

ASSESSMENT OF THE IMPACT OF WATER AND SEDIMENT QUALITY ON
AQUATIC MACROINVERTEBRATE ASSEMBLAGES IN THE BLYDE RIVER OF
THE OLIFANTS RIVER SYSTEM, LIMPOPO PROVINCE

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DECLARATION

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

.....

Ms KC Malakane

.....

Date

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Abstract

The Olifants River is one of the major river systems in South Africa, providing water for industries, mines, agricultural and domestic activities. Consequently, the river has turned out to be one of the most polluted rivers in South Africa. The Blyde River is an important tributary of the Olifants River, known for its continuous flow and good water quality. However, the recent increase in human activities such as, commercial agricultural and large-scale forestry practices in the catchment may have a negative impact on the quality of the water, which may consequently affect the aquatic biota community structure. The aim of the study was to assess the water and sediment quality of the Blyde River using aquatic macroinvertebrates as bioindicators and the South African Scoring System version 5 (SASS5) protocol was followed.

Seven sampling sites were selected at Blyde River and five samples were collected per site. Collected macroinvertebrates were identified to family level and classified according to their tolerance levels to pollution at the University of Limpopo Biodiversity Laboratory. In addition, seasonal readings of the physico-chemical parameters such as, pH, dissolved oxygen (DO), temperature, salinity, total dissolved solutes (TDS) and electrical conductivity (EC) were recorded *in situ* using YSI Model 554 Datalogger with a 4 m multiprobe and a Mettler Toledo SevenGo™. Most of the physico-chemical parameters recorded at Blyde River were within the South African water quality guidelines for aquatic ecosystems.

Metals such as, As, Al, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Sr, Sn, Ti, V, and Zn were tested from the water column, sediments and macroinvertebrate tissue. Almost all the metals analysed from the water column and sediments were within the quality guidelines. Out of the twenty tested metals, As, Ag, Cu, and Zn exceeded the recommended quality guidelines in the water column at some sites. Only As, Cr and Cu exceeded the recommended quality guidelines in the sediments.

The most abundant macroinvertebrates were from the orders Ephemeroptera, Diptera, and Trichoptera at all sites. There was a similar macroinvertebrate community structure across the sites, supported by the positive correlation analysis among the sites ($r > 0.4$). Wildlife Estate and Wildrivers Estate had the highest and lowest numbers of sensitive macroinvertebrates respectively. The highest percentage of

sensitive macroinvertebrate taxa was recorded in winter and the highest percentage of tolerant species was recorded in summer. This was also supported by the SASS indices, the SASS score, number of taxa and average score per taxon (ASPT) were highest in winter, followed by autumn, spring and lowest in summer. The overall SASS indices showed that the water quality condition at most of the sites at Blyde River could be considered as good. All the seven sites on average fell under ecological band A and B, except for the Wildrivers Estate, thus, the water quality status of the river could be described as unmodified and largely natural with few modifications.

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Chapter 1

1.1 Introduction

The growing human population, together with increasing urbanisation, industrialisation and the high demand for food has a negative impact on the environment (Odume 2011). Human activities over the years have led to an overexploitation of freshwater resources and habitat loss for aquatic organisms. Freshwater is one of the most important but limited resources in the world. Ninety-seven percent of the earth's water is salt water, only the remaining 3% is freshwater and it is not evenly distributed (Kale 2016). Out of the 3%, only about 1% is available and accessible for use to the world's population in the form of rivers, lakes, and ponds (Davies & Day 1998, Kale 2016).

Water has unique values and therefore needs to be available to its users in good quality and quantity (Davies & Day 1998). Water quality is defined as the biological, physical, chemical and the aesthetic properties of water that determines its fitness for different uses and for the health and integrity of aquatic ecosystems (DWA 1996). However, many water sources in the world are polluted to an extent that they are no longer fit for their intended purpose (Munyika *et al.* 2014). Anthropogenic activities in river catchments have mostly been associated with the deterioration of the ecological integrity and water quality of the rivers (Al-Shami *et al.* 2010). Though freshwater is a scarce resource, it is being contaminated at a faster rate in many countries due to the increasing population, industrialisation, and agriculture (Munyika *et al.* 2014).

To a certain extent any waterbody is capable of assimilating wastes without serious impacts because of self-purification and dilution (Field *et al.* 2011). If more wastes are deposited in the system, more than the system can dilute then, ecological damage will occur (Field *et al.* 2011). The cleansing capacity of the water resources is getting overwhelmed due to the increased waste generation from the increased human activities. Some wastes may be degraded by natural self-purification and dilution while some are not (Carpenter *et al.* 1998). Contaminants such as, trace and heavy metals are persistent, they are not affected by natural purification, therefore, their presence in the aquatic systems may limit the use and pose a threat to aquatic organisms (Sanders 1993, Dallas & Day 2004). Some African countries have already passed the threshold of water stress, whereby their economic development is already threatened due to lack of water supply (Ashton 2002). The rapid population growth in Africa may

lead many countries to long-term water shortage by the year 2025 (Ashton 2002). The water shortage issues in the continent are further fuelled by pollution, lack of management strategies and political instabilities (Odume 2011). The challenges of water quantity and quality in Africa may pose a serious threat to the economy of many southern and northern countries, where continuous variable and erratic rainfall patterns, extreme temperatures and high evaporation rates are experienced.

South Africa is a semi-arid country with a rapidly increasing population (DWAF 1991, DWAF 2004). The country is facing a shortage in water supply due to the low rainfall and high evaporation rates (Vörösmarty *et al.* 2010). There is a high-water demand for agriculture, mining, industrialisation, recreational and domestic uses. The limited nature of freshwater in South Africa makes this resource very much important in terms of sustainable economic and social development (Van Vuren *et al.* 1994. Basson & Rossouw 2003). Most of the rivers in South Africa are already water stressed, modified and vulnerable to change (Ashton & Dabrowski 2011). It has been predicted that South Africa will be facing serious water scarcity by 2030, because of its climate, water demand, growing population, and pollution rate (Odume 2011). River systems are being exploited through damming, channeling and via direct abstraction of water to meet the people's needs (Davies & Day 1998). The overexploitation has severe effects on the aquatic organisms and the ecological integrity (Odume 2011).

The Olifants River is one of the major river systems in South Africa, which provides water for industries, mining, agricultural and domestic uses (Machete *et al.* 2004). However, due to the various activities in its catchment, the river has turned out to be one of the most polluted rivers in South Africa (Grobler 1994, Addo-Bediako *et al.* 2014). Many of the impoundments and tributaries of the Olifants River System are said to be contaminated with heavy metals, inorganic nutrients such as, sulphates and nitrates from agricultural areas, mine drainages and water waste treatment works (Oberholster *et al.* 2013). The Olifants River was previously known to be a perennial river, but the river has been 'abused' that some of its parts are dry and characterised by pools that are connected by narrow flows during winter (Botha *et al.* 2011). Some of the tributaries that were previously said to be moderately impacted include the Mhlapitsi River, Tongwane River, and the Blyde River, where most of the time natural conditions were maintained (Ashton *et al.* 2001).

The Blyde River is one of the important tributaries of the Olifants River (Ashton *et al.* 2001). The river was known for its continuous flow and good water quality (DWAF 2004). The river is very important to the Olifants River as it provides good water quality and quantity (Ashton *et al.* 2001). The Blyde River originates in the Drakensberg Mountain near Sabie in Mpumalanga and it flows down to join the Olifants River in Limpopo Province (Singh & van Veelen 2001). The sub-catchment of the Blyde River lies partly on an escarpment and therefore it experiences more rainfall as compared to other sub-catchments in the Olifants Basin (DWAF 2004, Ashton *et al.* 2001).

1.2 Literature Review

The Blyde River geology mainly comprises of granite rock formation (Ashton *et al.* 2001). The land use of the Blyde River is mainly characterised by agricultural, industrial, domestic (DWAF 2004), and large-scale commercial forestry practices (Singh & van Veelen 2001). The Blyde River is considered to have its natural conditions maintained and its lower reaches were said to be protected by conservation activities (Ballance *et al.* 2001, Ashton *et al.* 2001). For that reason, it was reported to be inhabited by a high number of sensitive macroinvertebrate families (Ballance *et al.* 2001). However, there were few metal concentrations recorded in the river exceeding the DWAF guideline limits in the upper parts of the Blyde River in Mpumalanga in a study by Van Jaarsveldt (2004).

1.2.1 Water quality parameters

Many aquatic organisms are sensitive to changes in their environment such as, water temperature, pH, dissolved oxygen, electrical conductivity, and turbidity (Kale 2016). Temperature is an important water quality parameter and is relatively easy to measure (Bellingham 2006, Kale 2016). Water bodies naturally show daily and seasonal changes in temperature. However, anthropogenic changes to river water temperature will affect the aquatic organism's survival (Kale 2016). Some streams will increase in temperature as the river water moves down through urban, industrial and agricultural areas (Davies & Day 1998). The same might happen to the pH levels as the river water flows along the catchment with different land use activities.

The pH of water can give important information about the chemical and biological processes that occur in the water (Davies & Day 1998, Patel *et al.* 2016). Most metals become more soluble in water as the pH decreases (Kale 2016). The excess of

dissolved metals in water will negatively affect the health of aquatic organisms. Alterations in pH can indicate industrial pollution and high photosynthetic activities in the water (Munyika *et al.* 2014). The pH is typically monitored for assessments of aquatic ecosystem health as well as dissolved oxygen (Bellingham 2006).

Dissolved oxygen (DO) is important to all forms of aquatic life including the decomposers that break down materials (Trivedi *et al.* 2009). Solubility of oxygen in water increases with temperature decreases (Davies & Day 1998, Patel *et al.* 2016). The DO of freshwater at sea level will range from 15 mg/l at 0°C to 8 mg/l at 25°C (Bellingham 2006). Concentrations of unpolluted freshwater should be close to 10 mg/l (DWAF 1996). Generally, the concentration of dissolved oxygen is the product of biological activities. Photosynthesis of aquatic plants increase the DO during the day and decrease during the night (Davies & Day 1998). Anthropogenic contamination or natural organic material are consumed by microorganisms using oxygen (Bellingham 2006, Kale 2016). As this microbial activity increases, more oxygen is consumed by the organisms to facilitate their digestion process (Munyika *et al.* 2014), and as a result, the water will be depleted of oxygen. Microorganisms such as, bacteria can break down the contaminants in waters contaminated with fertilizers, suspended material, or petroleum waste, and oxygen is used (Davies & Day 1998). Typically DO levels less than 2 mg/l will cause death to many aquatic organisms such as, fish and sensitive macroinvertebrates (DWAF 1996, Bellingham 2006).

Electrical conductivity (EC) is the extent of the water's ability to conduct electric current (Davies & Day 1998, Kale 2016). The source of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from rainwater runoff, or other discharges (Davies & Day 1998). Most freshwater sources will range between 0.001 to 0.1 mS/m (Aihoon *et al.* 1997). Electrical conductivity also determines total dissolved solids and salinity. The EC of 0.3 mS/m is the level at which the health of some crops and freshwater aquatic organisms will be affected by the salinity (Aihoon *et al.* 1997). The salinity levels for most of the South African rivers has rendered some of the water unfit for irrigation (Bellingham 2006).

Turbidity occurs because of concentrations of suspended substances in water that affects the transparency or light scattering of the water (Davies & Day 1998). The range for natural water is 1 to 2000 NTU (DWAF 1996). Turbidity is typically made of

fine particles from different materials, organic or inorganic (Davies & Day 1998). These suspended particles range in size from 10 nm to 0.1 mm (Trivedi *et al.* 2009). The amount of suspended matter that remains in the water column depends on the current speed, when the current is slow, they settle down on the river bed (Trivedi *et al.* 2009). The anthropogenic sources of turbidity include erosion, industrial discharges, and eutrophication (Bellingham 2006). Many freshwater organisms are sensitive to prolonged exposure to turbidity, therefore monitoring of turbidity is an important criterion for assessing the quality of water (Bellingham 2006).

1.2.2 Nutrients

There are many chemicals, nutrients, and elements that are important for the growth and survival of living organisms. Nitrogen (N) and phosphorus (P) are essential nutrients for plant growth (Smith & Schinder 2009). They can be found in both a liquid phase and particulate phase. In water, they support the growth of plants. The major source of P and N is runoff from agricultural areas and cities (DWA 2011). Other anthropogenic sources of nutrients include sewage discharges, animal wastes, feeding lots and agricultural fertilizers (Davies & Day 1998). Phosphorus can be released from rock weathering, acid rain, and airborne pollutants.

When rivers are over-enriched with N and P, a range of adverse effects may occur including toxic algal blooms, eutrophication, fish kills, loss of macroinvertebrates, habitat loss and overall loss of biodiversity (Vitousek *et al.* 1997). Inorganic nitrogen includes all the major nitrogen components present in water; nitrite (NO_2^-) nitrate (NO_3^-) and ammonium (NH_4^+) (DWAF 1996). Ammonia and ammonium are reduced forms of inorganic nitrogen and their relative proportions in water are controlled by the temperature and pH (DWAF 1996).

Inorganic nitrogen contributes to the nutrition of rivers according to its abundance in the water (DWAF 1996). Concentrations lower than 0.5 mg/l may lead to oligotrophic conditions, which result in low productivity and without any growth of blue-green algae. Concentrations from 0.5 mg/l to 2.5 mg/l result in mesotrophic conditions, the system will be productive but even more productive when the concentrations are above 2.5 mg/l to 10 mg/l (DWAF 1996, Davies & Day 1998). Such increased concentrations of inorganic nitrogen cause eutrophication in the river system (DWAF 1996). As a result, there will be algal blooms which may include species that are toxic to aquatic life.

The presence of nitrates does not have a direct effect on some aquatic organisms such as, insects and fish, unlike oxygen and temperature, excess nitrates can make their conditions unsuitable for survival (Smith & Schinder 2009). There are many sources of nitrates including excreted of living aquatic organisms, dead plants and animals (Davies & Day 1998, Smith & Schinder 2009). Nitrates are sources of food for algae and excess nitrates result in high concentrations of algae. Large amounts of algae have many negative impacts on water quality (Vitousek *et al.* 1997, Dallas & Day 2004). It reduces the integrity of the river, causes eutrophication and introduces anoxic events (DWAFF 1996). Reduction in oxygen levels can induce stress to aquatic organisms which can affect their normal behavior and reproduction (DWAFF 1996). Some can choose to move to other areas, and some can die if the conditions persist for a long time (Carpenter *et al.* 1998).

The sources of phosphates entering the water systems are mainly from anthropogenic activities. Other sources of phosphates include animal wastes and bedrock (Smith & Schinder 2009). High concentrations of phosphates in water may result from point source discharges such as, industrial effluents, sewage effluents and domestic effluents (Davies & Day 1998). The non-point sources include agricultural runoff where fertilizers are applied, urban runoff and atmospheric precipitation (Davies & Day 1998). Phosphorus is an important macronutrient to plants, and it is accumulated by many organisms, and it can occur in organic and inorganic forms (DWAFF 1996). It can be present in water in a liquid form (dissolved form) or particulate form. It is one of the building blocks of DNA. It is a major nutrient in controlling the degree of eutrophication in aquatic ecosystems (Vitousek *et al.* 1997).

Phosphorous concentrations less than 0.005 mg/l are low enough to reduce the probability of eutrophication (DWAFF 1996). When the concentrations are above 0.25 mg/l it may result in eutrophic conditions in the river system stimulating the growth of aquatic plants and blue-green algal blooms (DWAFF 1996, Smith & Schinder 2009), and consume more oxygen resulting in the death of many aquatic organisms (Davies & Day 1998). These make the water unsuitable habitat for some aquatic organisms such as, fish and some groups of macro-organisms (Vitousek *et al.* 1997).

1.2.3 Metals in water and sediments

The metals found in most river systems have a natural origin but some of them are due to anthropogenic activities (Taghinia-Hejabi *et al.* 2010). Many of the rivers contain high concentrations of metals such as, cadmium (Cd), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) (Barakat *et al.* 2012). These metals when discharged in the rivers settle within the sediments by the process of adsorption, therefore the sediments serve as a sink that retains the metals (Marchard *et al.* 2006). Industrial, agricultural and mining activities are among the leading sources of metal pollution in the aquatic ecosystems (Chandrasekaran *et al.* 2013). Exposure to an excessive concentration of metals can be toxic to aquatic organisms and humans, however, some metals in the right concentrations have a very important contribution to life, some serve as nutrients, fertilizers or even form part of the living organisms' physiology (Chandrasekaran *et al.* 2013).

The disadvantages that come with a high concentration of metals in the aquatic ecosystems is that they are non-biodegradable and they become toxic to aquatic organisms as they accumulate over time (Taghinia-Hejabi *et al.* 2010). Heavy metals are associated with the deterioration of water and sediment quality as well as organism's health (Barakat *et al.* 2012). Toxic metals and pollutants accumulate in the bottom sediment where some sedentary organisms' dwell (Sola *et al.* 2004). These usually affect the health of aquatic organisms and may lead to disruptions in the food chains which can cause a decrease in biodiversity and degrade the ecological integrity (Vörösmarty *et al.* 2010).

Metal distribution and accumulation are influenced by mineralogical composition, adsorption, sediment texture and desorption (Taghinia-Hejabi *et al.* 2010). They get attached to fine substances in the water column and later settle on the sediments (Taghinia-Hejabi *et al.* 2010). Bellucci *et al.* (2002) have indicated that the concentrations of metals in sediments can be sensitive indicators of a contaminant in aquatic systems. The rivers flowing through developing areas and communities are highly susceptible to heavy metal pollution.

In South Africa, activities such as industrialisation, urbanisation, agriculture, and mining are the main cause of water pollution. Along the rivers, most of the un-impacted sites with metals are more likely to be situated above any major impact site like in

mountainous areas (Sola *et al.* 2004), whereas moderately impacted sites are found far downstream of an impact where there is evidence of restoration towards the pre-impacted conditions or found in areas of low agricultural activities (Dallas 1997). Severely impacted sites are more likely to be situated below a point source of pollution such as, sewage treatment works, industrial effluents, mining discharges and in areas of intensive farming activities (Dallas *et al.* 1998).

Metals are among the main pollutants in South African surface water resources (Sanders 1993, Dallas & Day 2004). Most of the rivers in South Africa receive metal contaminants from the increasing mining, agricultural and industrial releases into the rivers (Jooste *et al.* 2013). The Olifants River, for example, is one of the most polluted rivers in South Africa due to such anthropogenic activities taking place in the catchment (Ashton & Dabrowski 2011). The quality of water in this river is said to be “not desirable” and the pollution is progressively worsening (Wolmarans *et al.* 2014). The main contributors to metal pollution in this river are the mining activities in the upper part of the Olifants River catchment (Ballance *et al.* 2001, Wolmarans *et al.* 2014).

1.2.4 Aquatic Macroinvertebrates as bioindicators

It is crucial to conserve the ecological integrity of South African rivers because many species are under threat of extinction due to the continuous loss of their habitats (Ollis 2005). It is also important to assess the ecological state of the rivers before conservation strategies can be applied (Ollis 2005). River health programmes characterise different effects of disturbances on the aquatic environment by monitoring the response of biota. The aquatic biota provides a measure of the ecological integrity of the river as a whole (Ollis 2005, Dickens & Grahams 2002). Aquatic communities reflect and integrate the effects of physical and chemical disturbances (Davies & Day 1998, Dallas 2007). Fish communities, aquatic macroinvertebrates, and riparian vegetation are mostly used as bio-indicators for river health (Dallas 1997, Dickens & Grahams 2002).

Even though many types of organisms are used for bio-assessment of water quality and ecological integrity of freshwater ecosystems, aquatic macroinvertebrates are most widely used especially in lotic systems (Harker *et al.* 1989, Dickens & Grahams 2002, Wolmarans *et al.* 2014). Macroinvertebrates occupy diverse types of habitats

(Harker *et al.* 1989, Munyika *et al.* 2012). They can live in all different kinds of freshwater habitats including temporary ponds or rivers, deep or shallow systems and in clean or polluted waters (Wolmarans *et al.* 2014). They may reside on sediments, within sediments or on aquatic vegetation (Dickens & Graham 2002). They can be found in different kinds of water chemistry ranges, whether acidic or alkaline (Harker *et al.* 1989, Dallas 1997). They are important components of aquatic ecosystems, and they form part of the functional feeding groups such as, grazers, predators, shredders and filter feeders (Davies & Day 1998, Bredenhand 2005).

Macroinvertebrates have different community species with different sensitivities to a variety of stresses and they are quick to respond to such stresses (Dallas 1997, Davies & Day 1998, Dickens & Graham 2002, Grab 2013). They have a well-established taxonomy, at least to family level (Gerber & Gabriel 2002). These organisms act as continuous monitors of the water they inhabit.

Macroinvertebrates are victims of water pollution because most of them are bottom dwellers, they occupy the sediment which acts as a sink for pollutants (Watson & O'Farrell 1991, Wolmarans *et al.* 2014). They are therefore well known to be important for bioassessment (Munyika *et al.* 2014). Alterations in the composition and structure of aquatic macroinvertebrate communities are signs of changes in overall river conditions (Corbet 1999, Ferreira *et al.* 2012). They are largely non-mobile, so they turn out to be the representatives of the sampled site (Watson & O'Farrell 1991, Jonker & van Staden 2014). Most macroinvertebrates have a short lifespan, and they spent most of their aquatic life phase in one place thus, they are good indicators of localised river conditions (Corbet 1999).

The South African scoring system (SASS) is a rapid assessment biotic index developed for South African conditions whereby the presence of certain families of aquatic macroinvertebrates are used to determine the river health (Dallas 1997, Gerber & Gabriel 2002). The SASS method was developed by Chutter in order to find a cost and time effective method of assessing riverine water quality (Chutter 1994, Corbet 1999, Jonker & van Staden 2014). It is an approach used for rapid assessment of the health of a running water system based on its resident aquatic macroinvertebrate assemblage. The South African Scoring System facilitates the direction of impairment of water quality (Dallas 1997). It is based on aquatic

macroinvertebrates whereby each and every macroinvertebrate species or taxon is allocated a sensitivity score according to the water quality conditions it is known to tolerate (Gerber & Gabriel 2002, Dallas 1997).

Different types of macroinvertebrate families have different tolerances to individual water quality variables (Dallas & Day 1993, Malan & Day 2003). For that reason, the water of suitable quality is required to maintain a healthy population of aquatic macroinvertebrates and other aquatic organisms (Wolmarans *et al.* 2014). There are many ways in which SASS can be applied in monitoring and managing water quality including the assessment of accidental spills, locating pollution sources and monitoring riverine rehabilitation effectiveness (Dallas 1997).

The South African Scoring System is incorporated by the national biomonitoring programme for riverine ecosystems as one of the primary tools to be used for bio-assessment (Dallas 1997). Different macroinvertebrate families are grouped and assigned sensitivity scores relative to their tolerance to pollution (Gerber & Gabriel 2002). The tolerance levels are on a scale between 1 and 15 whereby the higher the score the lower the tolerance level. Level 1 to 5 indicates high tolerance, 6 to 10 moderate tolerance and 11 to 15 indicates low tolerance to pollution (Gerber & Gabriel 2002). High score signifies that the taxa have high sensitivity and less tolerance to pollution.

The SASS sampling is designed to incorporate all the different kinds of biotopes that are available such as stones-in-current, gravel, sand and mud, stones-out-of current, aquatic vegetation and marginal vegetation (Dickens & Graham 2002). These are because some taxa are mostly associated with certain kind of biotopes than others, therefore it is likely that the number and type of biotope available for habitation or for sampling may affect the scores (Dallas 1997). The South African Scoring System has three values; the number of taxa, SASS score and average score per taxon (ASPT) which are calculated to interpret the score (Dickens & Graham 2002). The SASS score is the sum of taxon scores for taxa present at a site. Average score per taxon is the SASS score divided by the number of taxa (Dickens & Graham 2002). The South African Scoring System data interpretation is based on the different sites where sampling is done (Dallas 2004). These sites are assigned to different ecoregions and longitudinal zones based on whether they are found upland or in the low land region

(Dallas 2004). The upland sites include those in mountainous areas, the source zones, and the transitional and upper foothill areas, whereas the lowland sites include the lowland zones and the lower foothill areas. Dallas (2007) came up with a method which is used to generate an ecological band by using the SASS score and ASPT values for each spatial group to determine the percentiles and bandwidths. The ecological bands range from A-F, each band representing a certain ecological category. A site will fall in an ecological band A if the site has a SASS5 score of 160 upwards and ASPT of 7.2 upwards. A represents a natural ecosystem, B- good water quality, C- fair water quality, D- poor, E-seriously modified and lastly F-critically modified (Dallas 2007).

1.2.5 Bioaccumulation

Aquatic macroinvertebrates accumulate metals of different concentrations in their tissues depending on how much of the metal is found in their surroundings (Sola & Prat 2006, Cid *et al.* 2010). Metals may be incorporated into macroinvertebrates directly from the water, sediments and from their diet (Santoro *et al.* 2009). These accumulated metals in a way can be used to determine the degree of water quality in that environment. The total accumulation found in the macroinvertebrates also depends on their intake and out-take amount (Sola & Prat 2006). The species physiology and processes of controlling metal distribution in the body also play a role (Sola & Prat 2006).

Metal accumulation in aquatic macroinvertebrates may differ with size, life cycle and different bioaccumulation patterns (Cid *et al.* 2010). The exposure of the environment to contaminants changes the structure of the aquatic community and reduces biodiversity (Santoro *et al.* 2009). A polluted environment may increase death rate of sensitive macroinvertebrates species, changes the reproduction and growth rate of other organisms (Santoro *et al.* 2009). Macroinvertebrates have different feeding strategies, they can be collectors, gatherers, predators or filter feeders (Davies & Day 1998, Cid *et al.* 2010). In a way, these feeding strategies suggest how the organisms accumulate the metals. The biological uptake of collectors and gatherers maybe from immediate contact with the sediment or the substratum instead of bio-concentration from water (filter feeders) or bio-magnification by predators (Santoro *et al.* 2009). In this study, the larvae of Odonata (dragonflies), mainly predators were selected for metal bioaccumulation analysis due to their bigger size and abundance in the area.

Insect larvae have many useful properties which make them very important for freshwater quality assessment (Sola & Prat 2006). They are found in freshwater of all kinds and they are also able to tolerate moderate metal concentrations (Wolmarans *et al.* 2014). They are more like sentinel organisms in freshwater. Most of them are sedentary, they are useful bioindicators of the sediments. The insect larvae are close to the base of the food chain, they may, therefore, constitute an important way for the transfer of sedimentary metals to the food web (Santoro *et al.* 2009).

1.3 Aims and Objectives

1.3.1 Aim

The aim of the study was to assess the water and sediment quality of the Blyde River and their impact on aquatic macroinvertebrate communities.

1.3.2 Objectives

- i. Determine the physico-chemical parameters of the water at the selected sites of the river.
- ii. Determine the concentrations of selected metals (e.g. Al, As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Sb, and Zn) in the water and sediment.
- iii. Determine the nutrient levels in the water at various sites.
- iv. Assess the impact of the quality of water and sediment on the aquatic macroinvertebrate assemblages in the river.

1.4 Problem statement

South Africa is a semi-arid country facing water supply shortage due to low rainfall and high evaporation. The Olifants River System is one of the major river systems in South Africa, of much importance to human and other living organisms. However, due to the various activities taking place in its catchment, the river has turned out to be one of the most polluted rivers in South Africa. The Blyde River is an important tributary of the Olifants River known for its continuous flow and good water quality. Recently, there has been an increase in agricultural activities and human settlements in the Blyde River catchment. This might cause an alteration of the ecosystem and the water quality which may affect the lower Olifants River. It is therefore important to assess the current

status of the river and ascertain the impact of such activities on the water quality and the aquatic biota.

1.5 Significance of the study

Aquatic invertebrate diversity in the Blyde River has not been recently studied. Especially following the major changes in the catchment such as the continuous farming activities and settlements. Blyde River has been previously considered being of good water quality, therefore, there is a need to determine the present status of the river. The study will determine the effect of anthropogenic activities and possible source of pollution in the catchment. The information will be useful for ecological conservation and management of the rivers.

1.6 Study area

The Blyde River rises on the western slopes of the north-south trending Drakensberg Mountains (Mpumalanga) and flows northwards towards the escarpment edge where it is dammed, the Blydepoort Dam (Ashton *et al.* 2001). From the Blydepoort Dam, the Blyde River cascades down a steep series of rapids to its lower reaches, where the river again flows northwards to join the Olifants River at the town of Hoedspruit in Limpopo Province (DWAF *et al.* 2004). The Blydepoort Dam is the largest impoundment on the Blyde River and regulates flows in the lower reaches. The Blyde River catchment is approximately 2000 km² in size covering an area inclusive of Graskop and Pilgrim's Rest in the southeast, Ohrigstad in the center, and Hoedspruit in the east/north-east (Ashton *et al.* 2001).

Geologically, the northern parts of the sub-catchment, downstream of the Blydepoort Dam, is made up of crystalline gneissic and granitic rocks of the Basement Complex, underlie the catchment (DWAF 2001). The Blyde River sub-catchment lies partly on the escarpment and as a result experiences considerably higher rainfall than the other sub-catchments in the Olifants River Basin. On the escarpment mean annual precipitation sometimes exceeds 1,000 mm. However, both to the east and the west of the escarpment, mean annual precipitation is about 600 mm (DWAF 2004). Seven sampling sites were selected (Table 1.1, Figure 1.2; 1.8), the sites were spread along the whole portion of the Blyde River in Limpopo province until the confluence with the Olifants River (Figure 1.1).

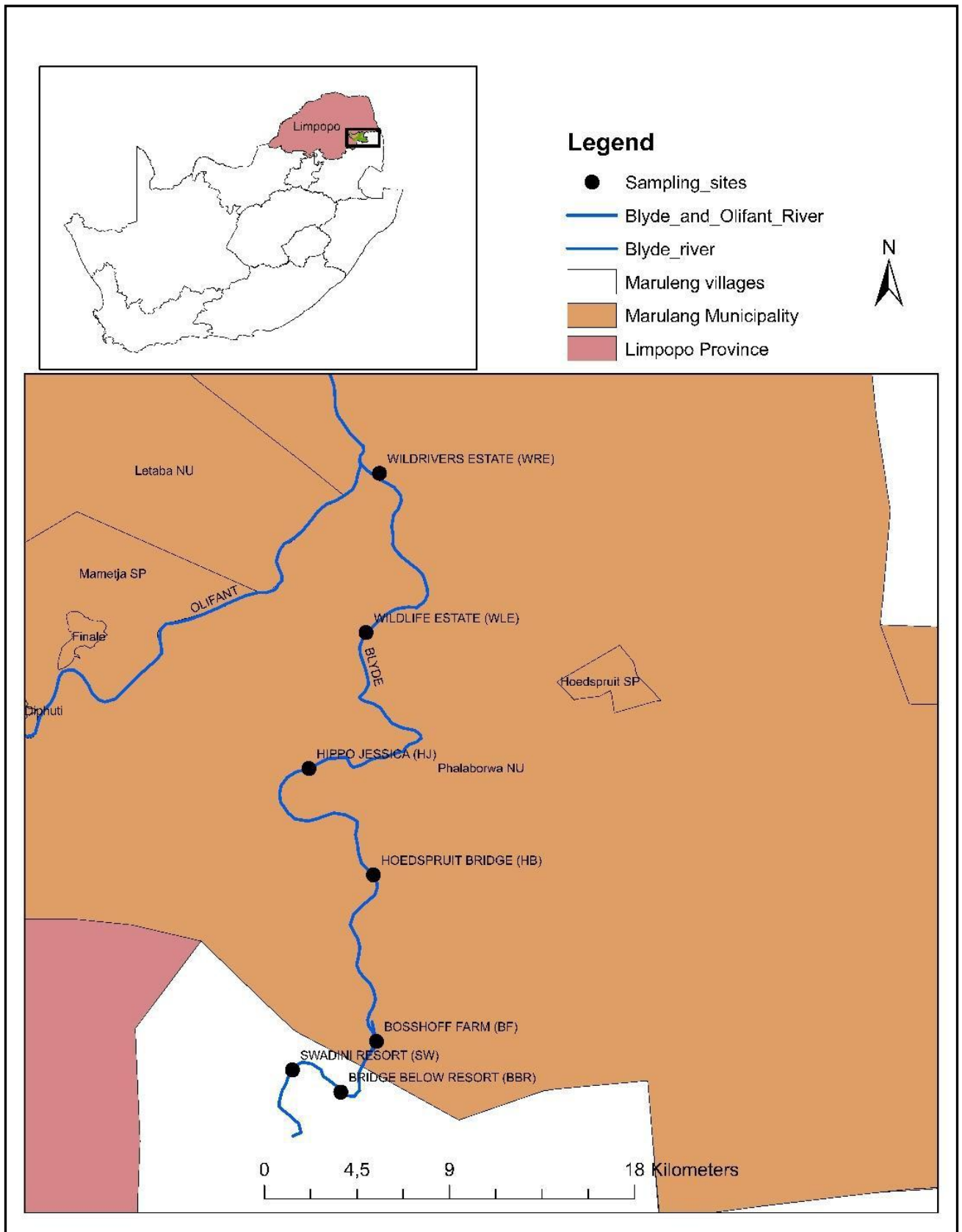


Figure 1. 1: The map showing the Blyde River and the selected sampling sites

Table 1. 1: Sampling sites selected at the Blyde River from up to downstream and their coordinates

Site Abbreviation	Site & Location	Coordinates
SR	Swadini Resort	24°30'59.46"S 30°47'56.14"E
BBR	Bridge below resort	24°30'14.42"S 30°50'08.49"E
BF	Bosshoff farm	24°25'52.45"S 30°50'03.59"E
HB	Hoedspruit bridge	24°24'19.03"S 30°47'54.19"E
HJ	Hippo Jessica	24°19'30.90"S 30°49'52.00"E
WLE	Wildlife Estate	24°23'04.94"S 30°48'22.09"E
WRE	Wild Rivers Estate	24°15'30.38"S 30°50'13.22"E

The first site SR was selected close to the Blyde canyon just next to the border that separates Mpumalanga and Limpopo. The last site WRE was near the river mouth where Blyde River joins the Olifants River, providing a final reference site, and an indication of the contrasting levels of contamination along the entire length of the river. The sites represent four different groups of land use; domestic (SR & BBR), agriculture (BF & HJ), industrial (HB) and 'pristine' areas (WLE & WRE).



Figure 1. 2: Swadini Resort (SR)



Figure 1. 3: Bridge Below Resort (BBR)



Figure 1. 4: Bosshoff farm (BF)



Figure 1. 5: Hoedspruit Bridge (HB)



Figure 1. 6: Hippo Jessica (HJ)



Figure 1. 7: Wildlife Estate (WLE)



Figure 1. 8: Wilddrivers Estate (WRE)

1.7 Dissertation outline

- Chapter 1 is the general introduction and literature review including the aims and objectives, problem statement and the significance of the study.
- Chapter 2 includes the water and sediment quality of the Blyde River based on the physico-chemical parameters and metals.
- Chapter 3 is about the macroinvertebrate structure and diversity of the Blyde River featuring the South African Scoring System version 5 (SASS5).
- Chapter 4 is the general discussion and conclusion of the dissertation.

Chapter 2: Water and Sediment quality

2.1 Introduction

Due to the various developmental projects embarked upon in South Africa such as, mining, industries, agriculture, domestic and aesthetic practices, this might have increased the level of pollutants in the river systems (Davies & Day 1998, Schwarzenbach *et al.* 2006). South Africa's water resources are very limited and crucial to the country's economy and biodiversity (Gyedu-Ababio & Venter 2013). Apart from its importance to human beings, the survival and diversity of many organisms depend on freshwater ecosystems, especially rivers (Gyedu-Ababio & Venter 2013).

To protect and manage these resources, it is necessary to conduct research to determine the status and conditions of these water resources. The Department of Water Affairs in South Africa, the then Department of Water Affairs and Forestry (DWAFF), developed a series of water quality guidelines that help in evaluating the fitness of water for certain uses such as, domestic, recreational, industrial, agricultural and aquatic ecosystems. (DWAFF 1996). The guidelines discuss factors such as, the physical water quality parameters, nutrients and toxic substances (e.g. metals). This chapter aims to evaluate the water and sediment quality of the Blyde River by determining the physico-chemical parameters including nutrients and concentration of metals.

The concentrations of the recorded levels of water constituents were compared to the available South African water quality guidelines or TWQR (Target Water Quality Ranges) to determine if the water quality of the Blyde River is still good. The South African water quality guidelines were supplemented by guidelines from other countries. This was done for the constituents that the South African guidelines do not cover, including sediments.

2.2 Materials and Method

2.2.1 Water and sediment sampling

Water and sediment samples were collected in 1000 ml acid pre-treated polyethylene bottles seasonally (winter and spring 2016 and summer and autumn 2017) in the

morning at each of the selected seven sampling sites at Blyde River. The sealed water and sediment bottles were stored in a cooler box with ice in the field until refrigeration at the University laboratory. The water was stored in a fridge (4°C) and the sediment was frozen until analyses. In addition, the physico-chemical constituents such as temperature, pH and dissolved oxygen were measured in the field using a YSI multiprobe meter (Model 554 Datalogger with a 4 m multiprobe). A Mettler Toledo SevenGo™ conductivity meter was also used to measure the total dissolved solids (TDS), electrical conductivity and salinity. The water samples were analysed for nutrients, sulphates, turbidity and hardness using a spectrophotometer (Merk Pharo 100 Spectroquant™) with Merck cell test kits at the University of Limpopo, Biodiversity Laboratory. Heavy and trace metals, metalloids and anions were analysed at an accredited (ISO 17025) chemical laboratory in Pretoria (WATERLAB (PTY) LTD). The ICP-MS scan analysis method was used at the water lab, together with the internal standards for quality assurance.

2.2.2 Statistical Analysis

The mean and standard deviation of the respective water variables and sediment metal concentrations were calculated. Analysis of variance (ANOVA) was performed using SPSS to determine whether there were significant differences among the different sites and seasons in water variables, metal concentrations of water and sediment. The significance level of $p \leq 0.05$ was used. Spearman's test was used to determine correlation between the physico-chemical parameters and metals recorded in the water column and sediments. Spearman correlation was performed to determine the correlations between the physico-chemical parameters and metals in the water column, and correlations between metals in the sediment.

2.3 Results

2.3.1 Physico-chemical water quality

pH, Water Temperature and Dissolved Oxygen (DO)

The recorded pH was slightly alkaline at all the sites and seasons. The lowest pH (8.33) was recorded at Hippo Jessica and the highest (8.67) was recorded at Swadini Resort (Table 2.1). There was no clear seasonal variation in the pH as it remained relatively constant throughout the sampling period and sites. The water temperature

at all the different sites showed almost the same seasonal trend with lower temperatures recorded in winter and higher temperatures in summer.

The highest mean temperature (25.98°C) was recorded at Wildrivers Estate and the lowest mean temperature (22.28°C) at Swadini Resort (Figure 2.1B). The mean DO concentration recorded in this study were all above 8 mg/l at all sites (Figure 2.1D). The oxygen levels were higher in the cooler seasons and lower in the warmer seasons. The highest mean DO concentration was recorded at Bridge Below Resort (11.85 mg/l) and the lowest mean concentration was recorded at Hippo Jessica (8.74 mg/l). The one-way ANOVA did not show any significant difference among sites nor seasons for the pH (df=6, F=1, $p > 0.05$), temperature (df=6, F=0.26, $p > 0.05$) and DO (df=6, F=0.367, $p > 0.05$).

Table 2. 1: Average physico-chemical values for the water at the different sites of Blyde River

Water quality parameters	SR		BBR		BF		HB		HJ		WLE		WRE		Water Quality Guidelines
	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	
pH	8.41-9.03	—	8.41-8.88	—	8.27-8.71	—	8.17-8.81	—	8.05-8.47	—	8.21-8.75	—	8.14-8.84	—	Should not vary by >5% ¹ 6.5-9.0 ³
Water Temperature (°C)	22.33	2.01	22.28	2.30	23.53	1.71	22.88	2.54	23.65	2.58	23.95	3.49	25.975	3.49	Should not vary by 10% from the natural value ¹
Electrical Conductivity (mS/m)	270.75	346.64	274.98	361.77	341.36	460.57	338.96	449.83	442.23	547.00	366.70	357.82	333.73	357.82	—
TDS (mg/l)	132.95	32.23	138.53	35.37	147.19	43.69	250.96	158.04	241.22	79.35	158.58	76.64	252.88	76.64	Should not vary by 15% from natural cycles ¹
Dissolved Oxygen (%)	118.05	13.55	132.1	7.7	111.05	26.95	119.05	11.65	94.05	22.75	111.45	14.95	115.85	9.85	80-120% ¹
Dissolved Oxygen (mg/l)	11.045	1.345	11.85	2.45	9.93	2.45	10.7	1.33	8.735	1.845	9.38	1.31	10.63	0.15	—
Salinity (‰)	0.25	0.34	0.27	0.37	0.35	0.47	0.38	0.51	0.46	0.59	0.38	0.51	0.49	0.63	<0.5‰ ¹
TWH	33.67	7.08	27.50	8.02	27.00	7.97	26.75	6.57	36.50	12.30	20.00	11.78	36.75	11.78	120 to 180 ³
Nitrite (mg/l)	0.02	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.005	0.00	0.06 ³
Nitrate (mg/l)	0.65	0.50	0.55	0.10	0.00	0.00	0.15	0.00	0.25	0.00	0.33	0.05	0.375	0.05	13.0 ³
Ammonium (mg/l)	0.06	0.00	0.05	0.00	0.04	0.00	0.05	0.00	0.03	0.00	0.03	0.03	0.065	0.03	<0.007 ¹ 0.354 ³
Total Nitrogen	0.77	0.79	0.61	0.58	0.04	0.06	0.20	0.00	0.30	0.52	0.35	0.35	0.445	0.35	<0,5 Oligotrophic & >10 Hypertrophic ¹
Phosphorous (mg/l)	0.22	0.00	0.16	0.32	0.40	0.00	0.25	0.00	0.03	0.00	0.18	0.20	0.1325	0.20	<0,005 (Oligotrophic) & >0,25 (Hypertrophic) ¹
Sulphate (mg/l)	17.35	5.27	16.90	4.75	18.03	4.92	17.05	4.14	24.00	3.92	14.93	6.27	25.95	6.27	—
Chloride (mg/l)	2.50	0.00	1.50	0.00	2.00	0.00	2.50	0.00	21.00	9.30	3.00	0.00	19	8.99	120 ³
Fluoride (mg/l)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.04	0.00	0.00	0.13	0.04	0.75 ¹ 0.12 ³
Turbidity NTU	14.00	8.69	14.00	12.88	6.75	2.05	7.50	2.69	13.25	10.99	7.75	5.24	10.25	3.34	8 (clear) to <50 (turbid) ²

■ Above Quality Guidelines

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

4-US-EPA (2012)- United States Environmental Protection Agency: Water Quality Guidelines-Aquatic Life

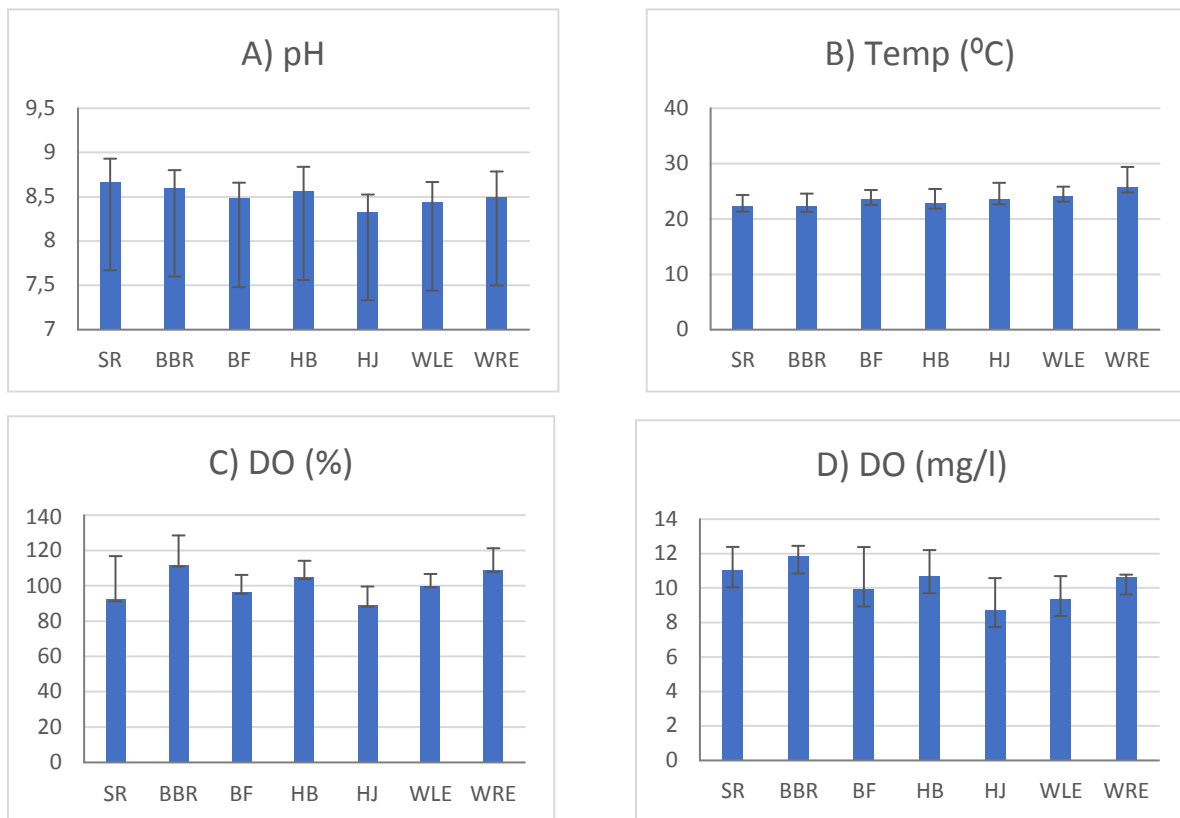


Figure 2. 1: Site variation in pH, water temperature, dissolved oxygen saturation and dissolved oxygen concentration from upstream to downstream at Blyde River

Total dissolved solids (TDS), Electrical conductivity (EC), Salinity and Turbidity

The mean total dissolved solids of the water varied between 132.95 mg/l and 252.88 mg/l at Swadini Resort and Wildrivers Estate respectively (Figure 2.2A). The highest readings were recorded in summer and the lowest in autumn at all sites. The mean electrical conductivity ranged from 270.75 mS/m at Swadini resort to 442.23 mS/m at Hippo Jessica. There was no clear seasonal variation in TDS and Electrical conductivity observed.

The salinity of the water at Blyde River was below the TWQR (<0.5‰), ranging from 0.25‰ to 0.49‰ at Swadini Resort and Wildrivers Estate respectively (Table 2.1). The one- way ANOVA indicated that there was a significant seasonal difference in the salinity (df=3, F=119.54, p<0.001), however, there was no significant difference between the sites (df=6, F=0.085, p>0.05). The Turbidity mean values varied between

6.75 and 14 NTU at Bosshoff farm and Swadini Resort respectively (Figure 2.2D). On average the maximum value was recorded in autumn and the minimum in spring.

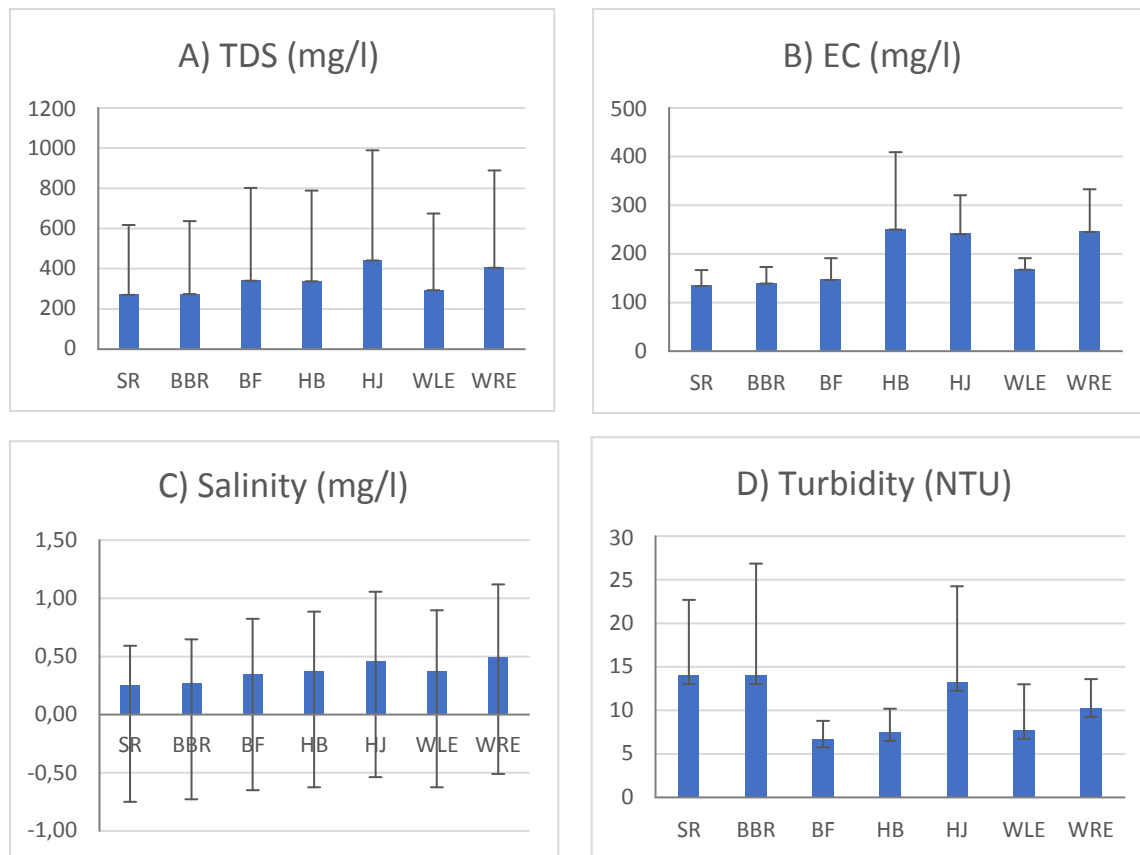


Figure 2. 2: The variation in total dissolved solutes, electrical conductivity, salinity and turbidity at different sites in the Blyde River

Nutrients and Ions

Nitrate, Nitrite, Total Nitrogen and Phosphorous

The mean nitrate recorded was highest at Swadini Resort (0.65 mg/l) but not detected at Bosshoff farm (Figure 2.3A). There was no clear seasonal variation or trend observed in the nitrate readings, however highest reading was recorded in summer. Most nitrate readings were recorded between 0.5 mg/l and 2.5 mg/l, indicating mesotrophic or oligotrophic conditions and below the CCME (2012) recommended guideline of 13 mg/l (Table 2.1).

The highest mean nitrite concentration was 0.02 mg/l at Swadini Resort, and the lowest mean concentration of 0.01 mg/l at Bosshoff Farm but was not detected at Wildlife Estate (Figure 2.3B). The highest concentration was recorded during spring at

0.03 mg/l and lowest was 0.02 mg/l detected in autumn and winter. All the recorded nitrite values were within the recommended guidelines, not exceeding the value of 0.06 mg/l (CCME 2012). The mean total nitrogen concentrations recorded at most of the sites were below 0.5 mg/l, thus the river can be classified as oligotrophic (DWAF 1996). The highest mean total nitrogen concentration recorded was 0.77 mg/l at Swadini Resort and the lowest was 0.20 mg/l at Hoedspruit Bridge. The highest values of total nitrogen were recorded in summer. The one-way ANOVA indicated a significant seasonal difference in the total nitrogen ($df=3$, $F=10.082$, $p<0.001$), but there was no significant difference between the sites ($df=6$, $F=0.723$, $p>0.05$).

The ammonium concentrations were below the detection limit (<0.01 mg/l) at most of the sites, however, the average concentration ranged between 0.03 mg/l and 0.07 mg/l at Wildlife Estate and Wilddrivers Estate respectively. The highest reading of ammonium was recorded in summer and on average, all the recorded readings were above the TWQR (<0.007 mg/l) however, the recorded values are within the CCME (2012) guidelines. The phosphorous concentrations recorded at Blyde River exceeded the TWQR of 0.005 mg/l indicating eutrophic conditions (Table 2.1). The highest mean concentration (0.40 mg/l) was recorded at Boshoff Farm and lowest (0.03 mg/l) at Hippo Jessica. The one-way ANOVA indicated a significant seasonal difference in phosphorous ($df=1$, $F=7.131$, $p<0.05$), however, there was no significant difference among the sites ($df=6$, $F=3.485$, $p>0.05$).

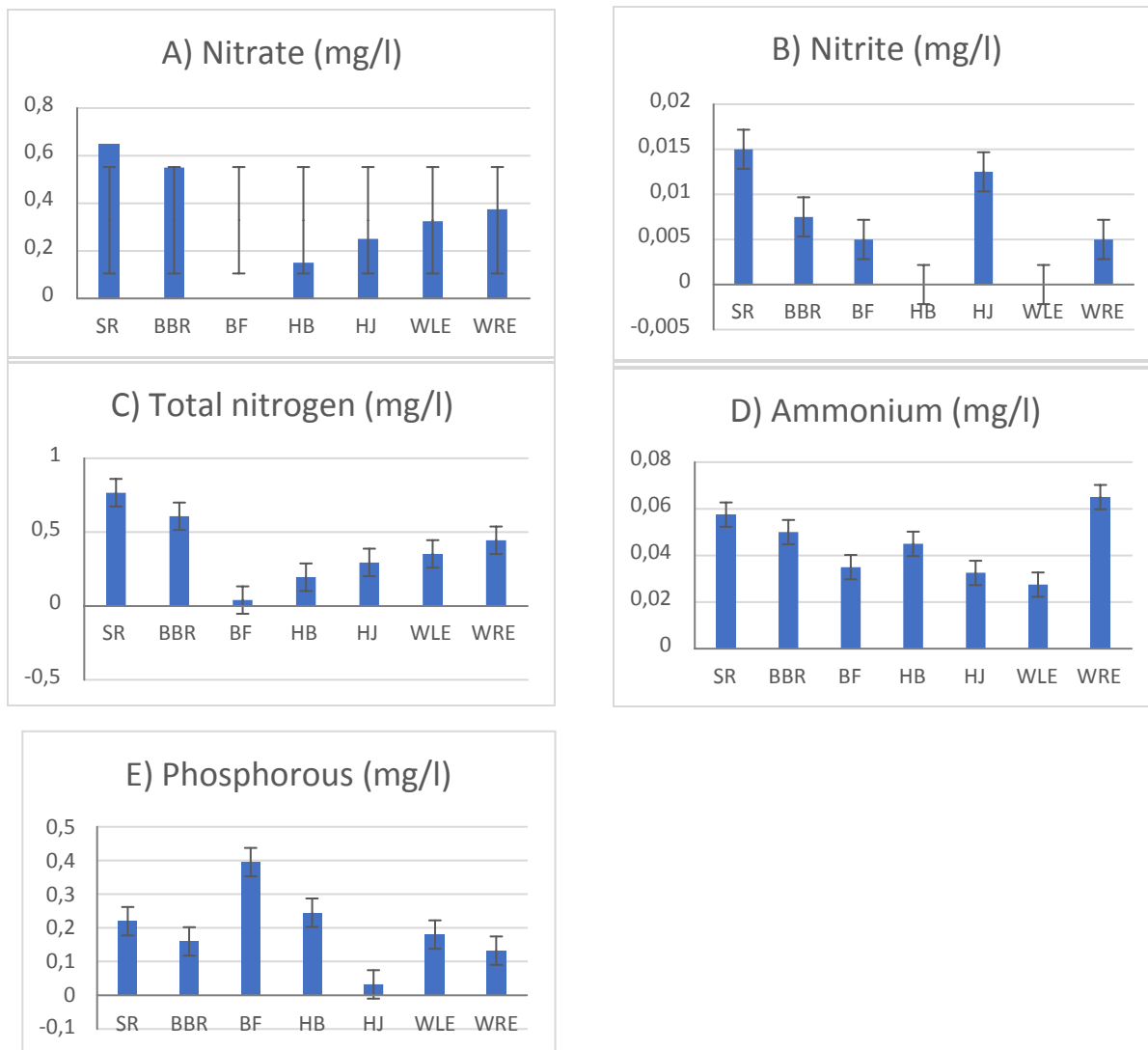


Figure 2. 3: The concentrations of nitrate, nitrite, total nitrogen, ammonium, and phosphorous at the different sites of the Blyde River

Sulphate Chloride, Fluoride and Total Water Hardness (TWH)

The highest mean sulphate concentration was recorded at Wilddrivers Estate (25.95 mg/l) and the lowest mean at Wildlife Estate (14.93 mg/l) (Figure 2.4A). The highest concentrations were recorded in summer and lowest in autumn. The one- way ANOVA indicated that there was a significant difference in sulphate concentrations among the sites (df=6, F=4.41, p<0.05) but there was no significant difference among the seasons (df=3, F=0.457, p>0.05). Most of the chloride concentrations were below detection limit (<0.01 mg/l), however, the mean concentrations varied between 1.50 mg/l and 21.00 mg/l at Bridge Below Resort and Hippo Jessica respectively. All the recorded concentrations were below the CCME guidelines of 120 mg/l (Table 2.1).

Fluoride concentrations were below detection limit at most of the sites. It was detected at Hippo Jessica (0.11 mg/l) and Wild Rivers Estate (0.13 mg/l) only (Figure 2.4C). Among the concentrations that were detected, the maximum was recorded in spring and the levels were below the TWQR (0.75 mg/l). The Total Water Hardness mean values ranged from 20.00 mg/l to 36.75 mg/l at Wildlife Estate and Wilddrivers Estate respectively (Figure 2.4D). The maximum hardness was recorded in spring and the lowest in summer at all the sites. The recorded hardness values were below the recommended value of 120 mg/l. The one- way ANOVA indicated a significant seasonal difference in the total water hardness ($df=3$, $F=20.59$, $p<0.001$), but there was no significant difference among the sites ($df=6$, $F=0.895$, $p>0.05$).

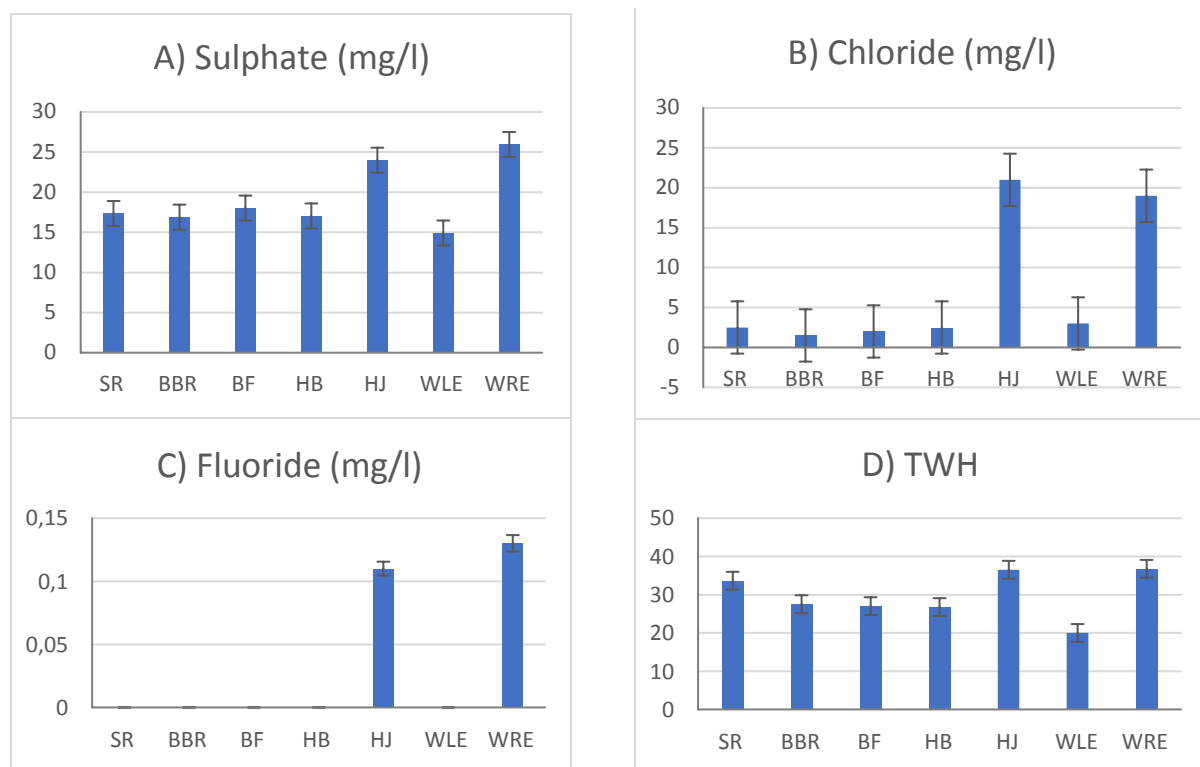


Figure 2. 4: The concentrations of sulphate chloride, fluoride and total water hardness at the different sites of the Blyde River.

2.3.2 Metals in the water column

Aluminium (Al)

The average concentration of Al ranged from 0.10 mg/l to 0.27 mg/l among the sites (Table 2.2). The Al concentration levels recorded were below the detection limit (<0.10 mg/l) at Bridge Below Resort (Table 2.2). The highest concentration of Al was recorded

in summer. Some of the recorded Al concentration levels were above the TWQR of 0.001 mg/l but at pH > 6.5.

Iron (Fe) and Barium (Ba)

Iron and barium were detected in all the seasons and at all the sites (Table 2.2). The mean concentration of Fe ranged from 0.12 mg/l to 0.37 mg/l. The water quality guidelines for Fe were exceeded only at the Wilddrivers Estate with a concentration greater than 0.3 mg/l (CCME 2012). Barium mean concentration ranged from 0.016 mg/l to 0.027 mg/l. All the recorded Ba concentrations were below the recommended limits of 0.7 mg/l (US-EPA 2012). The one-way ANOVA indicated a significant seasonal difference for both Ba (df=3, F=19.86, p<0.05) and Fe (df=3, F=3.71, p<0.05) but there was no significant difference in both metals among the sites.

Table 2. 2: Average metal concentrations of the water at Blyde River and water quality guidelines

Mg/l	SR		BBR		BF		HB		HJ		WLE		WRE		Water Quality Guidelines
	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	
Al	0.122	–	<0.100	–	0.106	–	0.1	–	0.188	0.07	0.108	–	0.2683	0.24	0.001*, (pH>6.5) ¹ , 0.1 ³
Sb	0.001	–	0.001	–	0.001	–	0.001	–	0.001	0	0.001	–	0.001	–	0.01 ⁴
As	0.001	–	0.007	–	0.004	–	0.008	–	0.015	0.01	0.01	0.01	0.011	–	0.01 ¹ , 0.005 ³ , 0.01 ⁴
Ba	0.018	–	0.016	–	0.018	0.01	0.018	0.01	0.027	0.01	0.021	0.01	0.026	0.01	0.7 ⁴
B	0.01	–	0.009	–	0.009	–	0.01	–	0.017	0.01	0.011	–	0.02	0.01	1.5 ³ , 1.2 ²
Cd	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	0.00015-0.004 ^{**1} , 0.000041 ³
Cr	<0.001	–	<0.001	–	0.0006	–	<0.001	–	0.0008	–	<0.001	–	0.0006	–	0.012 ^{*1} , 0.0089 ³
Co	<0.001	–	<0.001	–	<0.001	–	<0.001	–	0.0011	–	<0.001	–	<0.001	–	0.110 ²
Cu	0.002	–	0.003	–	0.003	–	0.004	–	0.016	–	0.004	–	0.005	–	0.0003-0.0014 ^{**1} , 0.00317 ³
Fe	0.125	0.06	0.1215	0.07	0.1515	0.10	0.1553	0.11	0.1695	0.44	0.1595	0.11	0.371	0.23	Vary<10% ¹ , 0.3 ³
Pb	<0.001	–	<0.001	–	<0.001	–	<0.001	–	0.0009	–	<0.001	–	0.0006	–	0.0002-0.0012 ^{**1} , 0.007 ³
Mn	0.0705	–	0.03	–	0.047	–	0.042	0.02	0.111	0.09	0.044	0.01	0.073	0.03	0.18 ^{**1} , <1.3 ²
Ni	0.001	–	0.003	–	0.001	–	0.001	–	0.002	–	<0.001	–	0.001	–	0.47 ⁴
Se	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	0.002 ¹ , 0.001 ³
Ag	0.035	–	<0.001	–	<0.001	–	0.011	–	<0.001	–	0.0328	–	<0.001	–	0.0001-0.003 ^{**2}
Sr	0.0159	–	0.0202	0.01	0.0237	–	0.0254	0.01	0.0604	0.02	0.0263	0.01	0.063	0.03	4.0 ⁴
Sn	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	<0.001	–	–
Ti	0.026	0.01	0.026	0.01	0.028	0.01	0.03	0.01	0.036	0.01	0.03	0.01	0.035	0.02	–
V	0.001	–	0.001	–	0.001	–	0.001	–	0.002	–	0.001	–	0.001	–	–
Zn	0.0359	0.01	0.073	0.02	0.042	0.01	0.0968	0.07	0.0525	0.01	0.0479	0.01	0.0475	0.01	0.002 ¹ , 0.03 ³ , <0.12 ⁴

— Above Quality Guidelines

* - pH-dependent

** - Water Hardness dependent

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

4-US-EPA (2012)-United States Environmental Protection Agency: Water Quality Guidelines-Aquatic Life

Strontium (Sr) and Titanium (Ti)

Strontium and Ti were detected at all sites in the Blyde River water samples. The mean concentrations of Sr ranged from 0.015 mg/l to 0.063 mg/l at Swadini Resort and Wilddrivers Estate respectively (Table 2.2). All Sr levels were below the recommended levels for drinking water (4.0 mg/l) according to BC-EPD (2006). The mean concentrations of Ti ranged from 0.026 mg/l at Bridge Below Resort to 0.036 mg/l at Hippo Jessica (Table 2.2). The one-way ANOVA indicated a significant seasonal difference in Sr ($df=3$, $F=6.39$, $p<0.05$) and Ti ($df=3$, $F=175.53$, $p<0.05$), but there was no significant difference in the two metals among the sites.

Antimony (Sb) and Arsenic (As)

The mean concentration of Sb recorded was 0.001 mg/l. The concentration was below the recommended limit (0.01 mg/l) according to US-EPA (2012). The Sb levels were only detected in summer. Arsenic was also mostly below the detection limit (<0.100 mg/l) in autumn, winter and spring. The mean concentrations ranged from 0.001 mg/l to 0.015 mg/l (Table 2.2). The detected As levels were below the TWQR (0.01 mg/l) at all the sites except at Wilddrivers Estate and Hippo Jessica.

Cadmium (Cd) and Chromium (Cr)

All the recorded Cd levels were below the detection limit at all sites (<0.001). Chromium was only detected at Hippo Jessica (0.008 mg/l), Wilddrivers Estate (0.0006 mg/l) and at Boshoff Farm (0.0006 mg/l) (Table 2.2) and all the detected Cr concentrations were below the TWQR (0.012 mg/l).

Cobalt (Co) and Copper (Cu)

Cobalt and Cu concentration levels were below the detection limit in the water samples in autumn, winter, and spring. Cobalt was only detected at Hippo Jessica (0.001 mg/l) in summer and was below the recommended levels (BC-EPD 2006). Copper was detected at all the sites in summer only, the levels ranged from 0.002 mg/l (Swadini Resort) to 0.016 mg/l (Hippo Jessica) (Table 2.3.2.1). The recorded Cu concentrations were all above the TWQR (0.0003-0.0014 mg/l).

Lead (Pb) and Silver (Ag)

Lead concentrations were below the detection limit (<0.010 mg/l) at all sites except at Hippo Jessica (0.0009 mg/l) and Wilddrivers Estate (0.0006 mg/l) in summer (Table 2.2). The recorded Pb levels were within the TWQR of 0.0002 mg/l - 0.0012 mg/l.

Silver was only recorded in winter at Swadini Resort (0.035 mg/l), in spring at Hoedspruit Bridge (0.011 mg/l) and Wildlife Estate (0.0328 mg/l). The recorded Ag concentrations were above the recommended BC-EPD guidelines of 0.0001 mg/l - 0.003 mg/l.

Boron (B) and Manganese (Mn)

The mean B concentration ranged from 0.009 mg/l (Bridge Below Resort) to 0.017 mg/l (Hippo Jessica) and none of the recorded B concentrations exceeded the recommended limits of 1.2 mg/l (BC-EPD 2006, CCME 2012). The concentration of Mn varied between 0.03 mg/l and 0.111 mg/l at Bridge Below Resort and Hippo Jessica respectively (Table 2.2). None of the recorded concentrations exceeded the TWQR of 0.18 mg/l.

Vanadium (V) and Zinc (Zn)

There are no guidelines for V concentrations in water, however, its mean concentration varied between 0.001 mg/l and 0.002 mg/l (Table 2.2). The highest concentration of V was recorded at Hippo Jessica. Zinc concentrations varied between 0.036 mg/l and 0.096 mg/l at Swadini Resort and Hoedspruit Bridge respectively (Table 2.2). The detected concentration levels of Zn were slightly above the TWQR (>0.002 mg/l).

Table 2. 3: Correlation in the physico-chemical parameters and metals recorded in the water column of the Blyde River.

	pH	EC	TDS	DO	Sulphate	Al	As	Cr	Cu	Fe	Zn
pH	1.00	-0.91	-0.25	0.92	-0.14	-0.21	-0.71	-0.48	0.03	0.03	0.13
EC		1.00	0.57	-0.93	0.43	0.47	0.80	0.65	0.16	0.16	-0.04
TDS			1.00	-0.32	0.68	0.67	0.71	0.45	0.62	0.62	0.41
DO				1.00	-0.26	-0.45	-0.59	-0.58	-0.06	-0.06	0.32
Sulphate					1.00	0.84	0.57	0.81	0.77	0.77	-0.22
Al						1.00	0.46	0.66	0.83	0.83	-0.39
As							1.00	0.50	0.42	0.42	0.19
Cr								1.00	0.48	0.48	-0.38
Cu									1.00	0.92	-0.17
Fe										1.00	-0.17
Zn											1.00

There was a strong correlation between pH and dissolved oxygen with a correlation coefficient of $r = 0.92$. A stronger correlation was also found between conductivity and As in the water column, whilst a weaker correlation was found between conductivity,

TDS and Chromium. Sulphate correlated strongly with Aluminium ($r=0.84$) and Chromium ($r=0.81$). Aluminium had a positive correlation with copper and iron, while Copper had a strong correlation with iron ($r=0.92$) (Table 2.3).

2.3.3 Metals in the sediment

Antimony (Sb) and Arsenic (As)

Antimony and As were detected at all the sampling sites and seasons in the sediments. Antimony levels varied between 0.40 mg/kg and 24 mg/kg at Wilddrivers Estate and Bosshoff Farm respectively (Table 2.5). Arsenic mean concentration levels varied from 6.23 mg/kg to 107.57 mg/kg at Wilddrivers Estate and Bosshoff Farm respectively (Figure 2.5D). The arsenic levels were higher than the recommended CCME (2012) guidelines at all the sites (>5.9 mg/kg). The one-way ANOVA indicated that there were no significant differences in Sb and As concentrations among the sites ($df=6$, $F=1.099$, $p>0.05$), As ($df=6$, $F=0.523$, $p>0.05$) but there was a seasonal significant difference in Sb ($df=3$, $F=4.824$, $p<0.05$).

Boron (B) and Barium (Ba)

Boron and Ba were detected in almost all the sediment samples. The average B concentration varied from 103.22 mg/kg to 138.77 mg/kg at Wilddrivers Estate and Bosshoff Farm respectively (Table 2.4). Barium levels varied between 257.18 mg/kg and 331.10 mg/kg at Hippo Jessica and Bridge Below Resort respectively (Figure 2.5C). The one-way ANOVA indicated that there was a significant seasonal difference in B ($df=3$, $F=7.86$, $p<0.05$) and Ba ($df=3$, $F=5.957$, $p<0.05$) concentrations. However, there was no significant difference among the sites B ($df=6$, $F=0.505$, $p>0.05$), Ba ($df=6$, $F=0.549$, $p>0.05$).

Cadmium (Cd) and Chromium (Cr)

Chromium was detected in all the seasons and sampling sites, whereas cadmium was only detected in autumn and winter. The mean concentration of Cr ranged from 41.50 mg/kg (Wildlife Estate) to 761.10 mg/kg (Wilddrivers Estate) (Table 2.4). The one-way ANOVA indicated that there was a significant seasonal difference in Cr concentrations ($df=3$, $F=6.503$, $p<0.05$). All the detected Cr mean concentrations were above the recommended CCME (2012) guidelines (>37.3 mg/kg). The mean concentration of Cd ranged from 0.01 mg/kg (Hoedspruit Bridge) to 0.11 mg/kg (Hippo Jessica) (Figure

2.5G). The Cd concentrations at Wildrivers Estate and Swadini Resort were below the detection limit (<0.001 mg/l).

Table 2. 4: The average concentration of metals in the sediments collected at the different sites of the Blyde River

Mg/kg	SR		BBR		BF		HB		HJ		WLE		WRE		Sediment Quality Guidelines (CCME 2012)
	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	AVE	±SD	
Al	29482.01	3623.22	37488.2	4112.57	46195.96	12932.07	40526.99	8545.63	39750.94	7571.91	36062.91	2156.28	41090.69	5103.2	—
Sb	1,48	1.13	8.19	9.71	24.74	6.74	6.3	5.59	7.24	7	7.11	8.42	0.4	0.69	—
As	29.04	19.55	57.2	59.18	107.57	49.32	51.03	40.46	44.88	46.56	50.79	46.03	6.23	3.61	5.9 mg/kg
Ba	270.22	55.04	331.1	46.69	269.33	50.72	280.67	114.69	257.18	90.21	289.71	79.36	287.21	74.93	—
B	121.79	168.13	119.75	182.84	138.77	186.13	105.84	169.54	136.15	207.99	105.9	168.5	103.22	168.62	—
Cd	0	0	0.04	0.05	0.09	0.1	0.01	0.02	0.11	0.18	0.41	0.67	0	0	0.6 mg/kg
Cr	56.33	15.48	48.9	16.79	98.24	42.46	80.44	50.32	108.01	73.88	41.5	12.82	761.1	449.43	37.3 mg/kg
Co	10.83	6	10.08	6.17	15.88	7.98	11.01	6.86	10.17	3.97	8.17	5.49	28.31	17.27	—
Cu	36.74	20.44	82	90.22	274.34	148.3	73.99	50.78	63.46	52.85	63.62	62.05	15.23	8.93	35.7 mg/kg
Fe	30309.86	4325.62	30298.8	5194.6	48221.26	15599.52	28108.78	12282.69	46210.48	35911.5	25332.63	6938.73	60590.44	30738.77	—
Pb	4.94	0.57	7.23	1.68	16.13	4.09	7.18	1.83	7.36	1.13	7.49	2.14	6.57	0.75	35.0 mg/kg
Mn	494.59	69.09	748.7	530.38	1175.65	490.46	949.8	635.99	1298.85	776.57	685.31	263.27	984.31	404.48	—
Ni	137.44	118.59	126.92	111.18	166.43	104.8	115.05	101.97	281.69	329.23	109.9	104.46	288.14	300.93	—
Se	0	0	0	0	0.01	0.01	0	0	0	0	0	0	0	0	—
Ag	0	0	0	0	3.56	5.73	1.12	1.87	0.73	1.26	13.9	24.07	0	0	—
Sr	44.34	25.09	94.6	42.86	70.02	39.61	112.41	51.99	106	71.09	87.51	31.97	96.65	39.85	—
Sn	0.56	0.44	1.26	1.15	3.44	1.24	0.93	0.91	1.46	1.3	1.12	1.22	0.23	0.38	—
Ti	3095.31	1299.86	2685.95	878.12	2850.22	400.98	2288.04	1034.5	4166.23	4397.34	1610.98	406.43	6527.55	3888.74	—
V	77.43	40.07	63.55	24.83	75.03	24.21	63.05	35.12	155.8	189.76	41.29	14.08	267.36	173.62	—
Zn	29.19	24.23	30.05	22.19	75.68	62.24	48.26	25.01	38.58	23.64	42.83	38.72	45.58	40.97	123.0 mg/kg

■ Above Quality Guidelines

CCME (2012): Canadian Council of Ministers of the Environment: Sediment Quality Guidelines-aquatic life

Cobalt (Co) and Copper (Cu)

Cobalt mean concentration levels ranged from 8.17 mg/kg (Wildlife Estate) to 28.31 mg/kg (Wildrivers Estate) (Table 2.4). The Cu concentration levels were between 15.23 mg/kg (Wildrivers Estate) and 274.34 mg/kg (Bosshoff Farm) (Figure 2.5I). The one-way ANOVA indicated that there was a significant seasonal difference in the Co and Cu concentrations Co (df=3, F=4.113, $p<0.05$), Cu (df=3, F=3.587, $p<0.05$), however, there was no significant difference in both Co and Cu concentrations among the sites. Almost all the recorded mean concentrations of Cu were above the recommended CCME (2012) guidelines (35.7 mg/kg).

Iron (Fe) and Lead (Pb)

Iron and Pb concentrations were recorded at all the sites and seasons in the sediments. High Fe concentration levels were detected in all the seasons and sites. Iron concentrations were between 25332.63 mg/kg (Wildlife Estate) and 60590.44 mg/kg (Wildrivers Estate) (Table 2.4). The Pb concentrations varied between 4.94 mg/kg (Swadini Resort) and 16.13 mg/kg (Bosshoff Farm). All the detected Pb concentrations were below the CCME (2012) quality guidelines (<35 mg/kg). The one-way ANOVA indicated a significant seasonal difference in Fe and Pb concentrations Fe (df=3, F=3.660, $p<0.05$), Pb (df=3, F=3.730, $p<0.05$), however, there was no significant difference in the metals among the sites.

Manganese (Mn) and Nickel (Ni)

Manganese concentration levels were between 494.59 mg/kg (Swadini Resort) and 1298.85 mg/kg (Hippo Jessica) (Table 2.4). The Ni concentration ranged from 109.90 mg/kg (Wildlife Estate) to 288.14 mg/kg (Wildrivers Estate) (Figure 2.5M). The one-way ANOVA indicated a significant seasonal difference in Ni (df=3, F=5.026, $p<0.05$), however, there was no significant difference in Ni among the sites (df=6, F=0.467, $p>0.05$).

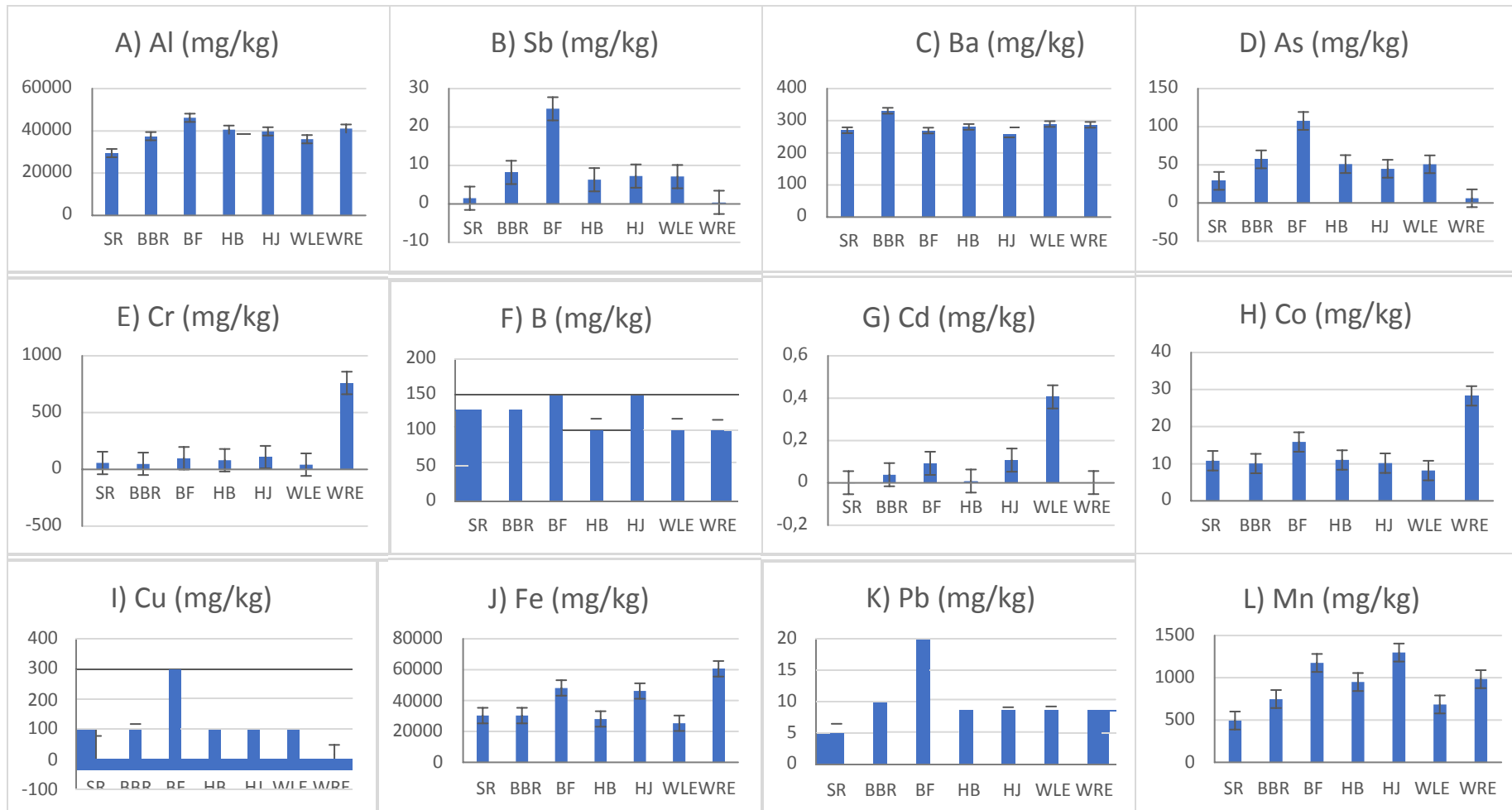


Figure 2. 5: The mean concentrations of metals in the sediment from the different sites of the Blyde River

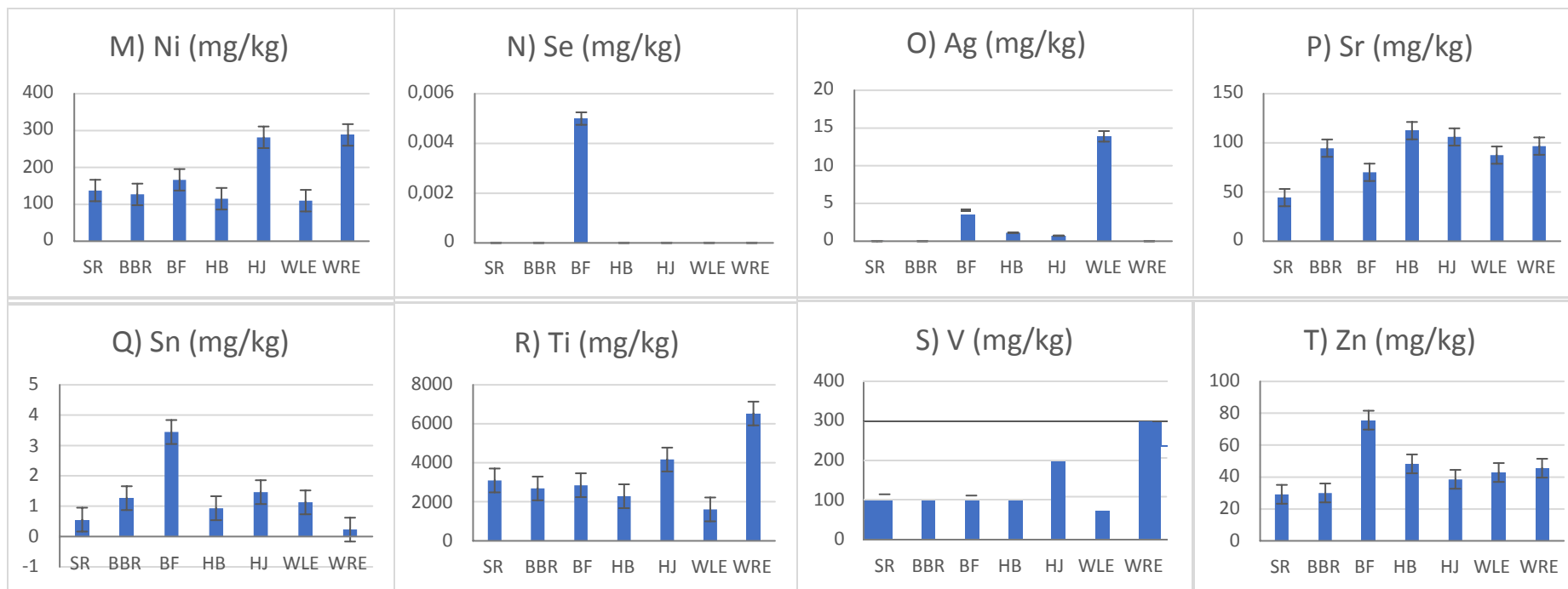


Figure 2. 6: The mean concentrations of metals in the sediment from the different sites of the Blyde River.

Selenium (Se) and Silver (Ag)

Selenium was only detected at Bosshoff Farm in spring at 0.02 mg/kg. The highest mean concentration of Ag was recorded at Wildlife Estate (Table 2.4). Silver was not detected at Bridge Below Resort, Wilddrivers Estate, and Swadini Resort throughout the sampling period (Figure 2.6O). The one-way ANOVA indicated a seasonal significant difference of Ag concentrations ($df=3$, $F=5.470$, $p<0.05$), but there was no significant difference in Ag concentration among the sites.

Strontium (Sr) and Tin (Sn)

The mean concentration of Sr varied between 44.34 mg/kg and 112.41 mg/kg at Swadini Resort and Hoedspruit Bridge respectively (Table 2.4). Tin was only recorded at Wildlife Estate, Bridge Below Resort, and Bosshoff Farm in summer. The mean concentration of Sn ranged from 0.23 mg/kg (Wilddrivers Estate) to 1.46 mg/kg (Hippo Jessica) (Figure 2.6Q). The one-way ANOVA indicated a significant seasonal difference in the Sr and Sn concentrations Sr ($df=3$, $F=3.572$, $p<0.05$), Sn ($df=3$, $F=7.398$, $p<0.05$), however, there was no significant difference in Sr and tin concentration among the sites.

Titanium (Ti) and Vanadium (V)

The highest mean concentration of Ti was recorded at Wilddrivers Estate (6527.55 mg/kg) and the lowest was recorded at Wildlife Estate (1610.98 mg/kg) (Table 2.4). Both Ti and V were recorded at all the sites and seasons. The mean concentration of V ranged from 41.29 mg/kg (Wildlife Estate) to 267.36 mg/kg (Wilddrivers Estate) (Figure 2.6V). The one-way ANOVA indicated that there was a significant seasonal difference in the Ti and V concentrations Ti ($df=3$, $F=4.987$, $p<0.05$), V ($df=3$, $F=5.579$, $p<0.05$), however, there was no significant difference among the sites.

Zinc (Zn) and Aluminium (Al)

Aluminium was recorded at all the sites and seasons throughout the study. Zinc levels were not detected in winter at all the sites. The mean concentration of Al ranged from 29482.01 mg/kg (Swadini Resort) to 46195.96 mg/kg (Bosshoff Farm) (Table 2.4). The highest Zn concentration was recorded at Bosshoff Farm (75.68 mg/kg) and the lowest was recorded at Swadini Resort (24.23 mg/kg) (Figure 2.6T). All the recorded Zn values were below the TWQR (123.0 mg/kg). The one-way ANOVA indicated a significant seasonal difference in the Al ($df=3$, $F=5.049$, $p<0.05$) and Zn ($df=3$,

F=3.291, p<0.05) concentrations, however, there was no significant difference in Al and Zn among the sites.

Table 2. 5: Correlation between metals in the sediments Blyde River

	Al	Sb	As	Cd	Cr	Cu	Fe	Pb	Mn	Ni	Ag	Zn
Al	1.00	0.67	0.53	-0.06	0.27	0.65	0.57	0.76	0.83	0.33	-0.04	0.83
Sb		1.00	0.97	0.16	-0.37	0.99	0.11	0.97	0.47	-0.19	0.19	0.81
As			1.00	0.21	-0.58	0.94	-0.14	0.88	0.33	-0.39	0.24	0.69
Cd				1.00	-0.31	0.06	-0.34	0.11	-0.11	-0.27	0.97	0.09
Cr					1.00	-0.33	0.79	-0.14	0.21	0.70	-0.28	0.09
Cu						1.00	0.14	0.97	0.43	-0.20	0.12	0.84
Fe							1.00	0.31	0.64	0.88	-0.38	0.41
Pb								1.00	0.53	-0.04	0.16	0.91
Mn									1.00	0.64	-0.20	0.58
Ni										1.00	-0.39	0.05
Ag											1.00	0.19
Zn												1.00

Aluminium had a positive relationship with most of the tested metals in the sediments ($r>0$), however, its strongest correlation was with Mn and Zn $r=0.83$. Zinc had a correlation with Al, Sb, As, Cu, Pb, and Mn, while Ag had a strong positive correlation with Cd only ($r=0.97$). Arsenic, Cu, and Pb had a strong positive relationship (Table 2.5).

2.4 Discussion

2.4.1 Physico-chemical water quality

Most of the physico-chemical parameters recorded at Blyde River were within the South African water quality guidelines for aquatic ecosystems (Table 2.1) (DWA 1996). The maximum water temperatures were recorded in summer as expected. The pH was found to be slightly alkaline at the sites throughout the sampling period. The possible explanation for the alkalinity might be the photosynthetic activities taking place in the river. When carbon dioxide is extracted from the water, the water pH level rises (Tucker & D'Abramo 2008, Munyika 2014). The dissolved oxygen levels were above 8 mg/l at all sites with higher levels recorded in the cooler seasons (winter and spring) and the lower levels recorded in warmer seasons (summer and autumn). This is because dissolved oxygen is temperature dependent, it dissolves better in cooler

waters than warmer waters (Davies & Day 1998, Lu *et al.* 2017). The water samples collected indicated that the Blyde River is well oxygenated with reference to the South African water quality guidelines (DWA 1996). There was a very strong positive correlation between pH and DO ($r=0.92$) (Table 2.3), probably because the changes in pH and DO are both affected by similar factors such as, the river productivity, photosynthesis activities and temperature. As pH decreases the dissolved oxygen decreases as a result (You *et al.* 2007, Zang *et al.* 2011).

There was a positive relationship between electrical conductivity and TDS ($r=0.57$) (Table 2.3), these results were in accordance with those of Thirumalini & Joseph (2009) in Malaysian freshwater. The relationship between EC and TDS is said to be the function of the type and nature of dissolved ions in the water (Thirumalini & Joseph 2009). Sulphate ion also had a positive correlation with TDS ($r=0.68$). Ions, such as sulphate, chloride, calcium, sodium, and magnesium are usually more correlated to TDS (Thirumalini & Joseph 2009). Turbidity revealed a trend between the seasons, whereby higher turbidity readings were recorded in summer during high flow period with the average of 14.57 NTU, and the lowest reading was recorded in winter (6.42 NTU). The possible reason might be due to the large volumes of particulate matter brought in by flooding during high flow seasons, thereby increasing turbidity (Matlou *et al.* 2017).

Nutrients and salts such as, nitrite, nitrate, total nitrogen, phosphorous and sulphate are important for life and growth of living organisms (Davies & Day 1998, Iqba *et al.* 2015). These nutrients are generally nontoxic if present in acceptable amounts in the aquatic ecosystem. However, excess nutrients such as, nitrogen and phosphorous can result in eutrophication (Dallas & Day 2004, Dodds & Smith 2016). Excess amounts are mostly enhanced by anthropogenic activities such as, fertilizers from agricultural, sewage and industrial discharges (Whipker & Cavins 2000). All the recorded nutrients and ions were within the recommended levels for aquatic ecosystems, except for phosphorous. The phosphorous levels indicated eutrophic conditions in the river with levels above 0.005 mg/l. Agricultural fertilizers and pesticides could be the main sources of phosphorus in the catchment. The Blyde River catchment is dominated by intensive agricultural activities in its lower reaches (Marr *et al.* 2017b). Nitrates detected at Blyde River were found to be in acceptable concentrations and the findings are in accordance with the study done by Marr *et al.*

(2017b). Ashton and Dabrowski (2011) stated that the intensive agricultural activities in the mid-reaches of the river pose negative impacts on the ecological conditions of the river through nutrient enrichment, however, the current study only found phosphorous enrichment.

The possible explanation for the acceptable quantities of nutrients in the Blyde River might be that the farmers are using good agricultural practice. The Blyde River is known for its good water quality and continuous flow (Ashton *et al.* 2001, Marr *et al.* 2017a). Even though the Blyde River catchment is dominated by large-scale farming, most of the nutrient levels detected in the water indicated that the river is still in good condition. The dilution effects due to high flow might have kept the concentrations relatively low (Magala 2012).

2.4.2 Metals in the water column

The concentration of metals in water is one of the leading water quality constituents that determines the fitness of water for certain uses (DWAF 1996, Lu *et al.* 2015). Some metals (trace and heavy) occur naturally in water and sediments in less destructive amounts. However, as the human population grows, anthropogenic activities become a source of excessive metal pollution into the water resources (Barakat *et al.* 2012). Although the presence of metals can be useful to the aquatic ecosystem, they turn to be toxic when they accumulate in higher concentrations (Davies & Day 1998, Fakoti *et al.* 2002). These metal accumulation problems are however magnified by the lack of a natural way of eradicating metals in the water and sediments (Chapman & Kimstach 1996).

The metals that were tested in the Blyde River include Al, Sb, As, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Sr, Sn, Ti, V, and Zn. However, most of the metals in the water column were below the detection limit or in concentrations below the TWQR (Table 2.2). Some of the metals including Al, Cu, Fe, Ag, and Zn were detected in slightly elevated concentrations exceeding the TWQR. Similar results were observed in a study by Jooste *et al.* (2013) in the Olifants River and Matlou *et al.* (2017) in the Steelpoort River. The higher concentrations of Al, Cu, Fe, Ag and Zn in the water column might be from both natural and artificial sources.

The natural sources may include weathering of minerals and soils and atmospheric deposition (Fakoti *et al.* 2002). The artificial sources could be from agricultural and domestic activities around the Blyde catchment. The recorded levels of Cu, Fe, and Zn in high concentrations may be because they are usually incorporated in the phosphorus fertilizers as micronutrients. However, Al and Fe are part of the top ten most abundant elements in the earth crust and Fe appears to be on top of the list (Forstner & Wittmann 1983). Therefore, it is not surprising to find Fe and Al abundant in the Blyde River. Some of the recorded Al concentration levels were above the TWQR of 0.001 mg/l, however, in Blyde River, such concentrations are less toxic as the pH of the water at all the sites is above 6.5.

Silver was found to be below detection level at many sites, it was only detected at Swadini resort in winter, at Hoedspruit Bridge and Wildlife Estate in spring. Even though Ag may not be a threat to human health, it is, however, second to Hg in its toxicity to aquatic invertebrates (Ranville *et al.* 2010). There was a strong positive correlation between Cu and Fe in the water column ($r=0.92$), Al with Cu ($r=0.83$) and Al with Fe ($r=0.83$). The positive correlation between these metals might be an indication that these heavy metals are from a common source (Salah *et al.* 2012).

2.4.3 Metals in the sediment

River sediments form an important part of the aquatic ecosystem, they provide habitat and food substances for some aquatic organisms and may serve as reservoirs for a variety of pollutants. Sediments are known to absorb persistent and toxic substances at higher levels as compared to the water column. The pollutants associated with sediments have a huge impact on the concentration of metals in the water column and accumulation in aquatic organisms (Barakat *et al.* 2012). The Blyde River catchment is dominated by agricultural activities; therefore, the river system is largely used for irrigation. Wastewaters from the agricultural fields run off and pollutants from the surrounding rural communities end up in this river.

Among all the tested metals, the correlation analysis of mean concentrations showed that Al had a strong correlation with most of the metals including Sb, As, Cu, Fe, Pb, Mn and Zn (Table 2.5). The positive relationship between these metals might be an indication that they have common contamination sources, or they behave the same way under certain levels of pH and water hardness.

Out of all the tested metals in the sediments only As, Cr and Cu exceeded the quality guideline values (CCME 2012). The probable reasons for the high concentrations of As, Cr, and Cu in the Blyde River could be due to natural sources, geology and the weathering of rocks. Furthermore, fertilizers from agricultural activities in the area could be largely contributing to the high concentrations of these metals (Fakoti *et al.* 2002). For example, the accumulation of Cu in soils and surface water is mainly due to anthropogenic activities such as, industrial activities. However, in an intensive farming area like the Blyde River catchment, the use of agricultural products containing Cu could be common especially in the application of pesticides (Toth *et al.* 2016). The general multipurpose usage of the above-mentioned heavy metals may lead to difficulties in tracing the source of origin of water pollution in that regard. The high Cr concentration could be from the cement used to build bridges, weirs and other buildings along the Blyde River. The highest Cr concentration in the sediment was recorded at Wildrivers Estate, where a lot of construction work is going on near the river.

The As levels in the sediments were found to be extremely high (Table 2.4), the maximum concentration recorded was 107.57 mg/kg at Bosshoff farm and the recommended sediment quality guideline for As is 5.9 mg/kg (CCME 2012). Arsenic is generally known for its geological origin and its high concentrations in clay soils, however, agricultural areas tend to have higher As content than the recommended levels (Toth *et al.* 2016) due to agricultural pesticides (DWAF 1996, Magala 2012). Arsenic fumes can also be dispersed from smelters (Forstner & Wittman 1983). Arsenic had a strong positive correlation with Sb ($r=0.97$), Cu ($r=0.94$), Pb ($r=0.88$), Zn ($r=0.69$) and Al ($r=0.53$), this relationship could suggest that these metals behave the same way under certain conditions such as, pH and temperature.

Most of the elevated metal concentrations were recorded at Bosshoff Farm and Wildrivers Estate. The Wildrivers Estate, the confluence of the Blyde River and the Olifants River, had the highest of most of the physicochemical and nutrient levels, suggesting that the Olifants River is contributing to the pollution at this site. Generally, the results of the physico-chemical parameters, nutrients and metals affirm that the Blyde River still could be said to have a good water quality. However, it is important to monitor changes in nutrient levels e.g. phosphorous and metals such as Al, As, Cu, Cr, Fe and Zn levels in the river.

Chapter 3: Macroinvertebrates

3.1 Introduction

Macroinvertebrates are frequently used for biomonitoring in streams and rivers (Wolmarans *et al.* 2014, Aschalew & Otto 2015). They are good indicators of pollution and other anthropogenic activities that might be causing stress to the water systems (Dickens & Grahams 2002, Ferreira *et al.* 2012, Aschalew & Otto 2015). The aquatic macroinvertebrates are used worldwide as bioindicators because they are abundant and found everywhere. Most of them are less-mobile, therefore good representatives of the area sampled (Ollis 2005). They provide information about the area they occupy and the pollution activities that might be taking place in that area (Oberholster *et al.* 2005).

Aquatic macroinvertebrates consist of a variety of taxa with different tolerance levels to pollution (Gerber and Gabriel 2002, Dallas 2004, Wolmarans *et al.* 2014). The advantage of using macroinvertebrates is that they can be seen with the naked eye and therefore easy to identify and classify (Dickens & Grahams 2002, M'Erimba *et al.* 2014). The bio-assessment technique that was applied in this study was the South African scoring system version 5 (SASS5). The technique is specifically developed for the assessment of the health and ecological integrity of South African river systems (Dallas 2007). The SASS5 method is rapid, inexpensive but scientifically sound to assess the aquatic ecological conditions in South Africa. The aim of this chapter was to assess the impact of water and sediment quality on aquatic macroinvertebrate communities along the Blyde River.

3.2 Materials and methods

Seven sampling sites were selected along the Blyde River based on the different activities in the catchment. Five samples of aquatic macroinvertebrates were collected at each selected site. The substrate was disturbed for a period of five minutes to free macroinvertebrates, they were then collected using a 30 by 30 cm SASS net with a 1 mm mesh size. Some specimens of the Order, Odonata (macroinvertebrate with a bigger size) were selected and frozen for bioaccumulation tests. The frozen

bioaccumulation samples were then taken to an accredited (ISO 17025) chemical laboratory in Pretoria (WATERLAB (PTY) LTD) for metal analysis. The samples were analysed using the ICP-MS Internal standards at the laboratory. Aquatic macroinvertebrate assessment was conducted using the South African scoring system version 5 (SASS 5) bio-assessment protocol (Dickens & Graham 2002) at the sites. The macroinvertebrates that could not be identified on site were preserved in 70% ethanol in small buckets immediately after being sampled in the field. Collected macroinvertebrates were separated from the debris, carefully sorted and identified to their different families (Gerber & Gabriel 2002) at the University of Limpopo's Biodiversity Laboratory using a stereomicroscope (LEICA EZ4). Macroinvertebrates were further classified per their tolerance levels to pollution, tolerant families have quality values (QV) ranging between 1-5, moderately tolerant 6-10 and sensitive to pollution 11-15 (Gerber & Gabriel 2002, Appendix A).

SASS score was calculated by adding all recorded macroinvertebrates per site, then the SASS score value was divided by the number of recorded taxa to obtain the ASPT (Dickens & Grahams 2002, Dallas 2007). There are different possible spatial variations in the assemblage of macroinvertebrates in different ecoregions, therefore there are guidelines developed to take into consideration the geographical and longitudinal variations for the interpretation of SASS5 data (Dallas 2007). The Blyde River is located within the Eastern Bankenveld ecoregion (Dallas 2007) and the ecological bands for this region were used for the interpretations of SASS5 data in this study (Table 3.1).

Table 3. 1: Ecological bands for Eastern Bankenveld-Upper ecoregion (Dallas 2007)

ecological band	SASS5 score ranges	Range of ASPT	Water quality category name	Description
E/F	<120	<5.82	Seriously/ Critically modified	Seriously critically modified
D	121-148	5.82-6.19	Poor	Largely modified
C	148-175	6.19-6.5	Fair	Moderately modified
B	175-205	6.5-7	Good	Largely natural with few modification
A	205-260	7-8	Natural	Unmodified

The mean and standard deviation of the aquatic invertebrate family abundance was calculated for each site per season. A one-way ANOVA was used to determine the differences in SASS 5 scores and ASPT among the sites and seasons. Spearman correlation was used to determine the relationship among sites in terms of the taxa.

Canonical correspondence analysis (CCA) was used to explore the relationship between macroinvertebrates and water quality parameters. The data were $\log_{(x+1)}$ transformed to stabilize the variance and the statistical package CANOCO 5 was used (Ter Braak & Smilauer 2012). A hierarchical cluster analysis was performed using statistica software, to group the sites based on the recorded macroinvertebrate taxa.

3.3 Results

3.3.1 Aquatic macroinvertebrate diversity and abundance and their tolerance levels to pollution

A total of 19 832 individuals belonging to 11 orders and 33 families were recorded at the sampling sites during the four seasons (autumn, winter, spring, and summer). The eleven orders were Ephemeroptera, Trichopteran, Coleoptera, Hemiptera, Odonata, Zygotera, Diptera, Plecoptera, Crustacea, Annelida and Mollusca (Figure 3.1). The order Diptera had the highest number of families (7) and Hemiptera, Crustacea, and Plecoptera had the lowest number of families (1 each) (Figure 3.1).

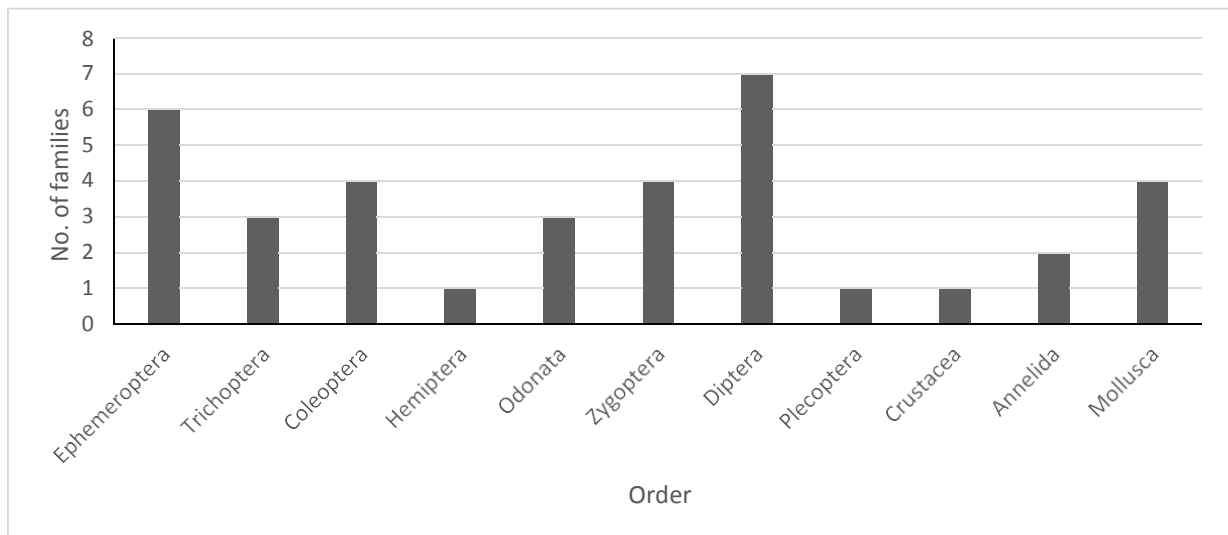


Figure 3. 1: The number of macroinvertebrate families in each order recorded in the Blyde River.

The highest number of orders was recorded at Bridge Below Resort (11) and Swadini Resort (11) and the lowest number of orders was recorded at Boshoff Farm (9). The highest number of families was recorded at Wildlife Estate (32) and the least number of families was recorded at Wilddrivers Estate (23) (Figure 3.2).

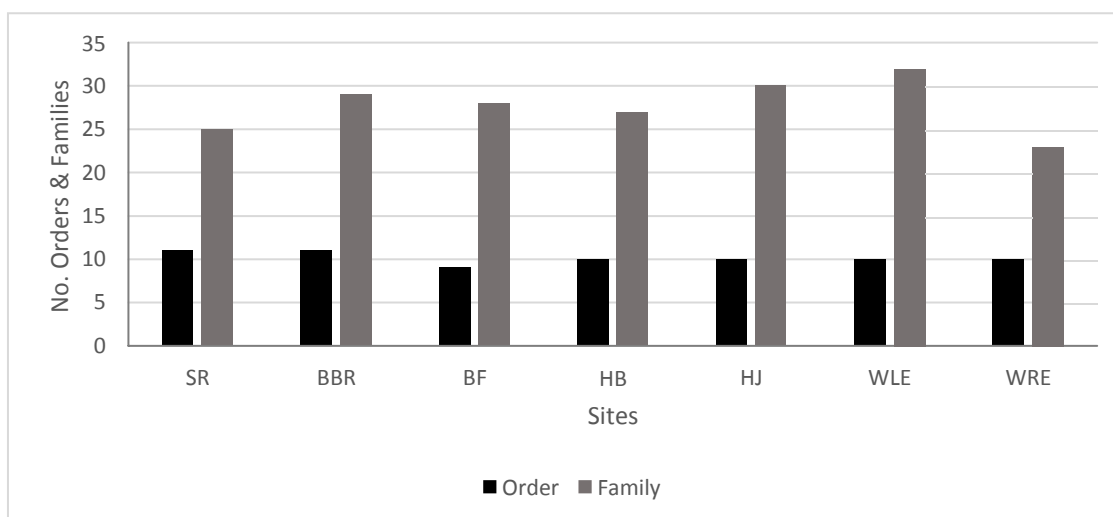


Figure 3. 2: The total number of macroinvertebrate orders and families recorded at the sampling sites of the Blyde River.

The highest number of individuals were recorded in Ephemeroptera and the lowest number of individuals were recorded in Hemiptera (Table 3.2). For the sites, the number of individuals recorded followed this order, from highest to lowest; Hippo Jessica 7426 (37%), Wildlife Estate 3651 (18%), Bridge Below Resort 2866 (15%), Wilddrivers Estate 2158 (11%), Hoedspruit Bridge 1390 (7%), Swadini Resort 1308 (7%) and Boshoff Farm 1033 (5%).

Table 3. 2: The total number of individual macroinvertebrates in the orders recorded at the different sites of the Blyde River

Total Order	SR		BBR		BF		HB		HJ		WLE		WRE	
	Tot	%	Tot	%	Tot	%	Tot	%	Tot	%	Tot	%	Tot	%
Ephemeroptera	768	59	1513	53	372	36	834	60	1887	25	1171	32	938	44
Trichoptera	207	16	768	27	412	40	146	11	1534	21	633	18	262	12
Coleoptera	48	4	92	3	72	7	76	5	928	12	78	2	14	0
Hemiptera	0	0	1	0	0	0	0	0	0	0	8	0	1	0
Odonata	86	7	41	1	9	1	9	1	53	1	6	0	25	1
Zygoptera	51	4	195	7	57	6	93	7	6	0	79	2	6	0
Diptera	85	6	179	6	60	6	171	12	2299	31	1173	32	86	4
Plecoptera	1	0	5	0	2	0	3	0	9	0	6	0	0	0
Crustacea	2	0	3	0	0	0	8	1	11	0	8	0	0	0
Annelida	18	1	7	0	3	0	2	0	40	1	89	2	42	2
Mollusca	42	3	62	2	46	4	48	3	659	9	408	11	784	36

Table 3. 3: Order, family and the number of individual macroinvertebrates recorded at the different sites of Blyde River

Order	Family		SR	BBR	BF	HB	HJ	WLE	WRE	Total
Ephemeroptera	Baetidae	BAE	227	594	138	417	501	330	276	2483
	caenidae	CAE	490	722	91	85	612	396	123	2519
	Heptageniidae	HEP	7	123	95	272	103	267	40	907
	Teloganodidae	TEL	39	68	13	20	139	101	295	675
	Leptophlebiidae	LEP	1	3	20	3		53	76	156
	Tricorythidae	TRI	4	3	15	37	532	24	128	743
Trichoptera	Hydropsychidae	HYD	207	754	392	131	1308	631	260	3683
	Philopotamidae	PHI		11	17	15	226	2	2	273
	Leptoceridae	LEP		3	3					6
Coleoptera	Gyrinidae	GYR	1	8	1	3	1	8	1	23
	Elmidae	ELM	23	72	31	52	917	52	13	1160
	Helodidae	HEL			2		9	1		12
	Psephenidae	PSE	24	12	38	21	1	17		113
Hemiptera	Naucoridae	NAU		1					1	2
Odonata	Libellulidae	LIB	10	12	6	5	50	4	10	97
	Aeshnidae	AES	8	8		2	1	1		20
	Gomphidae	GOM	68	21	3	2	2	1	15	112
Zygoptera	Chlorocyphidae	CHL	50	192	53	89	1	75	3	463
	Platycnemididae	PLA		3	2			1		6
	Coenagrionidae	COE	1		1	4	5	3	3	17
	Protoneuridae	PRO			1					1
Diptera	Athericidae	ATH	4	79	7	25	13	40	1	169
	Blephariceridae	BLE					2	8		10
	Tabanidae	TAB	11	8	5	7	79	19	11	140
	Dixidae	DIX			1	2	5	3		11
	Chironomidae	CHI	59	65	36	57	974	246	62	1499
	Muscidae	MUS		1			3	2		6
	Simuliidae	SIM	11	26	11	80	1223	855	12	2218
Plecoptera	Perlidae	PER	1	5	2	3	9	6		26
Crustacea	Potamonautidae	POT	2	3		8	11	8		32
Annelida	Hirudinea	HIR					36	57		93
	Oligochaeta	OLI	18	7	3	2	4	32	42	108
Mollusca	Physidae	PHY					1		2	3
	Planorbidae	PLA	1	3		2		17		23
	Thiaridae	THI	38	45	41	3	23	152	642	944
	Corbiculidae	COR	3	14	5	43	635	239	140	1079
Total number of individuals			1308	2866	1033	1390	7426	3651	2158	

The cluster analysis produced three main clusters of sites based on macroinvertebrate taxa (Figure 3.3). The first cluster was the Hippo Jessica, the second was the Wildlife Estate, the third was a cluster of Swadini Resort, Hoedspruit Bridge, Wilddrivers Estate, Boshoff Farm and Bridge Below Resort.

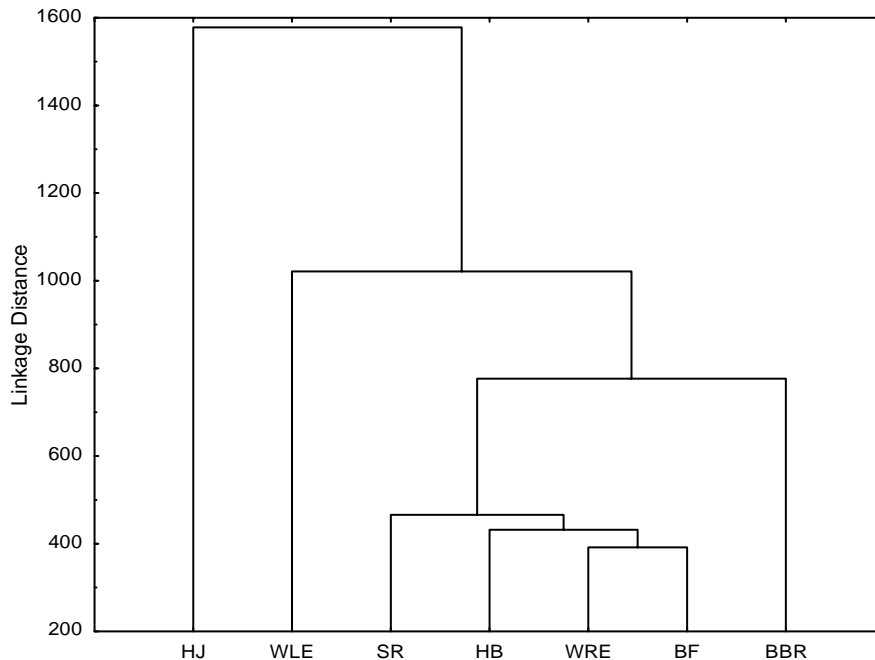


Figure 3. 3: Cluster analysis based on macroinvertebrates taxa collected across seven sampling sites in the Blyde River.

Spearman correlation yielded positive correlations ($r > 0$) among all the sites with respect to the macroinvertebrate families. The strongest correlations were between Swadini Resort and Bridge Below Resort ($r = 0.813$), Hoedspruit Bridge and Boshoff Farm ($r = 0.812$). The weakest correlation was between Swadini Resort and Hippo Jessica ($r = 0.405$) (Table 3.4).

Table 3. 4: Spearman correlation of macroinvertebrate taxa at the sampling sites

SITE	BBR	BF	HB	HJ	SR	WLE	WRE
BBR	1.000						
BF	0.768	1.000					
HB	0.804	0.812	1.000				
HJ	0.519	0.431	0.624	1.000			
SR	0.813	0.639	0.603	0.405	1.000		
WLE	0.674	0.640	0.781	0.576	0.608	1.000	
WRE	0.593	0.544	0.554	0.515	0.705	0.697	1.000

Comparing the numbers among the seasons, the highest numbers of orders and families were recorded in spring (11 orders and 32 families), followed by winter (10 orders and 27 families), autumn (10 orders and 25 families), and then summer (9 orders and 22 families) (Figure 3.4).

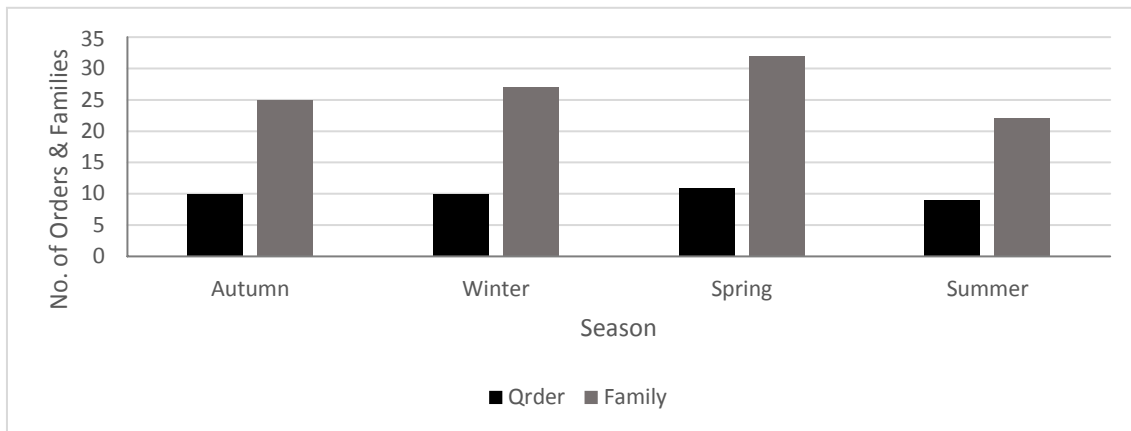


Figure 3. 4: The total number of macroinvertebrate orders and families recorded per season in the Blyde River.

It was observed that the highest total number of individuals was recorded in winter (10203) contributing up to 48%, followed by autumn with the total number of 5784 individuals (28%), then spring 3240 individuals, contributing 15% of the recorded

population, and summer with the least number of individuals (1818) contributing only 9% of the total abundance (Table 3.5).

Table 3. 5: The total number of individual macroinvertebrates in the orders recorded during the four seasons in the Blyde River

Order	Autumn	%	Winter	%	Spring	%	Summer	%
Ephemeroptera	2253	39	4195	41	896	28	601	33
Trichoptera	1490	26	1740	17	669	21	415	23
Coleoptera	703	12	357	3	355	11	40	2
Hemiptera	0	0	0	0	1	0	1	0
Odonata	94	2	105	1	46	1	22	1
Zygoptera	93	2	174	2	228	7	17	1
Diptera	512	9	3028	30	198	6	365	20
Plecoptera	21	0	6	0	7	0	0	0
Crustacea	24	0	5	0	4	0	7	0
Annelida	44	1	112	1	20	1	23	1
Mollusca	550	10	481	5	816	25	327	18

The macroinvertebrate families recorded in abundance were Hydropsychidae (3683), Caenidae (2519), Baetidae (2483), Simuliidae (2218), Chironomidae (1499), Elmidae (1160) and Corbiculidae (1079). Some were sensitive to pollution whereas others were moderately tolerant and tolerant to pollution (Figure 3.5). The highest number of tolerant families were recorded at Wildlife Estate (13) and the lowest was recorded at Wilddrivers Estate (10). Wildlife Estate also had the highest number of sensitive taxa (5) and the lowest number was recorded at Wilddrivers Estate (2) (Figure 3.5).

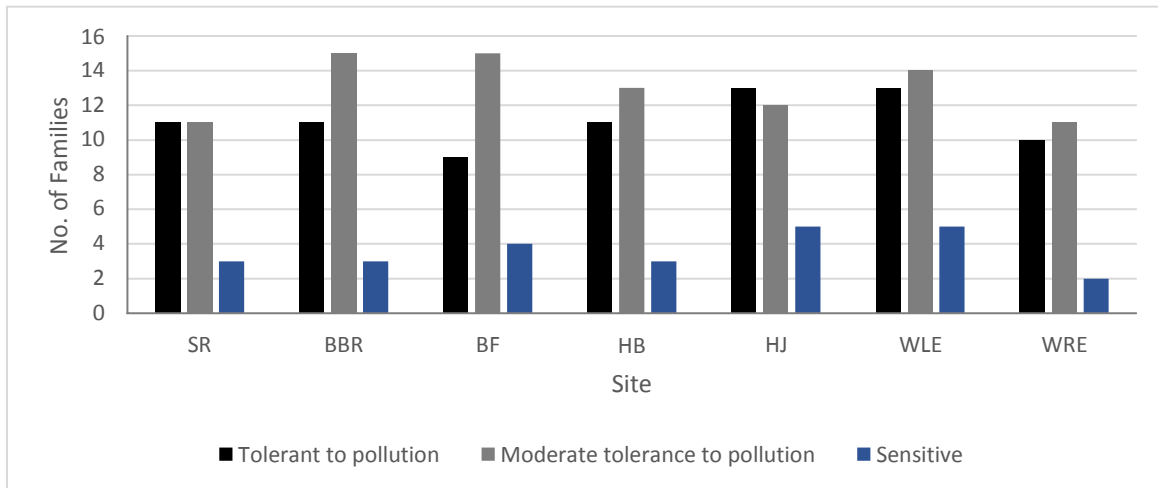


Figure 3. 5: Tolerant, moderate tolerant and sensitive macroinvertebrate taxa recorded at the different sampling sites.

Autumn samples had the least number (9) of tolerant families as compared to those of summer (10), winter (12) and spring (14). The highest number of sensitive families (6) was recorded in spring and the lowest number (3) was recorded in summer (Figure 3.6).

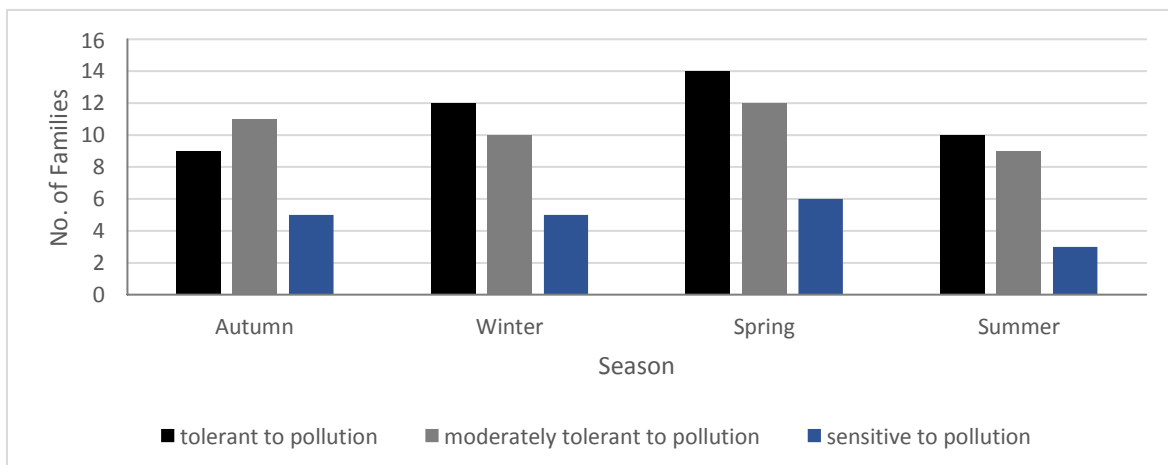


Figure 3. 6: The number of macroinvertebrates recorded within the different tolerance levels of pollution per season.

Table 3. 6: Analysis of variance of sensitive, moderate tolerant and tolerant to pollution macroinvertebrate taxa among the sites and seasons at the Blyde River

	Sum of Squares	df	Mean Square	F	Sig.
Sites					
Sensitive	78949.5	6	13158.2	0.410	0.864
Moderate	1856417.2	6	309402.9	1.883	0.131
Tolerant	1826183.4	6	304363.9	1.863	0.135
Seasons					
Sensitive	464560.7	3	154853.6	12.912	0.000
Moderate	795832.1	3	265277.4	1.411	0.264
Tolerant	799370.4	3	266456.8	1.435	0.257

Table 3.6 indicates that there were no significant differences ($p > 0.05$) among sites in all the three tolerant groups of macroinvertebrates, however, there was a significant seasonal difference ($p < 0.05$) in the sensitive macroinvertebrate group.

3.3.2 The South African Scoring System version 5 (SASS5)

Generally, the highest SASS indices values were recorded in winter and autumn but there are few exceptions at Wildlife Estate, Boshoff Farm and Hoedspruit Bridge, where the SASS score and the number of taxa were higher in spring (Table 3.7). On average, the Wildlife Estate had the highest values of SASS score, No. of taxa and ASPT, while the Wilddrivers Estate had the lowest values for all the three indices (Figure 3.7).

Table 3. 7: SASS indices calculated for data recorded from different sampling sites and seasons

	SASS score				No. of Taxa				ASPT			
	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum
SR	106	129	93	74	16	19	15	10	6.69	6.79	6.2	7.4
BBR	135	154	134	89	18	23	20	14	7.5	6.69	6.7	6.4
BF	123	119	148	51	17	17	21	10	7.24	7	7.05	5.1
HB	137	131	164	88	18	19	23	13	7.61	6.89	7.13	6.8
HJ	148	120	91	70	19	20	15	11	7.79	6	6.07	6.4
WLE	164	171	174	146	21	24	25	20	7.81	7.13	6.96	7.3
WRE	61	106	29	98	11	16	7	16	5.55	6.63	4.14	6.1

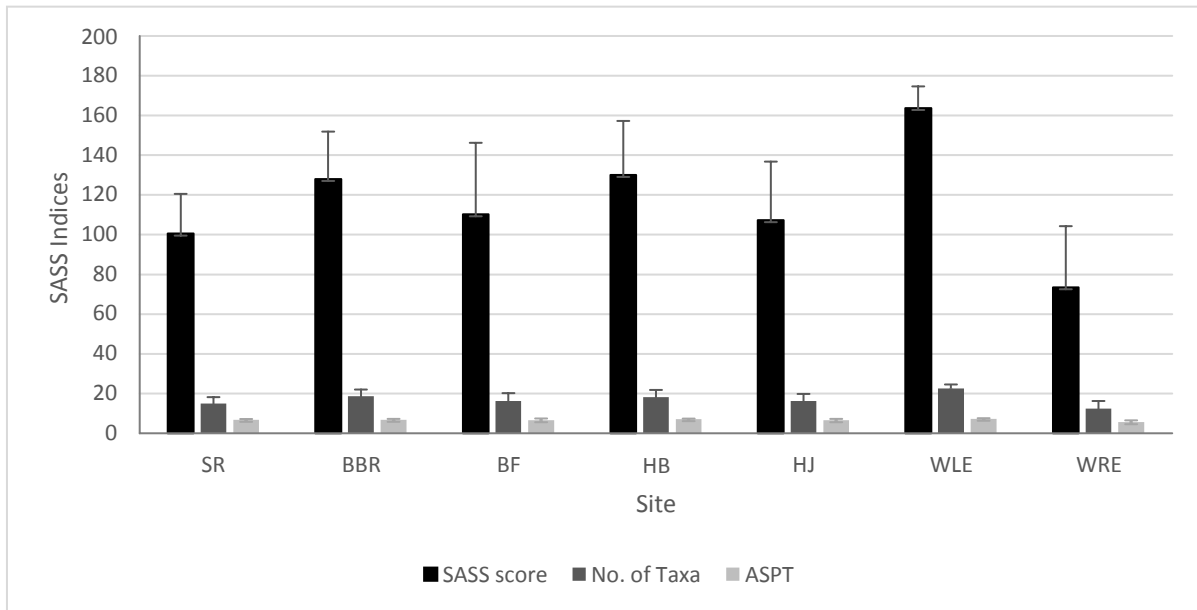


Figure 3. 7: The average SASS scores, Number of Taxa and ASPT for macroinvertebrates recorded at each site of the Blyde River.

The highest SASS score was recorded in winter (132.86) and the lowest was recorded in summer (88) (Figure 3.8). The highest number of taxa was recorded in winter (19.71) and the lowest was recorded in summer (13.43). However, the average ASPT value was highest in autumn (7.17) and lowest in spring (6.32).

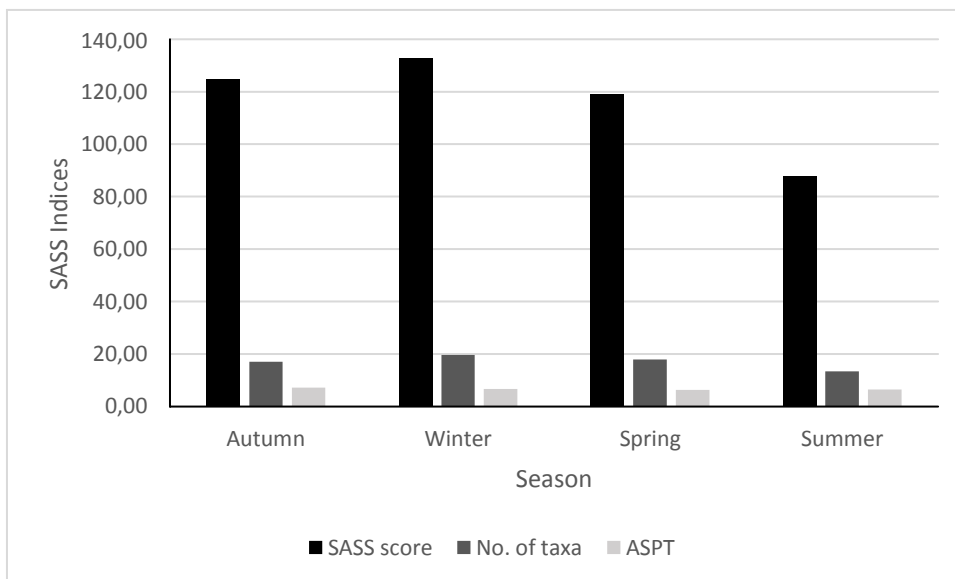


Figure 3. 8: The average SASS scores, Number of Taxa and ASPT for macroinvertebrates recorded at Blyde River per season.

The lowest ecological band reached was E/F with less than 5 ASPT value at the Wilddrivers Estate in spring. Swadini Resort was classified into ecological band C during spring, while in autumn and winter it was under band B, with an improvement to band A in summer due to a relatively high ASPT (Figure 3.9). Generally, Wildlife Estate and Hoedspruit Bridge were classified under ecological band A. Swadini Resort, Bridge Below Resort, Boshoff Farm and Hippo Jessica were grouped under ecological band B whereas, Wilddrivers Estate was grouped under the ecological band E/F. However, the overall ecological band of the Blyde River was B.

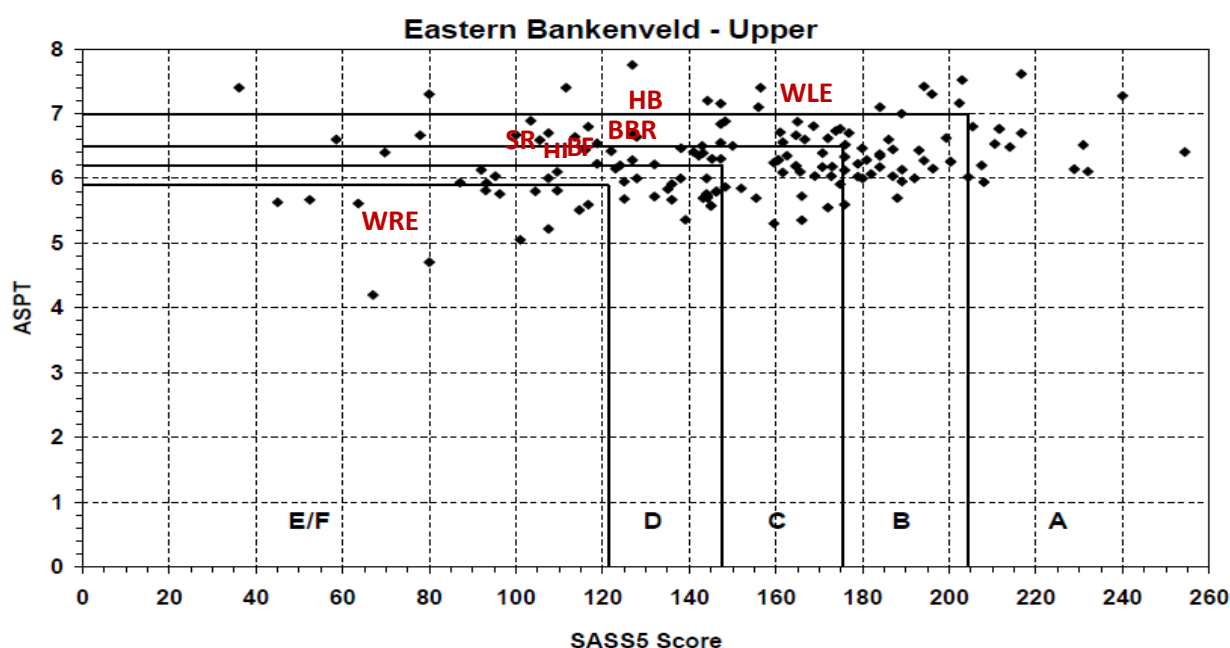


Figure 3. 9: The ecological bands of the sampled sites at Blyde River, Eastern Bankenveld-upper zones (Dallas 2007).

The CCA analysis revealed no specific arrangement or grouping of macroinvertebrates around the physicochemical parameters with respect to their tolerance levels to pollution. However, all the recorded families from the order Ephemeroptera (Baetidae, Heptageniidae and Caenidae) showed a strong correlation to the dissolved oxygen levels (Figure 3.10A). The figure 3.10B showed many nutrients concentrated in the first quadrant including total nitrogen, nitrate, ammonium, and sulphate. Most of the families in the first quadrant were very much tolerant to pollution with quality values ranging from 1 for the family Oligochaeta, Thiaridae (3),

Potamonautidae (3), Physidae (3), Coenagrionidae (4), and Naucoridae (7). The nutrients were positively correlated with the above-mentioned families. The tolerant families, for example, Physidae (PHY) and Naucoridae (NAU) were grouped together near most of the parameters (Figure 3.10A & B) (3.11A & B). The families were associated with metals such as, vanadium and titanium in the water column (Figure 3.11A). While in the sediments they showed an affinity towards iron (Fe), aluminium (Al) and manganese (Mn) (Figure 3.11B).

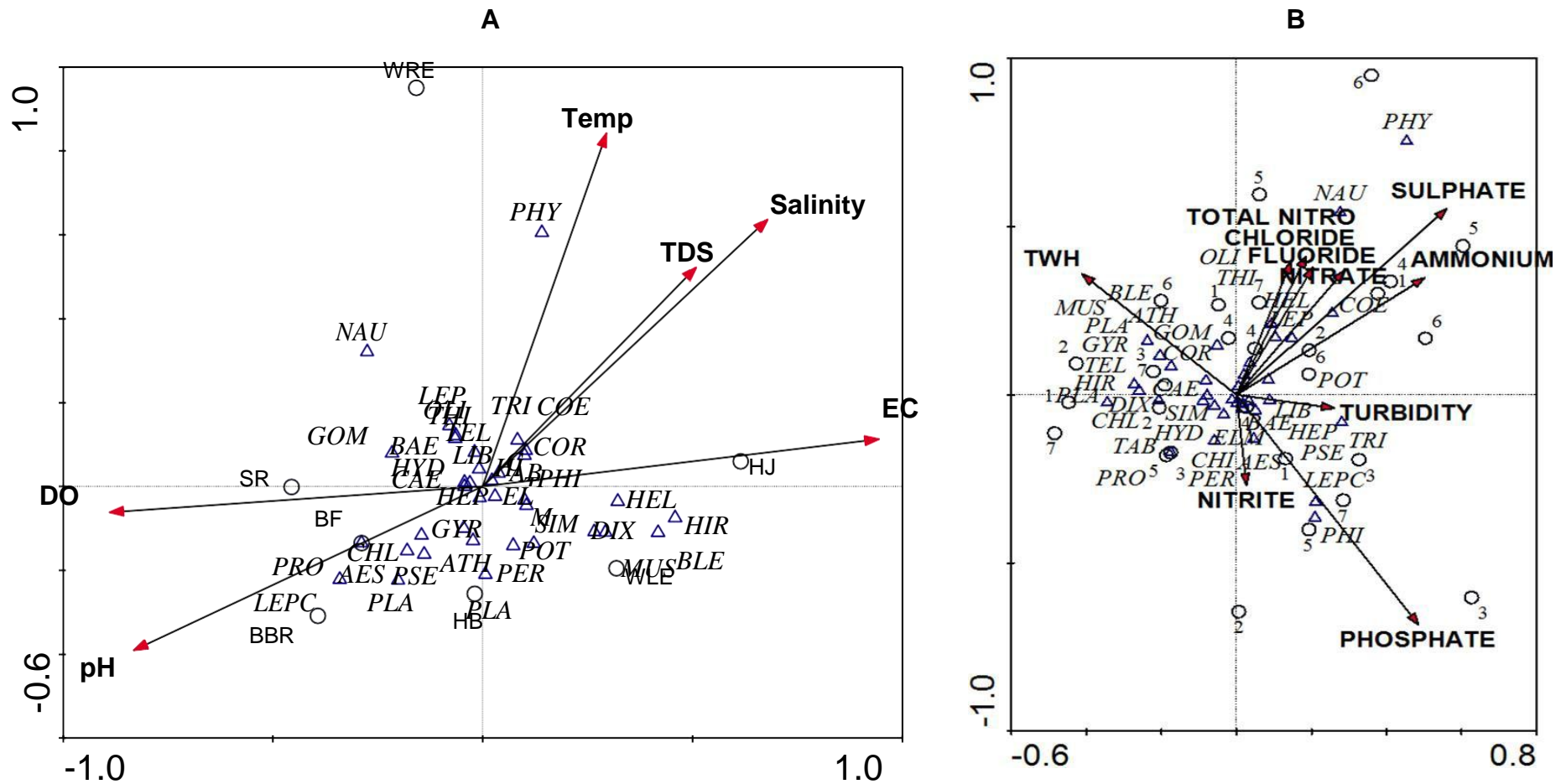


Figure 3. 10: CCA tri-plot of the relationship between recorded aquatic macroinvertebrates and water quality parameters (A) and CCA tri-plot of the relationship between recorded macroinvertebrates and nutrients at the Blyde River (B)

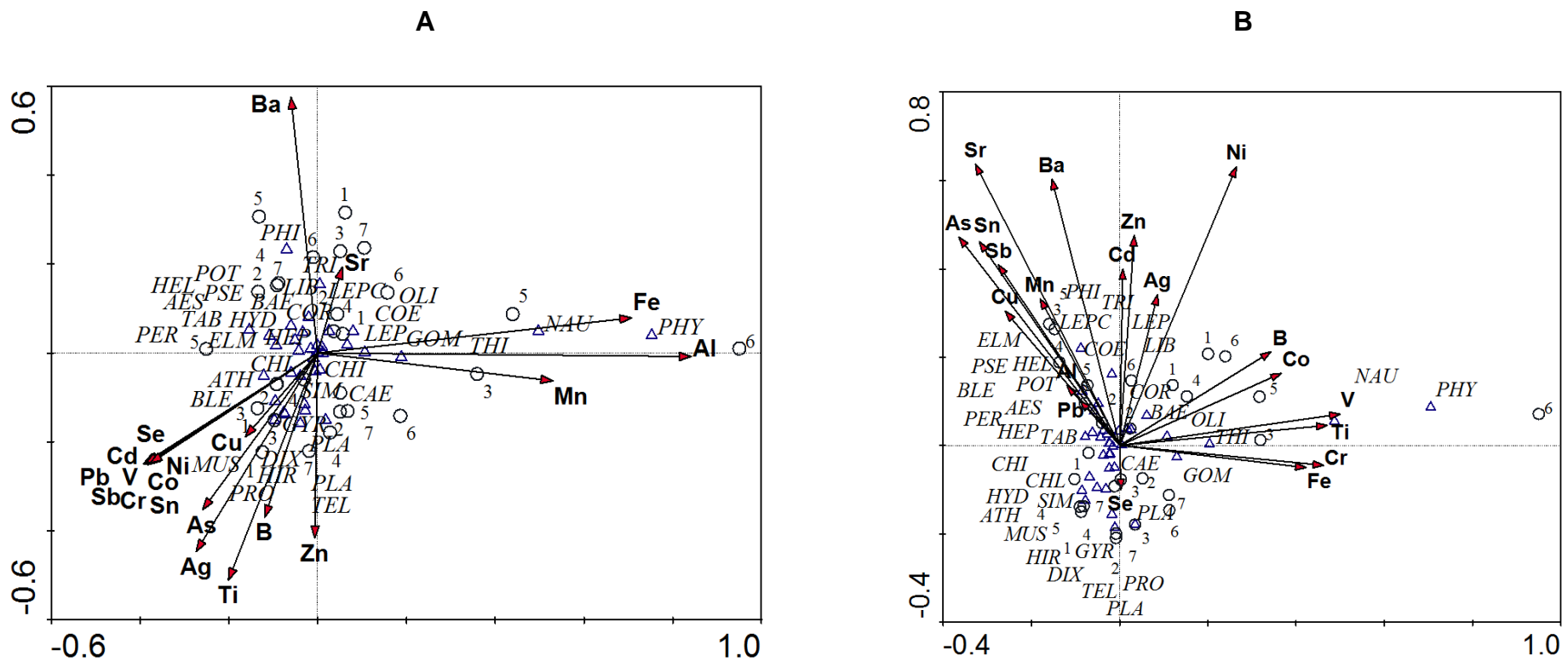


Figure 3. 11: CCA tri-plot of the relationship between recorded macroinvertebrates and metals in the water (A) and sediments (B) at the Blyde River.

3.3.3: Bioaccumulation

The following metals; As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, and Zn were detected in the tissues of the dragonflies (Odonata) at five sites of the Blyde River. The concentrations of metals in the body tissues of macroinvertebrates were compared with those in the sediments, some metals were found in higher concentrations in the sediments (As, Cr, Ni, Pb, Sb, and Zn), whereas some were found in higher concentrations in the macroinvertebrate tissue (Cd, Cu, Hg, Mn and Zn). There was a significant difference in Hg, Mn, Ni, Pb, and Zn recorded between sediments and body tissues of macroinvertebrates ($p < 0.05$). The metal concentrations in the macroinvertebrate tissue were found to be higher at Swadini Resort and lowest at Wildlife Estate (Table 3.8 and Figure 3.11).

Table 3. 8: Metal concentrations in the aquatic macroinvertebrates tissue collected at the different sites of the Blyde River

	SR		BBR		HB		HJ		WLE	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD	Ave	SD
As	32.26	-	19.81	2.44	12.32	-	16.59	3.49	7.3	4.3
Cd	0.56	-	0.28	0.03	0.17	-	0.09	0.09	0.25	0.25
Cr	13.81	-	4.59	1.78	2.05	-	5.55	2.14	1.82	0.53
Cu	187.13	-	101.07	28.8	78.18	-	61.06	39.92	52.89	26.26
Hg	0.32	-	0.18	0.02	0.17	-	0.26	0.05	0.06	0.06
Mn	3172.85	-	2105.68	395.37	3068.77	-	3637.48	2478.85	563.28	4.16
Ni	11.13	-	8.17	2.79	9.99	-	29.47	10.23	6.23	5.8
Pb	1.9	-	0.55	0.11	0.33	-	1.11	0.08	0.38	0.05
Sb	3.54	-	0.97	0.05	1.46	-	2.18	0.95	1.03	0.17
Zn	362.16	-	168.18	3.9	183.78	-	108.71	77.66	102.33	61.96

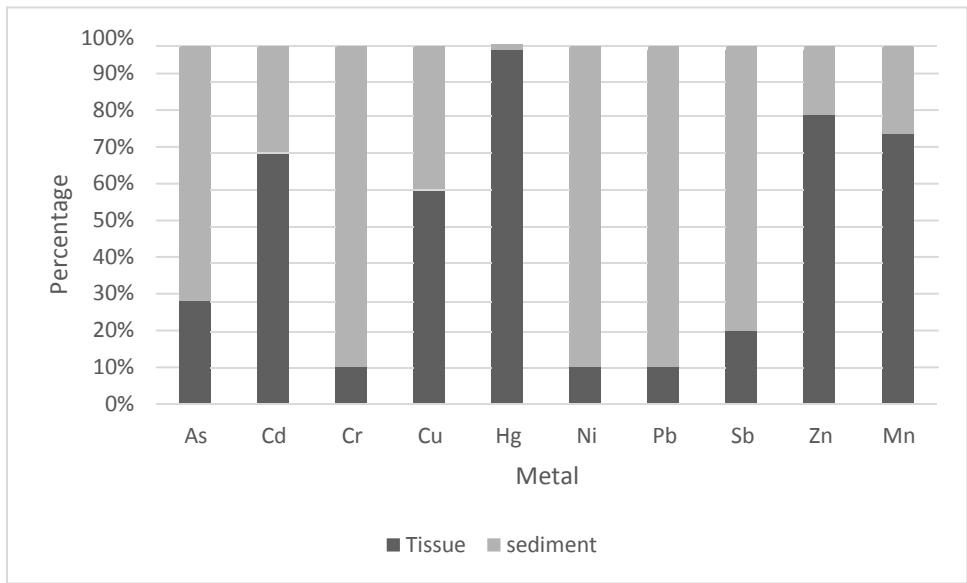


Figure 3. 12: Metal composition in the sediments and tissue of aquatic macroinvertebrates

3.4 Discussion

Eleven macroinvertebrate orders (Ephemeroptera, Trichoptera, Coleoptera, Hemiptera, Odonata, Zygoptera, Diptera, Plecoptera, Crustacea, Annelida, and Mollusca) were recorded at the Blyde River in the current study. However, the most abundant macroinvertebrates were from the orders Ephemeroptera (7 483), Diptera (4 053), and Trichoptera (3 962) (Figure 3.1), which are very common community structure of aquatic macroinvertebrates in streams (Jun *et al.* 2016, Matlou *et al.* 2017).

The order Ephemeroptera was mainly dominated by the families Baetidae, Caenidae, and Heptageniidae. These families were part of the most abundant families recorded throughout the sampling seasons and sites. They are well known for their high sensitivity to pollution, affinity to well-oxygenated waters and fast flowing waters (Jun *et al.* 2016). At Blyde River, the oxygen level was high, and the water was fast flowing, hence the high number of Ephemeroptera taxa. The presence of these macroinvertebrates at almost all the sites at the Blyde River indicates a high level of water quality (Olomukoro & Ezemonye 2007, Griffin *et al.* 2015). Hippo Jessica recorded the highest number of macroinvertebrates from the order Ephemeroptera and recorded the highest total overall abundance of macroinvertebrates as well (Table 3.4). It was followed by Wildlife Estate and Bridge Below Resort. The possible reasons for the abundance of macroinvertebrates at these sites could be due to high habitat diversity, besides ecological factors such as, water quality, substrate and food availability (Okarafor *et al.* 2012).

The order Diptera was dominated by the families Simuliidae, Chironomidae, and Athericidae. The families Simuliidae and Chironomidae are well known for their high tolerance to pollution, these families were found at all the sites, but the largest number was recorded at Hippo Jessica and the lowest was recorded at Bosshoff Farm. Most of the Dipteran families tolerate a wide range of water qualities, they can survive perfectly in good water quality as well as in very polluted waters (Bartlett-Healy *et al.* 2012). Most of them are not affected by the low dissolved oxygen levels, simply because some of the families such as, Chironomidae, Dixidae and Culicidae are capable of using the atmospheric oxygen and they are therefore less affected by the

water quality (Bartlett-Healy *et al.* 2012, Riens *et al.* 2013). If a river of balanced diversity of macroinvertebrates get contaminated, the pollution intolerant population will die out while the tolerant population such as, Diptera remains (Griffin *et al.* 2015). Therefore, the presence of the order Diptera together with other orders such as Ephemeroptera and Trichoptera, in considerable numbers is an indication of a balanced community (Griffin *et al.* 2015).

The third most abundant order was Trichoptera, dominated by the families; Hydropsychidae, Elmidae, and Philopotamidae. All the dominating families in the order Trichoptera were moderately tolerant to pollution and they were extensively encountered throughout the sampling sites. Members of the family are capable of surviving a wide range of water conditions, if found in abundance they may indicate average stream quality (Griffin *et al.* 2015). However, the sensitive and moderately tolerant macroinvertebrates were mostly dominating in number at every site. These could be an indication that the water quality is good and similar at almost all the sites.

The Wildlife Estate has the highest macroinvertebrate diversity with 32 families while the Wildrivers Estate recorded the lowest with 23 families. Species diversity is said to be variable in rivers in response to disturbance, suitable habitat availability and resource availability. Higher diversity is an indication of equal or close to equal opportunity of co-existence (Olomukoro & Ezemonye 2007). Therefore, higher diversity is better than higher abundance because a decreased diversity with a corresponding increased abundance of few taxa is an indication of community disturbance and imbalance (Morphin-Kani & Murugesan 2014). The high macroinvertebrate diversity at the Wildlife Estate could be an indication of a stress-free environment and the very low diversity at Wildrivers Estate could suggest that the environment is under some sort of strain or lack of habitat availability (Morphin-Kani & Murugesan 2014).

The differences between the sites based on the abundance and diversity of macroinvertebrates were observed through a cluster analysis (Figure 3.4). The cladogram grouped the sites into three clusters, the first cluster was Hippo Jessica which recorded the highest number of macroinvertebrates, followed by Wildrivers Estate. The other sites were grouped together because they did not show much difference in their abundance and diversity. The one-way ANOVA also did not show

any significant difference in recorded macroinvertebrate taxa among the sites ($p>0.05$). This was also supported by the correlation analysis (Table 3.5). There was a positive correlation between all the sites at Blyde River ($r>0.4$), indicating that the recorded taxa did not vary much among the sites. The strongest correlation was found between Swadini Resort and Bridge Below Resort ($r=0.813$), probably because Swadini Resort is close to Bridge Below Resort and may have similar conditions.

The CCA analysis revealed that the families Baetidae, Heptageniidae and Caenidae had a positive correlation with high dissolved oxygen recorded in the Blyde River. This could be because the families fall under the order Ephemeroptera which is known for its high affinity to dissolved oxygen (Dickens & Grahams 2002). On contrary, most of the tolerant families such as, Chironomidae and Hirudinea from orders Diptera and Annelida respectively had a negative correlation with the high dissolved oxygen. These are probably because these families are not affected much by the level of oxygen, because they can tolerate pollution (Riens *et al.* 2013).

The families Naucoridae and Physidae were always logged near the pollution factors indicating that they are less affected by the concentrations of metals and other water quality parameters. It could be because these families can tolerate pollution to a certain extent (Gerber & Gabriel 2002) or because the pollution level of the Blyde River was very low. The family Physidae has a high level of tolerance to pollution (Dickens & Grahams 2002, Appendix A). On the other hand, the family Naucoridae has the ability to live in moderately impaired streams because of their semi-aquatic lifestyle (Rios-Touma *et al.* 2014). However, these two families were recorded in very small numbers in the Blyde River, three individuals of Physidae and two individuals of Naucoridae. Therefore, these families could not possibly give reliable information about the total water quality of the Blyde River due to their minimum occurrence frequency (Gauch 1982, Cao *et al.* 2001). Lack of a clear trend in the distribution of macroinvertebrates in the CCA analysis further shows the similar nature of the conditions at the various sites, which also indicates that the river is generally of good quality. This could also explain why common taxa were found at all the sites.

Generally, the total macroinvertebrates abundance was higher in the cooler seasons (winter & spring) and lowest in summer. The seasonal differences may have been due to changes in abiotic factors such as, the amount of rainfall, water level, food

availability, habitat availability, temperature, dissolved oxygen as well as life history parameters (Dallas 2004, Meyer *et al.* 2007). For example, during summer there were fewer families of macroinvertebrates recorded at almost all the sampling sites. These might be due to the high rainfall that was experienced in the summer season that resulted in the change in flow regime and consequently altering the macroinvertebrate community (Olomukoro & Ezemonye 2007). During rainy seasons, rivers overflow and most macroinvertebrates get dislodged and washed away by the high current and velocity of water bodies, which may have impacted the availability of habitat and macroinvertebrates community structure (Dallas 2004). However, in the cooler seasons the water level was lower with weak currents, and therefore macroinvertebrates were able to cling on to substrates without being washed away by water currents.

In terms of SASS indices, the SASS score, number of taxa and average score per taxon (ASPT) were highest in winter followed by autumn, spring and then summer. However, the SASS score and number of taxa were highest in spring at Wildlife Estate (174), Hoedspruit Bridge (164) and Bosshoff Farm (148), this could be an indication of improved habitat diversity and river conditions due to the seasonal changes. Even though the SASS score and number of taxa were highest in spring at Wildlife Estate but its ASPT was lower during that season. This might be due to the fact that most of the families recorded in spring had lower quality values that contributed to the lower ASPT value (Gerber & Gabriel 2002, Dallas 2007). The overall SASS indices showed that the water quality at most of the sites at Blyde River could be considered as good. Most of the sites on average fall under ecological band A and B, thus, the water quality status can be described as unmodified and largely natural with few modifications respectively (Table 3.1).

The Wilddrivers Estate is the only site that falls under the average ASPT range of less than 6 and ecological band E/F which is described as seriously critically modified (Table 3.1). The reason behind these could be the lack of habitat diversity at Wilddrivers Estate. It is also the last site downstream before its confluence with the Olifants River, so there is a possible mixing of water from the Olifants River. Another cause could be the reduction of flow downstream, Wilddrivers Estate always had the lowest flow as compared to the other six sites. These might be largely due to off-stream farm dams and water abstraction by agricultural activities upstream (Young *et al.* 2004).

The metal concentrations in the tissue were higher than that in the water column but lower than some metals in the sediments (Goodyear & McNeill 1990). However, Cd, Cu, Hg, Mn, and Zn were found to be higher in the tissue than in the sediments. This is an indication of bioaccumulation (Chiba *et al.* 2011). The recorded Cd, Hg, Pb and Sb concentrations in the macroinvertebrate tissue were consistent to that of the study by Goodyear & McNeill (1990), and Amiard *et al.* (2006). The reason for the high concentrations might be the functional feeding group of the macroinvertebrate order selected (Odonata) which is mostly made up of predators found at the top of the food chain. Predators accumulate greater concentrations of metals than the other functional feeding groups through biomagnification (Davies & Day 1998, Farag *et al.* 1998).

The metal concentrations recorded in the macroinvertebrate tissues in the current study were comparable to the metal concentrations recorded in the macroinvertebrate tissue and fish muscle of other studies. Several studies indicated that macroinvertebrates accumulate more metals than fish (Michael & Mathis 1977, Caçador *et al.* 2012, Jooste *et al.* 2013). This could be because of the reduced mobility of most macroinvertebrates which makes their metal accumulation behavior different from that of fish which is more mobile. Most macroinvertebrates are also bottom-dwelling, sometimes buried in the bottom sediments, making themselves susceptible to metal accumulation from the sediments (Caçador *et al.* 2012). It was observed that Cd, Cu, and Zn recorded in the current study were extremely high as compared to the detected levels in the muscle tissue of fish (Caçador *et al.* 2012, Jooste *et al.* 2013). However, the detected metal concentration in the macroinvertebrate tissue of the current study did not differ much with those detected in macroinvertebrates of other studies (Chiba *et al.* 2011, Caçador *et al.* 2012).

Zinc and Cu are metabolically essential metals. However, any excess of these metals even in their metabolical form can be potentially toxic with a need for excretion or detoxification (Rainbow 2002). If the metal uptake of Zn and Cu exceeds the excretion and detoxification rate, the macroinvertebrates may experience toxic effects of the metal or death (Rainbow 2002). According to Chiba *et al.* 2011, metals like Zn are found in high concentrations in fertilizers used in agriculture, which is practice intensively in the Blyde River catchment. Zinc was recorded in high concentrations in macroinvertebrate tissue than in sediments and water,

Cadmium is also a metal of concern because it is very toxic, and it was detected in high concentrations in the macroinvertebrate tissue ranging from 0.09 mg/kg to 0.5 mg/kg in the current study. Cadmium is a cumulative poison that can be emitted from pesticides. Even though the metal can enter the aquatic ecosystem from land at a slower rate, it can accumulate in aquatic organisms in high concentrations (Cacodor *et al.* 2012). This is basically because the metal is not known to be metabolically functional in animals including macroinvertebrates, therefore, it cannot be used up metabolically or biodegraded naturally (Michael & Mathis 1977, Rainbow 2002, Cacodor *et al.* 2012). The toxic actions of Cd include disruption of oxygen uptake by organisms and consequently death (Michael & Mathis 1977).

Mercury was also detected in the macroinvertebrate tissue at Blyde River. It can be sourced from natural weathering of Hg bearing rocks and in many other anthropogenic activities including the burning of fossil fuels. Mercury accumulates in aquatic organisms and attains its highest concentrations in predatory organisms (Sinha *et al.* 2007). However, the recorded Hg concentration in the current study was lower compared with that recorded by Sinha *et al.* 2007 in the macroinvertebrate tissue.

Even though there are no quality guidelines for Mn in the sediments and macroinvertebrate tissue, the metal was detected in very high concentrations as compared to other studies (e.g. Jooste *et al.* 2013 and Magala 2012). The target water quality range of Mn in the water column is 0.18 mg/l, however, it was detected in the sediments at an average of 835.45 mg/kg and 2509.61 mg/kg in the body tissue of the macroinvertebrates. Manganese is a very important micronutrient for living organisms. Its absence or deficiency may cause chlorosis in plants, skeletal deformities and reduced reproductive capabilities in animals (DWA 1996). However, excess Mn in organisms or its surrounding may become toxic and consequently impair the functioning of the central nervous system (Niemic & Wiśniowska-Kielian 2015). The reason for the abundance of Mn in the Blyde River may be from natural sources (Niemic & Wiśniowska-Kielian 2015). Manganese is also used as a micro-nutrient fertilizer additive, which makes sense to find it in high concentrations in an intensive agricultural area like the Blyde River catchment. Swadini Resort was the site with the highest concentrations of metals recorded in the body tissue of macroinvertebrates, and the lowest readings were recorded at Wildlife Estate. This confirms that the

Wildlife Estate has very good water and sediment quality, hence the macroinvertebrates there bioaccumulate fewer metals in the tissues.

Chapter 4: General Discussion and Conclusions

4.1. Physico-chemical parameters

There was no significant difference among seasons or sites in most of the tested physico-chemical parameters except for salinity seasonally. This was an indication that the river's water quality did not differ much across the sites. The pH values recorded in this study were all slightly above 8.0 indicating an alkaline condition. These conditions might have been brought about by the photosynthetic activities taking place in the river (Matlou *et al.* 2017). The water was also found to be well oxygenated with average oxygen levels above 7 mg/l at all sites. The high oxygen levels in the water is an indication of good water quality (Davies & Day 1998). Despite the extensive farming that takes place in the Blyde River catchment, most of the nutrients were found to be within the recommended guidelines. It can, therefore, be concluded that the agricultural activities in the catchment have no significant negative impact on the Blyde River yet. Thus, the water from the Blyde River could be said to be providing water of good quality to the Olifants River (Ashton *et al.* 2001). However, phosphorous indicated eutrophic condition with concentrations above 0.005 mg/l (DWAF 1996).

4.2. Metals in the water

Twenty metal concentrations were tested from the water samples at Blyde River. Out of the twenty, six (Al, As, Cu, Fe, Ag, and Zn) were found to have exceeded the water quality guidelines at some sites (Table 2.2). However, the rest of the metals were within the recommended levels at all sites (Sb, Ba, B, Cd, Cr, Co, Pb, Mn, Ni, Se, Sr, Sn, Ti, and V) (DWAF 1996, CCME 2012, US-EPA 2012). Metals such as Al, Cu, and Zn if detected in higher concentrations, are likely to have detrimental effects on the aquatic ecosystem with lower pH (DWAF 1996, Fakoti *et al.* 2002). However, their presence may be rendered less toxic to the aquatic organisms due to the alkalinity of the Blyde River (Davies & Day 1998). The TWQR for Al is 0.001 mg/l at pH<6.5 and the pH values of the river ranged from 8.33 to 8.67. Therefore, Al is currently not a problem (DWAF 1996). In general, the results of the study indicated that the river is still in good ecological condition, this conclusion is in accordance with that of Marr *et al.* (2017b). The TWQR for Cu in the soft water like that of the Blyde River is 0.0003 mg/l at TWH<60. This limit was exceeded in the river making the water slightly

unsuitable for aquatic ecosystem use in terms of Cu. Iron was only found to be above the recommended guidelines at the Wildrivers Estate (CCME1995). The TWQR for Zn in water is 0.002 mg/l and the limits were exceeded at all sites in the Blyde River making the water unfavorable for the aquatic organisms (DWAF 1996) however, it was below the CCME guidelines (CCME 2012). Generally, the source of metal pollution in the Blyde River could be largely due to the dominating agricultural activities and agricultural runoffs, however, there could be contributions from natural sources as well

4.3. Metals in the sediments

Out of the 20 metal concentrations tested in the sediments, only three (As, Cr and Cu) exceeded the quality guidelines (CCME 2012). Arsenic levels in the sediments ranged from 29.04 mg/l to 107.57 mg/l and they exceeded the quality guidelines of 5,9 mg/kg for aquatic ecosystems at all sites. The high levels of As can cause detrimental effects on the aquatic organisms and livestock, the water can also be rendered unfit for irrigation due to the As levels. However, there are other factors that can determine the toxicity of As such as, pH, in this case, the pH of the Blyde River was slightly alkaline which is a good state to neutralise metal toxicity such as As (DWAF 1996). The main source of As could be from Agricultural activities. Furthermore, Cr and Cu were found to be slightly higher than the recommended guidelines in the sediments, probably because they are incorporated in fertilizers as macronutrients (Fakoti *et al.* 2002).

4.4. Aquatic Macroinvertebrates

Of the seven sites sampled during this study, most of the sites were similar in terms of macroinvertebrate assemblage and diversity. These could be an indication of uniform water quality conditions throughout the river. However, a slight difference was observed, and the variability might have been brought about by the types of microhabitats that were available at the different sites (Dallas 2007). Macroinvertebrates were sampled at stones in current biotope except at Wildrivers Estate which is dominated by sand and bedrock. It could be for that reason that the SASS score, No. of taxa and ASPT values were lower at Wildrivers Estate. Most importantly, the Wildrivers Estate is located at the confluence with the Olifants River, where there is a possible mixing of water from the two rivers. It is therefore not surprising to find water conditions and macroinvertebrate taxa slightly compromised

at that site, the Olifants River is one of the most polluted rivers in South Africa (Grobler 1994, Ashton & Dabrowski 2011, Addo-Bediako *et al.* 2014, Marr *et al.* 2017a).

The variability of macroinvertebrate assemblage says a lot about the conditions of a river at different sites because macroinvertebrates respond rapidly to changes in the water quality (Dickens & Grahams 2002, Matlou *et al.* 2017). However, the one-way ANOVA indicated no significant difference between the sites ($p > 0.05$) in terms of macroinvertebrates abundance and diversity. Aquatic macroinvertebrates were found in abundance in the cooler seasons than in the warmer seasons, and the most tolerant families were found in numbers in winter than in summer. The possible reason could be that the general condition of water in winter is more stable than in summer.

Overall, the macroinvertebrate assessment indicated good river health conditions with high ASPT values along the Blyde River. It was confirmed statistically by no significant differences among the sites, thus having similar river conditions. Secondly, the distribution showed the dispersal of macroinvertebrates at different sites randomly spread out, irrespective of their level of tolerance to pollution. This is an indication of a balanced community of macroinvertebrates and uniform water quality conditions in the river. The macroinvertebrate findings in the Blyde River were better than that of its main stem, the Olifants River, with lower ASPT values as compared to the Blyde River (Marr *et al.* 2017a & b). These imply that the water of the Blyde River is of good quality as compared to that of the Olifants River, thus the Blyde River as a tributary has a positive impact on the ecological conditions of the Olifants River.

Comparing with the concentrations in the tissue of macroinvertebrates, some of the metals (As, Cr, Ni, Pb, and Sb) were found in higher concentrations in the sediment than in the tissue of aquatic macroinvertebrates. While some metals (Cd, Cu, Hg, Mn, and Zn) were found in higher concentrations in the body tissue than in the sediments. The aquatic invertebrates (Odonata) may have bioaccumulated the metals from other species that process sediments as food sources (Demirak *et al.* 2005).

5. Conclusion

The aim of the study was to assess the water and sediment quality of the Blyde River and their impact on the aquatic macroinvertebrate communities. The study has shown that the water and sediment quality of the Blyde River is still good, despite the farming activities taking place in the catchment. The good quality of the river was proved by the diversity and abundance of aquatic macroinvertebrates at the different sites of the Blyde River which have shown a balanced community. Most of the recorded physico-chemical parameters, nutrients and metals were also within the recommended quality guidelines and target water quality ranges. The water of the Blyde River is therefore in better ecological condition than its main stem river (the Olifants River), and thus the Blyde River contributes positively to the ecological conditions of the Olifants River through its water quality and quantity. However, some metals were detected above the TWQR or recommended quality guidelines. This is an indication that the water quality of the river is slowly deteriorating.

6. Recommendations for future studies

The present study has indicated that the water quality of the Blyde River is still in a good condition, however, the few recorded elevated nutrients (phosphorous) and heavy metals (As, Ag, Cr, Cu, and Zn) is an indication of deterioration. Therefore, further studies and monitoring programmes should be conducted to start addressing the long-term potential risks. The studies should identify and address the possible sources of pollution in the catchment.

Detailed bioaccumulation studies of macroinvertebrates and other biota should be conducted as they are good reflectors of their environment, to determine the level of bioaccumulation of metals in these aquatic biotas. In the current study agricultural activities were identified as the main possible sources of nutrient and metal pollution. Therefore, proper methods of improving food production without causing environmental pollution through the use of agrochemicals should be implemented.

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Appendices

Appendix A

Table A 1: The quality values (QV) of aquatic macroinvertebrates (Dickens & Grahams 2002).

Order	Family		QV
Ephemeroptera	Baetidae	BAE	12
	caenidae	CAE	6
	Heptageniidae	HEP	13
	Teloganodidae	TEL	12
	Leptophlebiidae	LEP	9
	Tricorythidae	TRI	9
Trichoptera	Hydropsychidae	HYD	12
	Philopotamidae	PHI	10
	Leptoceridae	LEP	6
Coleoptera	Gyrinidae	GYR	5
	Elmidae	ELM	8
	Helodidae	HEL	12
	Psephenidae	PSE	10
Hemiptera	Naucoridae	NAU	7
Odonata	Libellulidae	LIB	4
	Aeshnidae	AES	8
	Gomphidae	GOM	6
Zygoptera	Chlorocyphidae	CHL	10
	Platycnemididae	PLA	10
	Coenagrionidae	COE	4
	Protoneuridae	PRO	8
Diptera	Athericidae	ATH	10
	Blephariceridae	BLE	15
	Tabanidae	TAB	5
	Dixidae	DIX	10
	Chironomidae	CHI	2
	Muscidae	MUS	1
	Simuliidae	SIM	5
Plecoptera	Perlidae	PER	12
Crustacea	Potamonautidae	POT	3
Annelida	Hirudinea	HIR	3
	Oligochaeta	OLI	1
Mollusca	Physidae	PHY	3
	Planorbidae	PLA	3
	Thiaridae	THI	3
	Corbiculidae	COR	5