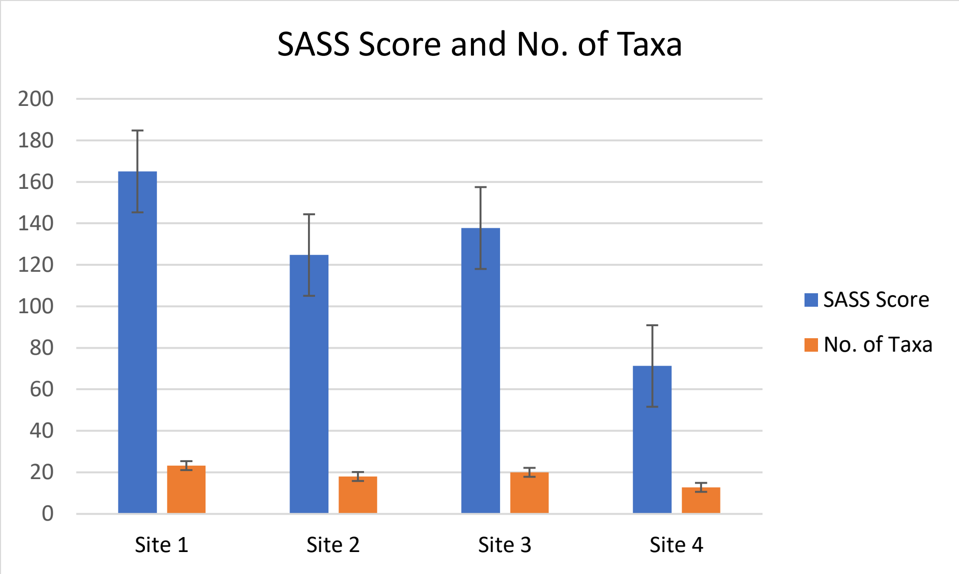

Figure 3.3 The SASS score and number of taxa at four Sites in the Dwars River.

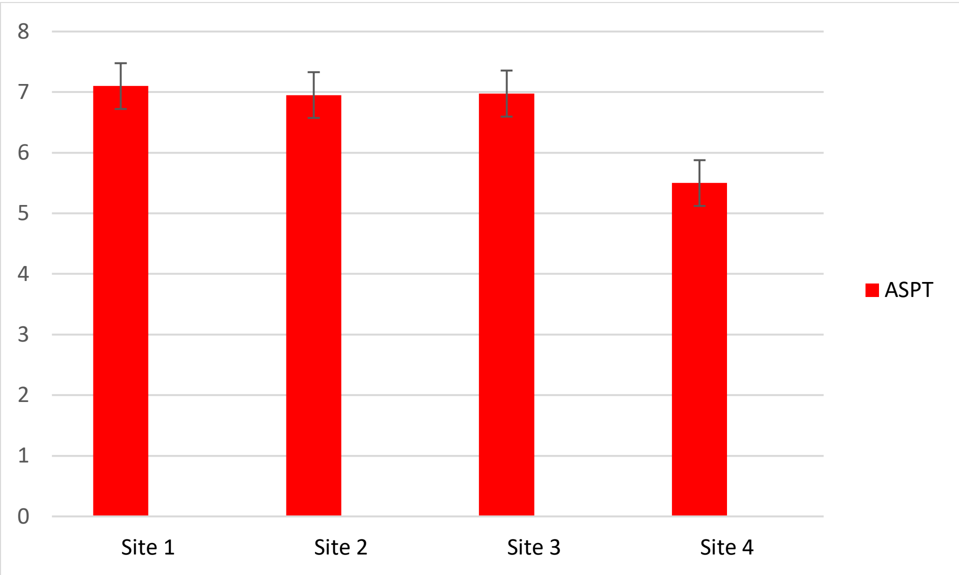

Figure 3.4 The ASPT calculated at four sampling Sites in the Dwars River.

3.3.4 CANONICAL CORRESPONDENCE ANALYSIS

The macroinvertebrates assemblages were well correlated to environmental factors which include DO, depth, width, temperature, salinity and TDS, which were the most important predictors of macroinvertebrate assemblage (Figure 3.4). Most aquatic macroinvertebrates, especially the sensitive taxa were observed at Site 1 (Figure 3.4). Low DO was correlated with moderate-tolerant and tolerant taxa such as Corduliidae, Leptoceridae, Aeshnidae Hydraenidae, Sphaeriidae, Muscidae, Hydrobiidae, Coenagrionidae and Sphaeriidae. While high TDS, salinity and EC, as an indication of poor water quality were correlated with the distribution of Ceratopogonidae, Corbiculidae, Elmidae, Chlorocyphidae, Chironomidae, Tricorythidae, Psephenidae, Perlidae and Prosopistomatidae (Figure 3.4). Salinity, TDS and EC condition prevailed at Site 3. An increase in temperature was strongly correlated with pollution tolerant taxa such as Simuliidae, Libellulidae, Lymnaeidae and Gyrinidae. The cumulative variance percentage explained by axis 1 and axis 2 of species-environment relationship was 49.4% and 81.3% respectively (Table 3.5). This is evident that the measured environmental variables were important in explaining variance in macroinvertebrate assemblages.

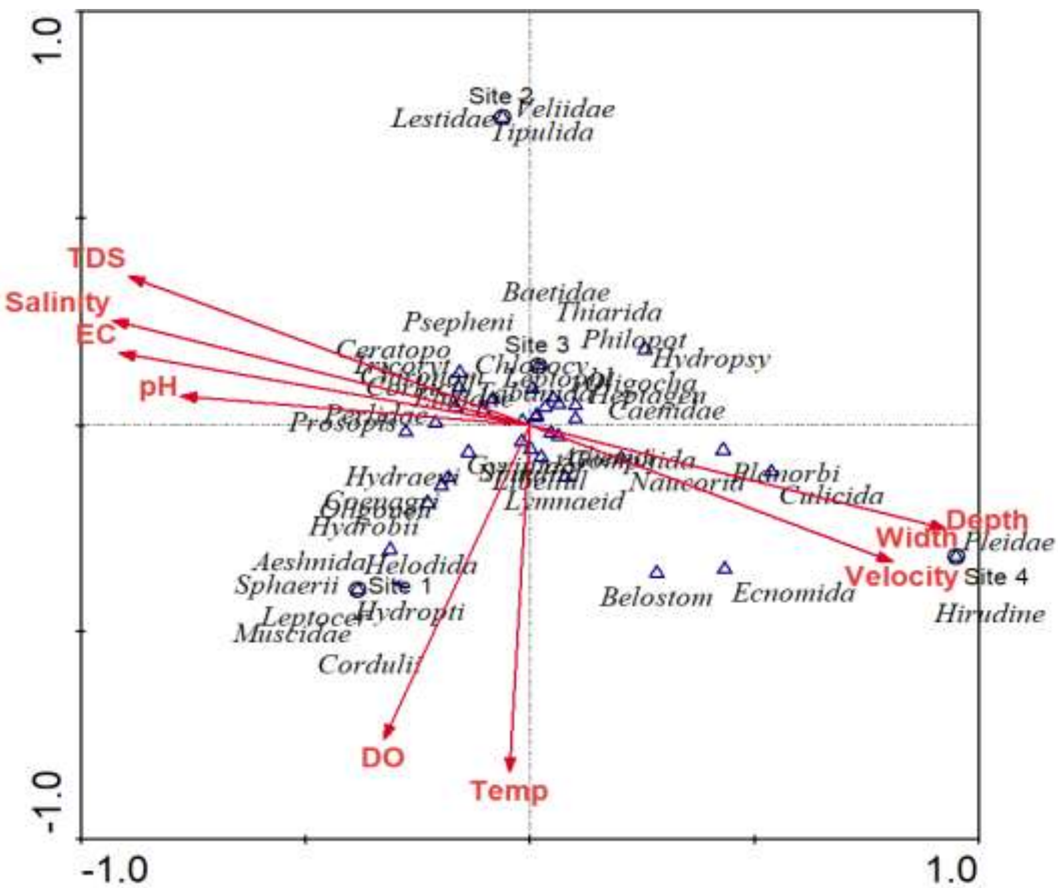

Figure 3.5 CCA plot depicting the relationship between physicochemical parameters and macroinvertebrates

Table 3.5 CCA results which indicate correlation between physicochemical parameters and macroinvertebrates

Axes	1	2	3	4	Total inertia
Eigenvalues	0.135	0.087	0.051	0	0.273
Species-environment correlations	1	1	1	0	
Cumulative percentage variance					
*of species data	49.4	81.3	100	0	
*of species-environment relation	49.4	81.3	100	0	
Sum of all eigenvalues					0.273
Sum of all canonical eigenvalues					0.273

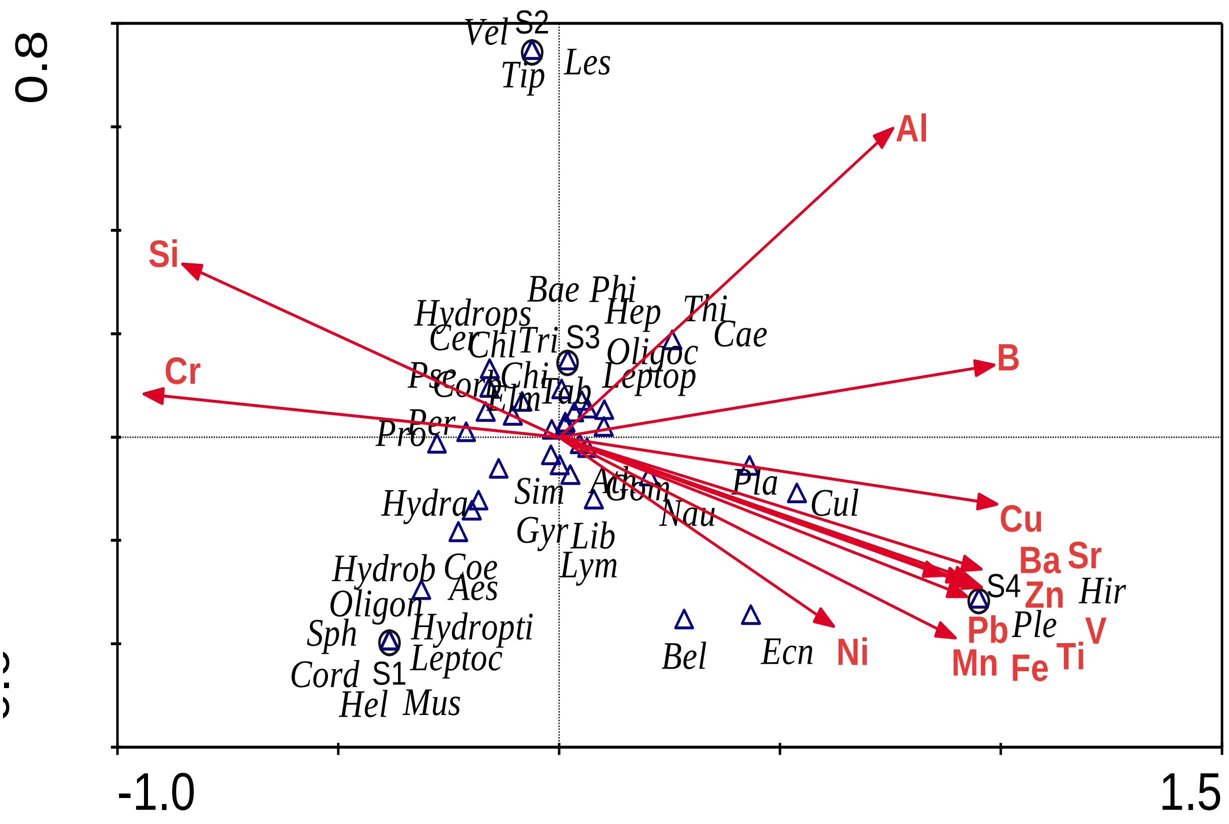

Figure 3.6 CCA plot depicting the relationship between macroinvertebrates and sediment metals

Canonical correspondence analysis results depicting the relationship between macroinvertebrates and sediment metals including metalloids are shown in Figure 3.5. The taxa-environment factor correlation (r) for factor 1 and factor 2 were both 1 (Table 3.5). Pollution tolerant families were mostly prevalent in Site 4 and they include families such as Hirudinea and Pleidae. Additionally, metals such as vanadium, iron, titanium, manganese, lead, nickel, zinc, barium, copper and strontium were strongly correlated with Hirudinea and Pleidae (Figure 3.5). This is evident that the measured environmental variables were efficient in explaining variance in macroinvertebrate assemblages.

3.4 DISCUSSION

Aquatic macroinvertebrates have been recognised to effectively assess and monitor the health of aquatic ecosystems (Dalu et al. 2017). This was because of their fixed mobility, varying tolerance levels to diverse stressors and their numerous life stages (Matlou et al. 2017). Different macroinvertebrates prefer different ranges of biotic and abiotic characteristics such as temperature, pH, flow rate and substrate composition. This implies that any change in their biotic and abiotic preference range due to pollutants can change their community structure even over a minor spatial measure (Rasifudi et al. 2018). Normally the disturbance will be encountered in at least one of its developmental stages which include the egg, larva, pupa and the adult and later these changes will be found in their community structure during sampling (Relyea et al. 2000).

Diptera is the most diverse order, whereas Plecoptera is the least diverse. The ranking from the highest to the lowest diverse order in terms of number of families is as follows: Diptera > Ephemeroptera and Odonata > Tricoptera and Coleoptera> Gastropoda and Hemiptera > Annelida and Pelecypoda > Plecoptera. The order Ephemeroptera was the richest as it had the highest number of individuals 2234. Ephemeropterans are known to be good indicators of water quality (Dalu et al. 2017). Site 1 had the highest number of orders and families (10 and 36 respectively) and contributed 65% of the total number of individuals (4933) sampled. The physicochemical results indicated that Site 1 experienced high nutrient and metal concentrations, which seemed to have no negative impact on the distribution and abundance of macroinvertebrate assemblage at this site. This could be due to the high dissolved oxygen experienced at this site and increased heterogeneity of the area which favoured the sensitive families.

Site 2 had 668 macroinvertebrate individuals contributing only 8% of the total number of individuals sampled. However, the H' diversity was the highest compared to all the sampling sites and this might be due to sufficient habitat diversity and enough dissolved oxygen to accommodate sensitive species. Site 3 had 1669 macroinvertebrate individuals contributing 22% of the total number of individuals sampled. The CCA plot depicting the relationship between physicochemical parameters and macroinvertebrate families indicated that Site 3 is mostly dominated by moderately tolerant families (Figure 3.5). This might be attributed to the lowest DO of 6.8 mg/ℓ recorded at this site.

Site 4 had the least number of taxa with 358 individuals contributing only 5% of the total number of individuals sampled. This could possibly be that nutrients and metals upstream are washed downstream where they are adsorbed on the sediment bed and later become bioavailable, which have negative impact on macroinvertebrate assemblages. The riparian vegetation during field campaigns was negatively impacted by frequent drinking and grazing of livestock. Furthermore, the substrate composition at Site 4 was 75% sand and 20% mud with low nutrient availability and high-water level, which is not conducive for macroinvertebrates (Dallas 2007). This might have contributed to the low number of individuals collected at this site. It is also at the confluence with the Steelpoort River which is known to be impacted (Matlou et al. 2017).

When comparing seasons, winter had the highest number of families and spring had the least number of families. The highest abundance of families collected during winter might be due to high nutrient availability and the low number of taxa during spring might be due low nutrient availability. Spring is the start of raining season in the study area. The family Hydropsychidae had the highest number of individuals with 1875 collected during the survey period. Hydropsychidae are from the order Tricoptera which has been known to be good indicators of oligotrophic conditions and are mostly found in well oxygenated and fast-flowing waters (Oliveira & Callisto 2010).

Regarding EPT taxa richness (Table 3.2), Site1 had the highest EPT value of 12, followed by Site 3 with an EPT of 9, followed by Site 2 with an EPT of 8 and then Site 4 with an EPT value of 6. The highest EPT value in Site 1 might be due to the diversity of substrate types and well aerated water with enough dissolved oxygen to accommodate the most sensitive taxa (Odume et al. 2015). The lowest EPT value at Site 4 might be attributed to habitat degradation because this site had highly modified riparian vegetation. The mean H' diversity values ranged from 2.3 (Site 4) to 2.6 (Site 2) across all the sampling sites. The highest Shannon Weiner (H') diversity value at Site 2, might be attributed to habitat diversity and good water quality which attracted the sensitive taxa.

The results obtained when using SASS5 bioassessment protocol indicated that the SASS score and No. of taxa are decreasing from upstream to downstream. The sequence from the highest SASS score and no. of taxa to the lowest is as follows Site 1> Site 3> Site 2> Site 4 (Table 3.4). This explains that Site 1 had more pollution sensitive species as compared to the other sites. Due to the presence of highly sensitive families such as Perlidae, Oligoneuridae, Prosopistomatidae, Heptageniidae, Helodidae and more than two species of Baetidae and Hydropsychidae, Site 1 can be said to be of better water quality condition compared to the other sites. According to Chutter (1995) interpretation, the SASS scores at Site 2 and Site 3 are greater than 100 with an ASPT of greater than 6 and this can be interpreted as natural water quality and high habitat diversity. There were very few pollution sensitive families collected at Site 4 and this contributed to less SASS score which affected the ASPT value at the site.

When adopting Chutter (1995) interpretation method (Table 3.5), it was observed that Site 1, Site 2 and Site 3 had the SASS Score of greater than 100 and ASPT value of greater than 6, which could be interpreted as water quality being natural and high habitat diversity (Table 3.5). Site 4 had the SASS Score within the range of 50-100 and ASPT value less than 6, thus the site is experiencing some deterioration in water quality (Chutter 1995).

Table 3.6 Interpretation of the SASS5 results (Chutter 1995)

SASS Score	ASPT	Interpretation
>100	>6	Water quality natural, habitat diversity high
<100	>6	Water quality natural, habitat diversity reduced
>100	<6	Borderline between water quality natural and some deterioration interpretation should be based on the extent by which the SASS exceeds 100 and the ASPT <6
50-100	<6	Some deterioration in water quality
<50	Variable	Major deterioration in water quality

At Site 4, during summer, autumn and spring the SASS Score was within the range of 50-100 and the ASPT value was less than 6, which could be interpreted as the site is experiencing deterioration in water quality (Chutter 1995). However, during winter at Site 4 the SASS Score was greater than 100 with an ASPT of greater than 6, thus, water quality is said to be natural and high habitat diversity (Table 3.6). Calculating the ASPT score in the river health assessment is crucial to analyse the health status of the aquatic environment (Dickens & Graham 2002).

The CCA results for physicochemical parameters and macroinvertebrates in axes 1 and axes 2, indicated negative loadings of DO and temperature. These parameters were associated with Leptoceridae, Muscidae, Hydroptilidae, Aeshnidae, Sphaeridae, Oligoneuridae, Hydraenidae and Coenagrionidae. Most families associated with Site 1 were pollution sensitive and pollution tolerant taxa. This might be due to good water quality experienced at this site which accommodated both sensitive taxa and tolerant taxa. Temperature was strongly correlated with pollution tolerant taxa such as Simuliidae, Libellulidae, Lymnaeidae and Gyrinidae. An increase in depth and width was strongly associated with pollution tolerant families such as Hirudinea and Pleidae at Site 4. This indicates that the conditions in the Dwars River is deteriorating downstream.

The CCA results depicting the relationship between macroinvertebrates and sediment metals including metalloids, indicated that at Site 4, the pollution tolerant taxa such as Hirudinea and Pleidae were associated with metals such as vanadium, iron, titanium, manganese, lead, nickel, zinc, barium, copper and strontium. Aluminium and Boron were correlated with families such as Oligochaeta, Leptophlebiidae, Thiaridae and Caenidae at Site 3. Most of the pollution sensitive taxa were associated with upstream of Site 1 while most pollution tolerant taxa were associated with downstream of Site 4.

In summary, the most abundant macroinvertebrates were collected at Site 1 and the least number were collected at Site 4. The highest EPT value was obtained from Site 1 and the lowest EPT value was from Site 4. The highest H' diversity index was obtained at Site 2, while the lowest was at Site 4. The highest SASS score, ASPT and No. of Taxa were at Site 1, while the lowest were at Site 4. Site 1 also had the highest number of sensitive families. This implies that Site 1 had relatively good water quality compared to Site 4. Furthermore, the ASPT and the SASS score results at Site 4 was very low and this was interpreted as some form of deterioration in water quality (Chutter 1995). This deterioration might be attributed to anthropogenic activities such as agricultural runoff and less habitat diversity. The CCA results confirmed that most pollution tolerant taxa were at the downstream site. Thus, the level of pollution is increasing from upstream to downstream sites. Furthermore, the high tolerant taxa at downstream site, which had high metal concentration in the sediment, but relatively good water quality confirms that sediment quality plays an important role in the distribution and diversity of benthic macroinvertebrates.

CHAPTER 4

4. GENERAL DISCUSSION AND CONCLUSION

4.1 Water and sediment quality

4.1.1 Physicochemical variables

Water quality changes can affect the distribution and diversity of aquatic biota and therefore they are used as bioindicators (Rasifudi et al 2018). The *in situ* water quality parameters such as pH, temperature, DO, conductivity, TDS and salinity were measured. One-way ANOVA was used to analyse variance of physicochemical variables among sites and seasons. The results indicated significant variation in most variables among the sites ($p<0.05$), with exception of conductivity. Seasonally, temperature, pH, salinity and TDS were not significantly different ($p>0.05$), However, conductivity and DO were significantly different ($p<0.05$). The water temperature during the study was within acceptable range with a value of 21.4ºC in summer (Site 3) and the minimum value of 17.6ºC in winter (Site 2). Kale (2016) has reported that the temperature patterns are expected to rise during summer and drop during winter seasons. This is because the solubility of oxygen decreases as the water temperature increases (Kale 2016). Toxicity of constituents and the rate of chemical reactions increase as the temperature increase (Dallas 2008).

The SAWQG for aquatic ecosystems has stipulated DO saturation limit of 80%-120% (DWAF 1996). Site 1 had the DO concentration of 9.13 mg/ℓ, while Site 3 had the DO concentration of 6.8 mg/ℓ. According to research the DO values are expected to rise in cooler temperature than in warmer temperature seasons (Kale 2016). In water bodies dissolved oxygen can be acquired through photosynthesis and diffusion during strong turbulence (Araoye 2009). In water bodies dissolved oxygen can be acquired through photosynthesis and diffusion during strong turbulence (Araoye 2009).

The pH was alkaline during the study, which ranged from 7.4 to 8.3 at all sites. Winter had the highest pH record while summer had the lowest pH record. Araoye (2009), has reported that pH fluctuations can directly or indirectly affect the conductivity, transparency, TDS and viscosity. Specifically, the pH is a measure of the concentration of the hydrogen (H+) and hydroxyl (H-) ions and conductivity depends on all ions present in a solution. Thus, a strong basic or acidic solution will have increased conductivity (Araoye 2009). Low pH levels can mobilise metals and dissolved salts which can be lethal to aquatic organisms (Dallas & Day 2004). The increase in pH values can be caused by leaching of calcium and magnesium from the soil.

Turbidity and TDS concentrations were within permissible limits for aquatic ecosystems (DWAF 1996b). However, EC and salinity concentrations were above permissible limits (DWAF 1996a; WHO 2006). The EC values ranged from 294.7-549.7 mS/m at all the sites during the study. Seasonally, the highest record of 559.1 mS/m was obtained during summer and the lowest record of 397.9 mS/m was obtained during autumn. Studies on inland freshwater indicated that conductivity ranging between 15 mS/m and 50 mS/m supports a variety of species (Abah et al. 2018). However, the WHO guideline has stipulated an acceptable limit of 100 mS/m (Ramalepe 2015). The TDS and EC are directly proportional; thus, DWAF (1996a), states that the TDS should not fluctuate by more than 15% from the background level as this could affect the biodiversity of species.

Salinity levels ranged from 0.21‰ at Site 4 to 0.56‰ at Site 1. The highest salinity mean concentration of 0.62‰ was recorded during winter and the lowest salinity mean of 0.2‰ was recorded during spring. Salinity as opposed to TDS, measures the inorganic dissolved content of water (DWAF 1996a). An increase in salinity might be due to tailings water from mines and low rainfall (Canedo-Arguelles et al. 2013). Salinity can affect the physiological and metabolic processes of an organism (Noyes et al. 2009).

Nutrients such as nitrate, nitrite and total nitrogen had the highest concentrations recorded at Site 1, with values above permissible limits (CCME 2012; DWAF 1996a). Phosphorus and nitrogen are regarded as the main nutrients which stimulate growth and when they are available in excess, they can cause eutrophication (Griffin 2017). The inorganic phosphorus was within the range of 0.025 and 0.25 mg/ℓ at all Sites, therefore, indicating eutrophic conditions. Nitrite is intermediate in conversion of ammonia and is known to be toxic to aquatic biota even at low levels (Smith et al. 1999). An increase in nutrients can be caused by industrial wastes, agriculture, and urban runoff. Over application of fertilizers in agriculture may enrich the aquatic systems which can lead to eutrophication and algal blooms (Griffin 2017).

The major ions, such as calcium, potassium and sodium were all within permissible limits (Chapman 1996; DWAF 1996a; c). However, magnesium concentrations at all sites and seasons were above permissible limits (DWAF 1996c). The magnesium concentration ranged from 14.25 mg/ℓ to 38.8 mg/ℓ at all the sites. An increase in magnesium concentration can be caused by weathering of rocks containing ferro-magnesium minerals and carbonate (Chapman 1996). Little is known about the effect of magnesium on the aquatic biota. However, it was reported in literature that when major ions are present in excess, they have the potential to affect the physiology of organisms (Dallas & Day 2004). There was no clear trend of physicochemical parameters observed from upstream to downstream.

4.1.2 Metals in water

Metals and metalloids such as iron, barium, manganese, nickel, strontium, boron and lead were within permissible limits in the water column (CCME 2012; DWAF 1996a; USEPA 2012; WHO 2003; WHO 2004). However, aluminium, chromium and zinc concentrations were above permissible limits (DWAF 1996a). The recorded aluminium concentration ranged from 0.16 mg/ℓ at Site 3 to 0.50 mg/ℓ at Site 1. Chromium concentration varied from 0.001 mg/ℓ at Site 4 to 0.02 mg/ℓ at Site 1. Zinc concentration ranged from 0.04 mg/ℓ at Site 2 and Site 4 to 0.08 mg/ℓ at Site 1. Generally, higher metal concentrations were observed at Site 1 compared to other sites. This might be caused by the intensity of the mining activities in the catchment near this site, coupled with domestic and industrial runoff. Studies have indicated that toxic constituents are detrimental in an aquatic environment, even when they are found in lower concentrations (Edokpayi et al. 2017; Naggar et al. 2018).

4.1.3 Metals in sediments

Copper and chromium in the sediment were above permissible limits (CCME 2012). Copper concentration ranged from 17.1 mg/kg at Site 1 to 48 mg/kg at Site 4. The toxicity of copper decreases when molybdenum, sulphates and zinc are present in an aquatic environment (Dallas & Day 2004). However, at low dose of 0.5 ppm, copper can be toxic to some algae (Dallas & Day 2004). Increased copper concentration might be attributed to industrial effluents and mine tailings runoff. Copper can affect both physiological and biochemical processes, when present in excess in an aquatic environment (Rai et al. 2015).

The recorded chromium concentration ranged from 0.3 mg/g at Site 4 to 5.5 mg/g at Site 1. Chromium can be released from different sources which include chromium plating, metal finishing industries, cooling towers, production of corrosion inhibitors and tanneries (Crafford & Avenant-Oldewage 2011). Chromium (VI) has been recognised as cancer causing constituent (Bojic et al. 2004; Krishnani et al. 2004).

4.2 Macroinvertebrates

4.2.1 Macroinvertebrate richness, abundance and diversity

Whenever pollution occurs in an aquatic system and it affects properties of water (chemical and physical), that alteration will be encountered in at least one of the stages of invertebrate's life cycle (Relyea et al. 2000). This renders aquatic macroinvertebrates as vital biomonitoring tools in rivers (Dalu et al. 2017). Ephemeropterans are known to be indicators of good water quality (Dalu et al. 2017). During the study, Ephemeroptera had the highest number of individuals from the total individuals sampled. However, Diptera was the most diverse order. Site 1 had the highest number of families and orders while Site 4 had the least families and orders. Literature indicates that habitat diversity creates several niches for various organisms (Odume et al. 2015).

The physicochemical results indicated that Site 1 experienced high nutrient and metal concentrations which seemed to have no negative impact on the distribution and abundance of macroinvertebrate assemblage as this site had the highest number of families and orders especially the most sensitive taxa. This could be due to the high level of dissolved oxygen experienced at this site and increased heterogeneity of the area which favoured very sensitive families. Site 4 had the least number of families and orders which could possibly be that the nutrients and metals upstream are washed downstream where they are adsorbed on the sediment bed and later become bioavailable, which have negative impact on macroinvertebrate assemblage especially the most sensitive taxa.

Seasonally, winter had the highest number of individuals collected and spring had the least count of individuals. The highest abundance of individuals collected in winter might be due to the good water quality conditions which is ideal for both sensitive and tolerant taxa (Day et al. 2001). Additionally, spring had an increase in metal concentrations such as aluminium, barium and vanadium which might affect the distribution and abundance of macroinvertebrates. The EPT taxa richness is obtained by the summation of the most sensitive families within the three orders Ephemeroptera, Plecoptera and Tricoptera. Site 1 had the highest EPT value while Site 4 had the lowest EPT value.

The EPT orders are known to have families which reside in cool, clean rivers which have enough dissolved oxygen content (Dalu et al. 2017). Site 2 had the highest H' diversity mean value while Site 4 had the lowest H' diversity value. The highest diversity might be due to suitable water quality conditions for sensitive species and reduced diversity might be due to altered water quality conditions. Altered water quality decreases biodiversity and abundance of species (Dalu et al. 2017; Rasifudi et al. 2018). It is known that good water quality is ideal for both sensitive and tolerant taxa, thus increasing species abundance and diversity (Mangadze et al. 2019).

4.2.2 SOUTH AFRICAN SCORING SYSTEM VERSION5 EDITION (SASS5)

The SASS5 forms the backbone of the River Ecostatus Monitoring Program in South Africa (Vos et al. 2001). Site 1 had the highest SASS score, number of taxa and ASPT while Site 4 had the lowest SASS score, number of taxa and ASPT. Site 1 is interpreted as natural water quality and high habitat diversity due to the highest SASS score and ASPT while Site 4 is interpreted as there is some deterioration in water quality (Chutter (1995). The highest SASS score and ASPT at Site 1 could be due to the highest dissolved oxygen experienced at this site and increased heterogeneity of the area which accommodated very sensitive families. The sensitive families at Site 1 include Perlidae, Oligoneuridae, Prosopistomatidae, Heptageniidae, Helodidae and more than two species of Baetidae and Hydropsychidae.

The results for seasonal variation indicated that during summer and autumn at Site 4, the SASS Score was within 50-100 and the ASPT value was less than 6, an indication of deterioration in water quality (Chutter 1995). Furthermore, during spring at Site 4 the SASS Score was less than 50 and the ASPT value was variable, which was interpreted as major deterioration in water quality (Chutter 1995). However, during winter at Site 4 the SASS Score was greater than 100 with an ASPT greater than 6, which can be interpreted as natural condition and high habitat diversity (Chutter 1995). At Site 1, Site 2 and Site 3 during all the seasons, SASS Score was greater than 100 with an ASPT value greater than 6, which is interpreted as natural water quality and high habitat diversity.

The CCA results showing the relationship between physicochemical parameters and aquatic macroinvertebrates indicated that, high number of macroinvertebrate families were associated with low temperature at Site 1. Increase in salinity, TDS and EC prevailed at Site 2. An increase in depth and width observed at Site 4 was strongly correlated with pollution tolerant taxa such as Simuliidae, Libellulidae, Lymnaeidae and Gyrinidae. The deterioration in water quality at Site 4 (downstream) might possibly be due to nutrients and metals washed downstream from upstream and are adsorbed in the sediment, which later may become bioavailable and impact sensitive families negatively. The CCA results which depict the relationship between macroinvertebrates and sediment metals, indicated that pollution tolerant families were mostly prevalent at Site 4. Families such as Hirudinea and Pleidae were correlated with metals such as vanadium, iron, titanium, manganese, lead, nickel, zinc, barium, copper and strontium. Pollution tolerant families at Site 4 caused the low SASS score and ASPT values, which was interpreted as deterioration in water quality (Chutter 1995).

CONCLUSION

Recent research paper by Magala (2015) has illustrated that there is an increase in nutrient content which affect the aquatic biota in the Dwars River, for this reason during the study the research results clarified that some nutrients (nitrate, nitrate, and total nitrogen) and metals (copper, lead, zinc, barium and titanium) are increased in the Dwars River especially at Site 4. This verifies that the water quality in the Dwars River is strongly influenced by nutrients and metals from anthropogenic activities in the catchment. Hence, the hypothesis was accepted, and the research aims, and objectives were met.

Overall, there is pollution gradient in the Dwars River, as indicated by an increase in water quality constituents such as salinity, EC, nitrate, nitrite, total nitrogen, magnesium, aluminium, chromium, zinc, and copper. During field survey there were signs of algae growth observed at all the sampling sites. Increased nutrient levels in an aquatic environment has been associated with algal bloom which can result in anoxic conditions that can have deleterious effect on the aquatic biota. Based on the SASS5 results, it may be concluded that downstream of Site 4 is mostly modified as compared to the upstream of Site 1. Low biotope diversity and poor water quality were the biggest cause of reduced biodiversity in the Dwars River. The low number of sensitive taxa downstream was the main cause of low SASS scores and ASPT, which is an indication of poor water quality. The increased metal concentration in both water and sediment implies that the contaminants have the potential to pose danger to the aquatic biota and humans when they are bioavailable. In summary, the results indicate that there is anthropogenic impact coupled with leaching of mineral sources of the underlying rocks taking place in the river.

RECOMMENDATIONS AND FUTURE STUDIES

Not enough studies have been conducted in the Dwars River, which implies that biomonitoring of the river should be prioritised to provide more information about the state of the river. Studies on sediment and water quality should be conducted more frequently to monitor the impact of mining and industrial activities in the river catchment. This will ensure protection of the most sensitive species and sustain the health and the integrity of the aquatic ecosystem. Enforcement of government regulations on effluents discharged from mining and industrial sectors. Human activities in the catchment area should be minimised because they are impacting water quality of the river. The frequent monitoring of the system will alert the conservation authorities to take preventative measures to protect and maintain the health of the Dwars River. Further negligence of the Dwars River, will result in more deterioration which will cause extinction of most of the sensitive species and this will collapse the ecosystem, ultimately affecting both humans and animals depending on the river.

REFERENCES

Abah J, Simasiku EK, Nakwaya DN. 2018. Assessment of Zambezi River water quality using macroinvertebrates population diversity. *Journal of Fisheries and Aquatic Science* 13: 12-20.

Addo-Bediako A, Matlou K, Makushu E. 2018. Heavy metal concentrations in water and sediment of the Steelpoort River, Olifants River System, South Africa. *African Journal of Aquatic Science* 43: 413-416.

Ahmed M, Rauf M, Mukhtar Z, Saeed NA. 2017. Excessive use of nitrogenous fertilizers: An unawareness causing serious threats to environment and human health. *Environmental Science and Pollution Research* 24 : 26983-26987.

Alade SM, Abdulazeez SS. 2012. Effects of calcite and dolomite mining on water and soil qualities: A case study of freedom group of companies, Ikpeshi, Edo State Nigeria. *Journal of Emerging Trends in Engineering and Applied Sciences* 3: 19-24.

Alavaisha E, Lyon SW, Lindborg R. 2019. Assessment of water quality across irrigation schemes: A case study of wetland agriculture impacts in Kilombero Valley, Tanzania. Water 11: 671.

Araoye PA. 2009. The seasonal variation of pH and dissolved oxygen (DO_2) concentration in Asa lake Ilorin, Nigeria. *International Journal of Physical Sciences* 4:271-274.

ATSDR (Agency for Toxic Substances and Disease Registry). 1997. *Toxicological profile for titanium chloride*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

ATSDR (Agency for Toxic Substances and Disease Registry). 2007. *Toxicological profile for Lead*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

BC-EPD (British Columbian Environmental Protection Division). 2006. Water quality- a compendium of working water quality guidelines for British Columbia. In: Government of British Columbia Environmental Protection Division. Available http://www.env.gov.bc.ca/wat/wq/BCguidelines/working.html [Accessed 08-06-2018].

Bervoets L, Blust R. 2003. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: Relationship with fish condition factor. *Environmental Pollution* 126: 9-19.

Bojic AL, Purenovic M, Bojic D. 2004. Removal of chromium (VI) from water by micro-alloyed aluminium composite (MAlC) under flow conditions. *Water SA* 30: 353-358.

Bouwman AF, Bierkens MFP, Griffioen J, Hefting MM, Middelburg JJ, Middelkoop H, Slomp CP. 2012. Nutrient dynamics, transfer and retention along the aquatic continuum from land to ocean: Towards integration of ecological and biogeochemical models. *Biogeosciences* 10: 1-16.

Brink I, Kamish W. 2017. Associations between stormwater retention pond parameters and pollutant (suspended solids and metals) removal efficiencies. *Water SA* 1: 45-53.

Camargo JA, Alonso A. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International* 32: 831-849.

Canedo-Arguelles MB, Kefford J, Piscart C, Prat N, Schafer RB, Schulz CJ. 2013. Salinisation of rivers: An urgent ecological issue. *Environmental Pollution* 173: 157-167.

CCME (Canadian Council of Ministers of the Environment). 2012. Environmental quality guidelines: Water quality guidelines for the protection of aquatic life and sediment quality guidelines for the protection of aquatic life. In: Canadian Council of Ministers of the Environment. Available http://ceqg rcqe.ccme.ca/ [Accessed 05-06-2018].

Chapman D. 1996. *Water Quality Assessments- A Guide to Use of Biota. Sediments and Water Monitoring* (2nd edn). Cambridge: Chapman and Hall Publishers. 60 pp.

Chutter FM. 1995: The role of aquatic organisms in the management of river basins for sustainable utilisation. *Water Science and Technology* 32: 283-291.

Chutter FM. 1998. Research on the rapid biological assessment of water quality impacts in streams and rivers. WRC Report No 422/1/98. South Africa: Pretoria, Water Research Commission. 230 pp.

Correia TG, Narcizo AM, Bianchini A, Moreira RG. 2010. Aluminium as an endocrine disruptor in female Nile Tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology C* 151: 461-466.

Crafford D, Avenant-Oldewage A. 2011. Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (Sharptooth catfish) from the Vaal River System-strontium, aluminium, lead and nickel. *Water SA* 36: 621-626.

Dalesman S, Lukowiak K. 2010. Effect of acute exposure to low environmental calcium on respiration and locomotion in Lymnaea stagnalis (L.). *Journal of Experimental Biology* 213: 1471-1476.

Dallas HF, Day JA. 2004. The effect of water quality variables on aquatic ecosystem: A review. WRC Report No. TT224/04. South Africa: Pretoria, Water Research Commission.

Dallas HF, Ross-Gillespie V. 2015. Sublethal effects of temperature on freshwater organisms, with special reference to aquatic insects. *Water SA* 41: 712-26.

Dallas HF. 2007. The influence of biotope availability on macroinvertebrate assemblages in South African Rivers: Implications for aquatic bioassessment. *Freshwater Biology* 52: 370-380.

Dallas, H. 2008. Water temperature and riverine ecosystems: An overview of knowledge and approaches for assessing biotic responses, with special reference to South Africa. *Water SA* 34: 393-401.

Dalu T, Wasserman RJ, Tonkin JD, Mwedzi T, Magoro ML , Weyl OLF. 2017. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream. *Science of the Total Environment* 607: 317-325.

Day JA, Stewart BA, De-moor IJ, Louw AE. 2001. Guides to the freshwater Invertebrates of Southern Africa-volume 3: Crustacea II-Ostracoda, Copepoda and Branchiura. WRC Report No. TT148/01. Water Research Commission, Pretoria, South Africa. 177 pp.

Dickens CW, Graham P. 2002. The South African Scoring System (SASS) version 5 rapid bio-assessment method for rivers. *African Journal of Aquatic Science* 27: 1-10.

Donald, DB. 2017. Trophic decline and distribution of barium in a freshwater ecosystem. *Hydrobiologia* 784: 237-247.

Du-Plessis JA, Schloms B. 2017. An investigation into the evidence of seasonal rainfall pattern shifts in the Western Cape, South Africa. *Journal of the South African Institution of Civil Engineering* 59: 47-55.

DWAF (Department of Water Affairs and Forestry). 1996a. South African Water Quality Guidelines Volume 7: Aquatic Ecosystems. Department of Water Affairs and Forestry, Pretoria, South Africa. 161 pp.

DWAF (Department of Water Affairs and Forestry). 1996b. South African Water Quality Guidelines: (2nd edn.) Volume 7: Aquaculture. Pretoria, South Africa.

DWAF (Department of Water Affairs and Forestry). 1996c. South African Water Quality Guidelines Volume 1: Domestic Water Use. Department of Water Affairs and Forestry, Pretoria, South Africa. 161 pp.

DWAF (Department of Water Affairs and Forestry). 2008. National Aquatic Ecosystem Health Monitoring Programme (NAEHMP): River Health Programme (RHP) Implementation Manual. Version 2. ISBN No. 978-0-621-383343-0. Department of Water Affairs and Forestry, Pretoria, South Africa.

Edokpayi JN, Odiyo JO, Popoola OE, Msagati TA. 2017. Assessment of trace metals contamination of surface water and sediment: A case study of Mvudi River, South Africa. *Sustainability* 8: 135.

Fatoki OS, Lujiza N, Ogunfowokan AO. 2002. Trace metal pollution in Umtata River. *Water SA* 28: 183-190.

Gerber A, Gabriel MJM. 2002. *Aquatic Invertebrates of South African Rivers: Field guide*. Department of Water Affairs and Forestry, Pretoria. 17-78 pp.

Gordon AK, Griffin NJ, Palmer CG. 2014. The relationship between concurrently measured SASS (South African Scoring System) and turbidity data archived in the South African River Health Programme's Rivers Database. *Water SA* 41: 21-25.

Griffin NJ. 2017. The rise and fall of dissolved phosphate in South African Rivers. *South African Journal of Science* 113:1-7.

Haggag AA, Mahmoud MA, Bream AS, Amer MS. 2018. Family variation of aquatic insects and water properties to assess freshwater quality in El-Mansouriya stream, Egypt. *African Entomology* 26: 162-173.

Hamza-Chaffai A., 2014. Usefulness of bioindicators and biomarkers in pollution biomonitoring. *International Journal of Biotechnology for Wellness Industries* 3: 19-26.

Hashim F, Rahman NAA, Amin NM. 2015. Morphological analysis on the toxic effect of manganese on *Acanthamoeba sp*. isolated from Setiu wetland, Terengganu: An in vitro study. *Procedia Environmental Sciences 30*: 15-20.

Jia XX, Varbanov PS, Walmsley TG, Yan Y. 2017. Water pollution impact assessment of Beijing from 2011 to 2015: Implication for degradation reduction. *Chemical Engineering Transactions* 61: 1525-1530.

Jooste A, Marr SM, Addo-Beddiako A, Luus-Powell WJ. 2015. Sharptooth catfish shows its metal: A case study of metal contamination at two impoundments in the Olifants River, Limpopo River System, South Africa. *Ecotoxicology and Environmental Safety* 112: 96-104.

Justice C, White SM, McCullough DA, Graves DS, Blanchard MR. 2017. Can stream and riparian restoration offset climate change impacts to salmon populations? *Journal of Environmental Management* 88: 212-227.

Kale VS. 2016. Consequence of temperature, pH, turbidity and dissolved oxygen water quality parameters. *International Advanced Research Journal in Science, Engineering and Technology* 3: 1-5.

Kalogianni E, Vourka A, Karaouzas I, Vardakas L, Laschou S, Skoulikidis NT. 2017. Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean Intermittent River. *Science of the Total Environment* 604: 639-650.

Koff T, Vandel E, Marzecová A, Avi E, Mikomagi A. 2016. Assessment of the effect of anthropogenic pollution on the ecology of small shallow lakes using the paleolimnological approach. *Estonian Journal of Earth Sciences* 65: 221-233.

Krishnani KK, Parimala V, Meng X. 2004. Detoxification of chromium (VI) in coastal water using lignocellulosic agricultural waste. *Water SA* 30: 541-544.

Kumar R. 2015. *Freshwater Ecosystem Ideas and Actions*. India: Random Publications. 12 pp.

Leske T, Buckley C. 2003. Towards the development of a salinity impact category for South African environmental lifecycle assessments: Part 1- A new impact category. *Water SA* 29: 289-296.

Liu Z, Li Y, Li Z. 2009. Surface water quality and land use in Wisconsin, USA - a GIS approach. *Journal of Integrative Environmental Sciences* 6: 69-89.

Magala M. 2015. Aquatic health assessment of Steelpoort River System, Limpopo, South Africa. Hons thesis, University of Johannesburg, South Africa. 3-13 pp.

Mangadze T, Dalu T, Froneman PW. 2019. Biological monitoring in Southern Africa: A review of the current status, challenges and prospects. *Science of the Total Environment* 648: 1492-1499.

Marchand C, Lalliet-Verges E, Baltzer F, Alberic P, Cossa D, Baillif P. 2006. Heavy metals distribution in mangrove sediments along the mobile coastline of French Guiana. *Marine Chemistry* 98: 1-17.

Marr SM, Mohlala TD, Swemmer A. 2017. The ecological integrity of the lower Olifants River, Limpopo province, South Africa: 2009-2015 - Part A: Olifants River main stem. *African Journal of Aquatic Science* 42: 1-8.

Maseti PP. 2005. Biomonitoring in to contrasting catchments. MSc thesis, Rhodes University, South Africa.

Mathers KL, Chadd RP, Extence CA, Rice SP, Wood PJ. 2016. The implications of an invasive species on the reliability of macroinvertebrate biomonitoring tools used in freshwater ecological assessments. *Ecological Indicators* 63: 23-28.

Matlou K, Addo-Bediako A, Jooste A. 2017. Benthic macroinvertebrate assemblage along a pollution gradient in the Steelpoort River, Olifants River System. *African Entomology* 25: 445-453.

Mondal I, Bandyopadhyay J, Paul AK. 2016. Water quality modeling for seasonal fluctuation of Ichamati River, West Bengal, India. *Modeling Earth Systems and Environment* 2:113.

Morrison G, Fatoki OS, Ekberg A. 2001. Assessment of the impact of point source pollution from the Keiskammahoek sewage treatment plant on the Keiskamma

River-pH, electrical conductivity, oxygen-demanding substances (COD) and nutrients. *Water SA* 27: 475-480.

Mosase E, Ahiablame L. 2018. Rainfall and temperature in the Limpopo River basin, Southern Africa: Means, variations and trends from 1979 to 2013. *Water* 10: 364.

Munir T, Hussain M, Naseem S. 2016. Water pollution, a menace of freshwater biodiversity: Review. Journal of Entomology and Zoology Studies 4: 578-580.

Mustafa S, Sibel S, Mustafa T, Durali M. 2005. Determination of trace metals in mushroom samples from Kayseri, Turkey. *Food Chemistry* 92: 649-652.

Naggar YAl, Khalil MS, Ghorab MA. 2018. Environmental pollution by heavy metals in the aquatic ecosystems of Egypt. *Open Access Journal of Toxicology* 3:555.

Naidoo K. 2005. The anthropogenic impacts of urbanisation and industrialisation on water quality, ecology and health status of the Palmiet River catchment in Durban, Kwazulu-Natal. MSc thesis, University of Kwazulu-Natal, South Africa. 14-15 pp.

Noyes PD, McElwee MK, Miller HD, Clark BW, Van-Tiem LA, Walcott KC, Erwin KN, Levin ED. 2009. The toxicology of climate change: Environmental contaminants in a warming world. *Environment International* 35: 971-986.

Oberholster PJ, Botha A, Chamier J, De-Klerk AR. 2013. Longitudinal trends in water chemistry and phytoplankton assemblage downstream of the rivers WWTP in the upper Olifants River. *Ecohydrology and Hydrobiology* 13: 41-51.

Oberholster PJ, Myburgh JG, Ashton PJ, Coetzee J, Botha AM. 2011. Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. *Ecotoxicology and Environmental Safety* 75: 134-141.

Odume ON, Muller WJ. 2011. Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River. *Physics and Chemistry of the Earth* 36: 929-938.

Odume ON, Palmer CG, Arimoro FO, Mensah PK. 2015. Influence of selected biotopes on chironomid-based bioassessment of the Swartkops river, Eastern Cape, South Africa. *Water SA* 41:343–358.

Oelofse S. 2008. *Emerging issues paper: Mine water pollution*. ISBN NO. 978-0-9814178-5-1. Pretoria: Department of Environmental Affairs and Tourism.

Oforka NC, Osuji LC, Onwuachu UI. 2012. Assessment of heavy metal pollution in muscles and internal organs of chickens raised in Rivers State, Nigeria. *Journal of Emerging Trends in Engineering and Applied Sciences* 3: 406-411.

Okeyo A, Nontongana N, Fadare T, Okoh A. 2018. Vibrio species in wastewater final effluents and receiving watershed in South Africa: Implications for Public Health. *International Journal of Environmental Research and Public Health* 15: 1266.

Oliveira A, Callisto M. 2010. Benthic macroinvertebrates as bioindicators of water quality in an Atlantic forest fragment. *Iheringia Série Zoologia* 100: 291-300.

Oliveira VM, Assis CR, Costa HM, Silva RP, Santos JF, Carvalho-Jr LB. 2016. Aluminium-Sulfate exposure: A set of effects on hydrolases from brain, muscle and digestive tract of juvenile Nile tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology* 191:101-108.

Owusu-Sekyere E, Scheepers ME, Jordaan H. 2016. Water footprint of milk produced and processed in the South Africa: Implications for policymakers and stakeholders along the dairy value chain. *Water SA* 8: 1-2.

Potasznik A, Szymczyk S. 2015. Magnesium and calcium concentrations in the surface water and bottom deposits of a river-lake system. *Journal of Elementology* 20: 677-692.

Rai AN, Ullah A, Haider J. 2015. Determination of acute toxicity of copper and cobalt for *Tilapia nilotica. Journal of Bioresource Management 2: 16-25.*

Ramalepe TP. 2015. Effects of pollution and metazoan parasites on the health and oxidative stress biomarkers of two cyprinid fish species in the Olifants River System, South Africa. MSc thesis, University of Limpopo, South Africa.

Ramollo PP. 2008. Bioassessing the impact of water quality on the health and parasite composition of *Oreochromis mossambicus* at the Phalaborwa industrial complex (PIC) and the barrage (Olifants River) in the Limpopo province, South Africa. MSc thesis, University of Limpopo, South Africa.

Rashid I, Romshoo SA. 2012. Impact of anthropogenic activities on water quality of Lidder River in Kashmir Himalayas. *Environmental Monitoring Assessment* 185: 4705-4719.

Rasifudi L, Addo-Bediako A, Bal K, Swemmer TM. 2018. Distribution of benthic macroinvertebrates in the Selati River of the Olifants River System, South Africa. *African Entomology* 26: 398-406.

Relyea CD, Minshall GW, Danehy RJ. 2000. Stream insects as bioindicators of fine sediment. *Proceedings of the ASCE Watershed Management 2000 Conference. Ft. Collins*. Colorado: United States of America.

Santoyo G, Hernández-Pacheco C, Hernández-Salmerón J, Hernández-León R. 2017. The role of abiotic factors modulating the plant-microbe-soil interactions: Toward sustainable agriculture: A review. *Spanish Journal of Agricultural Research* 15: 1-11.

Smith MJ, Kay WR, Edward DHD, Papas PJ, Richardson KJ, Simpson JC, Pinder AM, Cale DJ, Howitz PHJ, Davis JA, Yung FH, Norris RH, Halse SA. 1999. AusRivAS: Using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwater Biology* 41: 269-282.

Struijs J, Beusen A, De-Zwart D, Huijbregts M. 2010. Characterisation factors for inland water eutrophication at the damage level in lifecycle impact assessment. *International Journal of Life Cycle Assessment* 16: 59-64.

Szczerbinska N, Gatczynska M. 2015. Biological methods used to assess surface water quality. *Archives of Polish fisheries* 24:185-196.

Ter Braak CJF, Šmilauer P. 2012. *Canoco reference manual and user's guide*: Software for ordination, version 5.0. Microcomputer Power, Ithaca, NY. 118.

Teske PR, Wooldridge TH. 2004. Affinities of some common estuarine macroinvertebrates to salinity and sediment type: Empirical data from Eastern Cape estuaries, South Africa. *African Zoology* 39: 183-192.

Tredoux G, Cavé L, Engelbrecht P. 2004. Groundwater pollution: Are we monitoring appropriate parameters. *Water SA* 30: 662-667.

USEPA (United States Environmental Protection Agency). 2008. Health effects support document for boron. Health and Ecological Criteria Division. Washington DC 20460. Available www.epa.gov/safewater/ccl/pdf/boron.pdf [Accessed 20 January 2019].

USEPA (United States Environmental Protection Agency). 2012. National recommended water quality criteria: Aquatic life. Available http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm [Accessed 13 February 2019].

Vos P, Wepener V, Cyrus DP. 2001. Efficiency of the SASS4 rapid bioassessment protocol in determining river health: A case study on the Mhlathuze River, KwaZulu-Natal, South Africa. *Water SA* 28: 13-22.

Wepener V, Vermeulen LA. 2005. A note on the concentration and bioavailability of selected metals in sediment of Richards Bay Harbour, South Africa. *Water SA* 31: 589-595.

WHO (World Health Organisation). 2003. Background document for preparation of WHO Guidelines for drinking-water quality: Antimony in drinking water. WHO/SDE/WSH/03.04/74. Geneva. 22 pp.

WHO (World Health Organisation). 2004. Barium in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/76. Geneva. 21 pp .

WHO (World Health Organization). 2006. Guidelines for drinking water quality. Environmental Health Criteria. Geneva. 132 pp.

Wolmarans CT, Kemp M, De-Kock KN, Boets W, Van Rensburg L, Quinn L. 2014. A semi-quantitative survey of macroinvertebrates at selected sites to evaluate the ecosystem health of the Olifants River. *Water SA* 40: 245-254.

Younus M, Abbas T, Zafar M, Raza S, Khan A, Saleem AH, Idrees MA, Nisa QU, Akhtar R, Saleem G. 2016. Assessment of heavy metal contamination in raw milk for human consumption. *South African Journal of Animal Sci*ence 46: 16-169.

Zengeni R, Kakembo V, Nkongolo N. 2016. Historical rainfall variability in selected rainfall stations in Eastern Cape, South Africa. *South African Geographical Journal* 98: 118-137.

Zhou Z, Huang T, Ma W, Li Y, Zeng K, 2015. Impacts of water 589 quality variation and rainfall runoff on Jinpen Reservoir, in 590 Northwest China. *Water Science and Engineering* 8: 301-308.

APPENDIX A: WATER AND SEDIMENT QUALITY

Table 1: Seasonal variations of water quality parameters from the four sampling sites in the Dwars River.

Water Quality Parameters	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
Water temperature	27.9	22	26.8	24.3	16.5	13.3	17.3	19.2	14.7	11.6	14.6	15.8	26.1	23.6	26.7	23.8
DO (mg/ℓ)	7.13	6.04	7.39	9.06	10.16	7.31	5.69	6.19	5.25	4.38	5.05	4.96	8.73	7.52	5.59	7.7
pH	8.02	7.82	8.34	7.54	7.46	8.04	7.94	7.56	9.2	8.69	8.94	9.06	8.32	7.71	8.1	7.64
Conductivity (mS/m)	678	605	631	322.2	441.1	424	483.9	242.5	490	455.5	496	346.6	549	527	588	267.3
TDS (mg/ℓ)	416	416	396	212.6	341.9	354.9	369.2	176.8	6.28	12.78	6.32	11.6	344.5	351	370.5	181.4
Salinity (‰)	0.93	0.31	0.29	0.15	0.26	0.27	0.28	0.13	0.82	0.58	0.65	0.46	0.25	0.26	0.28	0.13
Calcium (mg/ℓ)	42	41	37	24	37	38	39	25	38	39	38	30	38	36	35	24
Magnesium (mg/ℓ)	45	45	40	12	34	35	40	14	38	38	37	20	33	34	38	11
Potassium (mg/ℓ)	1.9	1.7	1.9	1.7	0.8	0.8	1.1	1.6	1.1	1.4	1.2	1.6	0.7	0.8	0.7	1.7
Sodium (mg/ℓ)	27	25	24	12	18	19	22	13	20	20	21	17	18	21	22	12
Nitrate (mg/ℓ)	22	19	17	0.2	15	15	14	0.9	12.5	10.4	12.2	4.6	-	-	-	-
Nitrite (mg/ℓ)	0.46	<0.05	<0.05	<0.05	0.38	<0.05	<0.05	<0.05	0.2	0.03	0.03	0.02	0.3	-	-	-
Phosphate (mg/ℓ)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.04	0.05	0.04	0.04	-	-	-	-
Ammonia (mg/ℓ)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.04	-	0.04	0.04	-	-	-	-
Total Nitrogen (mg/ℓ)	22.06	19	17	0.2	15	15	14	0.9	12.58	10.43	12.27	4.66	-	-	-	-
Turbidity (NTU)	-	-	-	-	3.8	1	2.7	3.1	16	<25	17	<25	-	-	-	-

Table 2: Seasonal variations of metals and metalloids recorded from the water column of the Dwars River.

	Summer				Autumn				Winter				Spring			
Metals(mg/ℓ)	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
Aluminium	1,36	0,48	0,16	0,24	0,13	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	0,12	< 0.10	< 0.10	0,12
Iron	1,41	0,53	0,17	0,5	0,12	< 0.03	0,09	0,14	0,04	0,11	0,08	0,1	0,15	0,08	0,08	0,24
Titanium	0,04	0,04	0,03	0,02	0,03	0,04	0,03	0,02	0,05	0,05	0,05	0,01	0,03	0,02	0,03	0,02
Barium	0,04	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,28	0,08	0,02	0,04	0,02	0,02	0,02	0,03
Manganese	0,14	0,04	< 0.03	0,05	< 0.03	< 0.03	< 0.03	0,03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03
Nickel	0,02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.01	< 0.01	< 0.01	< 0.01
Vanadium	0,01	0,01	0,01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0,01	0,01	0,01	0	0,01	0,01	0,01	< 0.01
Chromium	0,03	0,01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0	0	0	0	< 0.01	< 0.01	< 0.01	< 0.01
Strontium	0,19	0,19	0,17	0,1	0,16	0,16	0,16	0,1	0,15	0,15	0,15	0,11	0,17	0,18	0,18	0,11
Zinc	0,27	0,12	0,12	0,09	0,03	0,02	0,05	0,05	0,02	0,02	0,01	0,01	0,01	0,01	0,02	0,02
Boron	0,02	0,01	0,02	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001	0,02	0,01	0,01	0,01

Table 3: Seasonal variations of sediment metals and metalloids (mg/kg or mg) recorded from the Dwars River

	Summer				Autumn				Winter				Spring			
Metals	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
Aluminium	18698,2	20188,8	39109,6	35729,7	56558,0	48538,6	56694,6	62917,7	23942,5	34962,9	13839,3	9921,0	30702,4	36819,2	30942,8	34150,9
Barium	24,8	24,8	183,1	191,6	136,7	104,3	171,2	514,2	69,1	77,8	17,2	36,3	146,9	160,5	119,8	233,1
Boron	0,0	0,0	16,0	4,0	0,0	0,0	0,0	0,0	22,4	70,3	67,2	160,5	0,0	0,0	0,0	0,0
Chromium	7931,3	8265,5	1886,4	231,1	7018,1	6361,6	4782,8	394,3	3287,1	2428,7	1571,0	227,1	3719,0	2723,2	3150,3	530,3
Copper	3,2	8,4	55,2	32,7	34,6	30,1	26,0	28,1	10,4	17,2	7,2	71,5	19,8	27,8	13,2	59,9
Iron	61707,0	63868,7	58453,0	162591,2	60418,0	56835,3	53954,9	92668,7	42399,0	46014,8	58963,2	310845,1	62461,4	71616,4	70348,3	251273,9
Manganese	1482,0	1524,2	1157,5	1275,0	1218,0	1332,3	1193,6	1101,1	1545,1	1297,4	1601,0	2521,0	1463,8	1434,8	1708,5	2166,5
Nickel	476,8	453,9	219,1	84,2	364,8	397,5	269,3	72,2	634,2	633,1	766,9	1812,0	380,5	354,7	430,2	138,6
Strontium	48,4	48,3	108,4	73,5	92,5	73,7	159,4	210,5	84,3	78,2	31,6	24,4	117,1	127,5	98,0	137,5
Titanium	1853,3	3110,6	4279,5	32235,5	1845,3	2163,5	4293,3	14660,6	1361,4	1542,1	1256,3	65744,1	2061,4	4564,3	2253,7	43432,1
Vanadium	167,1	227,1	0,0	1252,7	135,5	156,5	218,9	442,8	144,6	125,0	124,0	2431,1	172,9	319,3	162,0	2428,6
Zinc	0,0	0,0	0,0	0,0	0,0	0,0	60,3	82,7	65,1	68,3	82,0	863,9	55,3	94,5	75,3	233,8
Lead	0,8	1,6	2,8	4,4	5,5	4,6	4,8	5,3	3,6	5,2	1,6	2,4	2,4	2,4	2,3	4,5

APPENDIX B: MACROINVERTEBRATES BIOMONITORING

Table 1: The total count of invertebrate individuals within families per site and season recorded from the Dwars River

	Summer				Autumn				Winter				Spring			
Families	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	4	5	2	3	9	4	2	4	0	0	0	0	0	3	2	1
Hirudinea	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Perlidae	1	1	1	0	0	0	1	0	1	1	0	0	8	0	0	0
Baetidae 1sp	0	8	0	0	47	0	0	0	0	0	0	0	0	0	3	3
Baetidae 2sp	31	0	64	6	0	0	0	10	0	0	6	0	0	3	0	0
Baetidae >2sp	0	0	0	0	0	20	118	0	240	71	0	30	150	0	0	0
Caenidae	54	3	129	6	13	7	87	8	194	64	126	85	84	9	53	18
Heptageniidae	2	10	6	0	0	1	2	1	6	2	2	4	8	0	3	0
Leptophlebiidae	29	14	63	1	10	15	17	4	57	30	10	23	51	20	30	1
Oligoneuridae	3	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0
Prosopistomatidae	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0
Tricorythidae	2	0	15	0	2	3	2	0	0	0	0	0	21	5	1	0
Chlorocyphidae	0	3	5	0	0	2	6	0	3	1	2	2	7	2	6	0
Coenagrionidae	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lestidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aeshnidae	0	0	0	0	0	0	0	0	23	0	1	0	0	0	0	0
Corduliidae	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0
Gomphidae	27	5	13	3	34	5	7	10	316	10	40	27	42	8	15	1
Libellulidae	12	1	37	1	25	1	8	4	279	4	1	10	48	1	1	1
Belostomatidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Naucoridae	2	0	0	0	0	1	2	4	0	0	0	0	1	0	0	0
Pleidae	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Veliidae	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecnomidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2
Hydropsychidae 1sp	0	0	0	5	0	0	63	3	0	0	0	0	0	8	3	0
Hydropsychidae 2sp	160	21	191	0	0	61	0	0	0	0	0	22	272	0	0	0
Hydropsychidae >2sp	0	0	0	0	211	0	0	0	813	29	13	0	0	0	0	0
Philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Leptoceridae	2	0	0	0	1	0	0	0	3	0	0	0	5	0	0	0

Table 1: Continued

Elmidae	82	4	96	3	133	18	98	3	127	0	4	3	128	9	6	0
Gyrinidae	0	0	2	0	0	0	1	0	12	2	0	2	5	1	0	0
Helodidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0
Psephenidae	41	5	37	0	16	5	40	0	27	0	2	0	78	2	12	0
Athericidae	10	3	4	0	1	0	2	0	8	1	4	6	11	2	1	0
Ceratopogonidae	8	1	27	0	40	31	14	0	34	5	0	0	3	2	0	0
Chironomidae	7	0	19	0	16	30	47	1	202	14	2	0	57	2	0	1
Culicidae	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
Simuliidae	1	0	7	0	16	1	4	0	256	10	1	10	0	0	0	0
Tabanidae	49	5	28	1	33	14	8	0	112	15	2	11	21	5	13	0
Tipulidae	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0
Lymnaeidae	1	0	0	1	1	0	4	0	0	0	0	0	0	0	0	0
Planorbinae	0	0	1	1	0	0	2	1	0	0	0	0	0	0	0	0
Thiaridae	0	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0
Corbiculidae	23	4	2	0	20	4	8	0	3	1	0	1	12	2	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 2: Presence and absence of macroinvertebrates families at each sampling site in the Dwars River

	Site 1			
Taxon	July 2017	Nov 2017	Feb 2018	May 2018
Oligochaeta	x	x	@	@
Hirudinea	@	@	@	@
Perlidae	x	@	x	x
Baetidae 1sp	@	x	@	@
Baetidae 2sp	x	@	@	@
Baetidae >2sp	@	@	x	x
Caenidae	x	x	x	x
Heptageniidae	x	@	x	x
Leptophlebiidae	x	x	x	x
Oligoneuridae	x	x	@	@
Prosopistomatidae	@	@	@	x
Tricorythidae	x	x	@	x
Chlorocyphidae	@	@	x	x
Coenagrionidae	x	@	@	@
Lestidae	@	@	@	@
Aeshnidae	@	@	x	@
Corduliidae	@	x	@	x
Gomphidae	x	x	x	x
Libellulidae	x	x	x	x
Belostomatidae	x	@	@	@
Naucoridae	x	@	@	x
Pleidae	@	@	@	@
Veliidae	@	@	@	@
Ecnomidae	@	x	@	@
Hydropsychidae 1sp	@	@	@	@
Hydropsychidae 2sp	x	@	@	x
Hydropsychidae >2sp	@	x	x	@
Philopotamidae	@	@	@	@
Hydroptilidae	@	@	@	x
Leptoceridae	x	x	x	x
Elmidae	x	x	x	x
Gyrinidae	@	@	x	x
Helodidae	@	@	x	@
Hydraenidae	@	x	@	@
Psephenidae	x	x	x	x
Athericidae	x	x	x	x
Ceratopogonidae	x	x	x	x
Chironomidae	x	x	x	x
Culicidae	@	@	@	@
Muscidae	@	@	x	@
Simuliidae	x	x	x	@
Tabanidae	x	x	x	x
Tipulidae	@	@	@	@
Hydrobiidae	@	@	x	@
Lymnaeidae	x	x	@	@
Planorbinae	@	@	@	@
Thiaridae	@	@	@	@
Corbiculidae	x	x	x	x
Sphaeriidae	@	@	x	@

''X'' denotes family presence

''@'' denotes family absence

Table 2: Continued

Taxon	Site 2			
	July 2017	Nov 2017	Feb 2018	May 2018
Oligochaeta	x	x	@	x
Hirudinea	@	@	@	@
Perlidae	x	@	x	@
Baetidae 1sp	x	@	@	@
Baetidae 2sp	@	@	@	x
Baetidae >2sp	@	x	x	@
Caenidae	x	x	x	x
Heptageniidae	x	x	x	@
Leptophlebiidae	x	x	x	x
Oligoneuridae	@	@	@	@
Prosopistomatidae	@	@	@	x
Tricorythidae	@	x	@	x
Chlorocyphidae	x	x	x	x
Coenagrionidae	@	@	@	@
Lestidae	x	@	@	@
Aeshnidae	@	@	@	@
Corduliidae	@	@	@	@
Gomphidae	x	x	x	x
Libellulidae	x	x	x	x
Belostomatidae	@	@	@	@
Naucoridae	@	x	@	@
Pleidae	@	@	@	@
Veliidae	x	@	@	@
Ecnomidae	@	@	@	@
Hydropsychidae 1sp	@	@	@	x
Hydropsychidae 2sp	x	x	@	@
Hydropsychidae >2sp	@	@	x	@
Philopotamidae	@	@	@	@
Hydroptilidae	@	@	@	@
Leptoceridae	@	@	@	@
Elmidae	x	x	@	x
Gyrinidae	@	@	x	x
Helodidae	@	@	@	@
Hydraenidae	@	@	@	@
Psephenidae	x	x	@	x
Athericidae	x	@	x	x
Ceratopogonidae	x	x	x	x
Chironomidae	@	x	x	x
Culicidae	@	@	@	@
Muscidae	@	@	@	@
Simuliidae	@	x	x	@
Tabanidae	x	x	x	x
Tipulidae	@	x	@	@
Hydrobiidae	@	@	@	@
Lymnaeidae	@	@	@	@
Planorbinae	@	@	@	@
Thiaridae	x	@	@	@
Corbiculidae	x	x	x	x
Sphaeriidae	@	@	@	@

''X'' denotes family presence

''@'' denotes family absence

Table 2: Continued

	Site 3			
Taxon	July 2017	Nov 2017	Feb 2018	May 2018
Oligochaeta	x	x	@	x
Hirudinea	@	@	@	@
Perlidae	x	x	@	@
Baetidae 1sp	@	@	@	x
Baetidae 2sp	x	@	x	@
Baetidae >2sp	@	x	@	@
Caenidae	x	x	x	x
Heptageniidae	x	x	x	x
Leptophlebiidae	x	x	x	x
Oligoneuridae	x	@	@	@
Prosopistomatidae	@	@	@	@
Tricorythidae	x	x	@	x
Chlorocyphidae	x	x	x	x
Coenagrionidae	x	@	@	@
Lestidae	@	@	@	@
Aeshnidae	@	@	x	@
Corduliidae	@	@	@	@
Gomphidae	x	x	x	x
Libellulidae	x	x	x	x
Belostomatidae	@	@	@	@
Naucoridae	@	x	@	@
Pleidae	@	@	@	@
Veliidae	@	@	@	@
Ecnomidae	@	@	@	@
Hydropsychidae 1sp	@	x	@	x
Hydropsychidae 2sp	x	@	@	@
Hydropsychidae >2sp	@	@	x	@
Philopotamidae	@	@	@	x
Hydroptilidae	@	@	@	@
Leptoceridae	@	@	@	@
Elmidae	x	x	x	x
Gyrinidae	x	x	@	@
Helodidae	@	@	@	@
Hydraenidae	@	x	@	@
Psephenidae	x	x	x	x
Athericidae	x	x	x	x
Ceratopogonidae	x	x	@	@
Chironomidae	x	x	x	@
Culicidae	@	x	@	@
Muscidae	@	@	@	@
Simuliidae	x	x	x	@
Tabanidae	x	x	x	x
Tipulidae	@	@	@	@
Hydrobiidae	@	@	@	x
Lymnaeidae	@	x	@	@
Planorbinae	x	x	@	@
Thiaridae	x	@	@	@
Corbiculidae	x	x	@	@
Sphaeriidae	@	@	@	@

''X'' denotes family presence

''@'' denotes family absence

Table 2: Continued

	Site 4			
Taxon	July 2017	Nov 2017	Feb 2018	May 2018
Oligochaeta	x	x	@	x
Hirudinea	x	@	@	x
Perlidae	@	@	@	@
Baetidae 1sp	@	@	@	x
Baetidae 2sp	x	x	@	@
Baetidae >2sp	@	@	x	@
Caenidae	x	x	x	x
Heptageniidae	@	x	x	@
Leptophlebiidae	x	x	x	x
Oligoneuridae	@	@	@	@
Prosopistomatidae	@	@	@	@
Tricorythidae	@	@	@	@
Chlorocyphidae	@	@	x	@
Coenagrionidae	@	@	@	@
Lestidae	@	@	@	@
Aeshnidae	@	@	@	@
Corduliidae	@	@	@	@
Gomphidae	x	x	x	x
Libellulidae	x	x	x	x
Belostomatidae	@	@	@	x
Naucoridae	@	x	@	@
Pleidae	x	@	@	@
Veliidae	@	@	@	@
Ecnomidae	@	@	@	x
Hydropsychidae 1sp	x	x	@	@
Hydropsychidae 2sp	@	@	x	@
Hydropsychidae >2sp	@	@	@	@
Philopotamidae	@	@	@	@
Hydroptilidae	@	@	@	@
Leptoceridae	@	@	@	@
Elmidae	x	x	x	@
Gyrinidae	@	@	x	@
Helodidae	@	@	@	@
Hydraenidae	@	@	@	@
Psephenidae	@	@	@	@
Athericidae	@	@	x	@
Ceratopogonidae	@	@	@	@
Chironomidae	@	x	@	x
Culicidae	@	x	@	@
Muscidae	@	@	@	@
Simuliidae	@	@	x	@
Tabanidae	x	@	x	@
Tipulidae	@	@	@	@
Hydrobiidae	@	@	@	@
Lymnaeidae	x	@	@	@
Planorbinae	x	x	@	@
Thiaridae	x	@	@	@
Corbiculidae	@	@	x	@
Sphaeriidae	@	@	@	@

''X'' denotes family presence

''@'' denotes family absence