Exposure and effects of platinum and associated metals on the aquatic ecosystems within a platinum mining region

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SUMMARY

Platinum group elements are precious metals that occur in nature only in trace concentrations. These metals are indispensable for humans due to their unique catalytic properties while they are also resistant to corrosion. Platinum group elements are used in various applications from automotive catalytic converters to medical applications and are emitted to the environment to such an extent that anthropogenic fluxes exceed natural fluxes. Due to its exceptional properties and innovative applications, the demand for these metals exceed the supply and the demand is predicted to keep increasing in the future. Three-quarters of the world's platinum supply are from South Africa, and is situated in the Bushveld Igneous Complex. These intensive platinum mining activities have detrimental effects on aquatic ecosystems draining the mining regions. The Hex River is the main arterial drainage for this productive platinum mining region. However, mining activities are not the only stressors within this system, the city of Rustenburg and its industrial activities, as well as informal settlements also contribute metal and nutrient inputs to the Hex River.

Therefore, the aims of the present study were to assess the Hex River system by means of water and sediment quality, as well as bioassessments (*i.e.* aquatic macroinvertebrates, fish, and associated parasites). Firstly, the primary source of platinum and associated metal pollution to the system was determined. Due to seasonal changes and altered physicochemical variables, the behaviour of metals in water and sediments within this system were assessed. Secondly, the macroinvertebrate community structure was determined and evaluated how the mining activities alter it from reference to impacted conditions. Thereafter, the bioaccumulation of metals by macroinvertebrates was also assessed to determine whether these metals are bioavailable. Thirdly, the fish health and associated human health risk associated with the consumption of these fish were determined and lastly, the metal accumulation in associated fish parasites were assessed.

From the results it was confirmed that the intensive mining activities were the main source of Cr, Ni, Cu, As, and Pt contamination, while urban effluent contributed to Zn, Cd and Pb contamination in the Hex River. The metal behaviour was also influenced by several physicochemical variables and affected the bioavailability, uptake and toxicity of metals in the system. The intensive mining activities had a detrimental effect on macroinvertebrate community structures and decreased the species diversity from reference to impacted sites. Tolerant species thrived at the impacted sites and had positive relationships with the pollutants (Cl, SO_4, Cr, Ni, Pt) entering these sites. These metals were accumulated in several macroinvertebrate families, indicating that the metals were bioavailable to aquatic biota and the families Lymnaeidae, Baetidae, Tubificidae, and Chironomidae emerged as great biomonitoring organisms. The concentrations of platinum in the family Tubificidae were several times higher than freshwater clams from urban systems, indicating

that platinum from mining activities are to a similar or even higher degree bioavailable to biota than platinum from auto-catalysts.

Fish from this system also accumulated high metal concentrations and posed not only a threat to the fish health but also a health risk to humans that consume these fish. The fish from the impacted site were associated with a 10 – 90% higher human health risk compared to fish from the reference site and could potentially cause several carcinogenic and non-carcinogenic risks. The associated fish parasites accumulated higher metal concentrations than their fish host species and indicated which metals were bioavailable within the aquatic system, due to their lack of a digestive system. The parasites also accumulated non-essential metals more readily compared to the fish that accumulated essential metals more easily. It was also evident that platinum and associated metals from mining activities were as bioavailable to aquatic biota as platinum from auto-catalysts.

The present study concluded that the Hex River is subjected to various stressors especially intensive mining activities, while urban and industrial effluent also contributed to metal and nutrient contamination. The metals that entered the Hex River were bioavailable to several aquatic biota species and posed a threat not only to the ecosystem health but also to humans utilizing fish and water from this catchment. The present study provided valuable data and information on the exposure and effects of platinum and associated metals on the aquatic ecosystems within a platinum mining region.

Keywords:

Platinum, auto-catalyst, biomonitoring, bioaccumulation, bioavailability, human health risk

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Platinum group elements (PGE) are precious metals that are naturally found only in trace concentrations in the Earth's crust and consists of iridium (Ir), osmium (Os), palladium (Pd), platinum (Pt), rhodium (Rh), and ruthenium (Ru) (Peucker-Ehrenbrink and Jahn, 2001; Hoppstock and Sures, 2004; Mudd *et al*., 2018). These metals are indispensable to industrial applications and are commercially important due to unique properties such as their resistance to corrosion and oxidation, electrical conductivity, high melting points and excellent catalytic properties (Zereini and Wiseman, 2015; Zientek *et al*., 2017). The elements of most commercial significance are Pt, Pd, and to a lesser degree, Rh and these are also naturally found in higher concentrations (Pawlak *et al*., 2014). It occurs in sufficient quantities for mining, mainly in South Africa, Russia and the United States (Pawlak *et al*., 2014; Mudd *et al*., 2018). South Africa is the world's main producer of PGE with 73, 39 and 82% of Pt, Pd and Rh of global production in 2018, respectively (Johnson-Matthey, 2018).

Platinum group elements are mostly used in automotive catalytic converters (37%) (Pawlak *et al*., 2014) and are emitted into the environment through diffuse release from vehicles. Also, chemical (25%), electrical (12%), glass (16%) industries, medical and dental applications (10%), as well as mining are primary sources of PGE (Zereini and Wiseman, 2015; Johnson-Matthey, 2018). Anthropogenic activities are releasing PGE into the environment to such an extent that anthropogenic fluxes are surpassing natural fluxes (Rauch and Peucker-Ehrenbrink, 2015). This increase in PGE concentrations into the environment from anthropogenic activities prompted researchers to assess the exposure and effects of PGE on the environment. Consequently, considerable research has been done on PGE accumulation from automotive catalysts in road and airborne dust, as well as in soils in the vicinity of roads of different cities worldwide (summarized in Okorie *et al*., 2015; Zereini and Wiseman, 2015). Elevated concentrations of PGE in organisms living in and on contaminated soils, and sediments were found (summarized in Sobrova *et al*., 2012; Ruchter *et al*., 2015). These catalyst-derived PGE particles are introduced via surface runoff into sediments of rivers and other water bodies (Ruchter *et al*., 2015). The solubility of PGE is highly variable between the individual elements, as well as in different sample media, whereas PGE in environmental samples have a higher solubility than those in the original catalytic converter materials (Bruder *et al*., 2015). This is due to several substances that are present in the environment, such as complexing agents, which modulate the solubility of PGE post-emission, Pt especially has a higher solubility in the presence of organic compounds such as acetate (Zimmermann *et al*., 2003). Other environmental variables also influence the solubility of Pt such as pH, particle size, as well as the concentrations and type of anion species which makes it more bioavailable (Zereini *et al*., 2015). In the last three decades, research has increased on PGE and provided information on the increase of PGE concentrations

in the environment, the chemical behaviour of PGE that includes the mobility, solubility, bioaccesibility and its toxic potential (Zereini and Wiseman, 2015). However, according to Rauch and Fatoki (2015), only a few studies focused on PGE discharges from PGE mining and production activities, while data on the occurrence of PGE in biota near PGE production sites is still lacking.

In South Africa, the resources of PGE are located within the Bushveld Igneous Complex (Cawthorn, 2010). The Bushveld Igneous Complex is situated in the north-western part of South Africa and consists of a western and eastern limb. Intensive mining activities (PGE and Cr) in the Bushveld Igneous Complex require large volumes of water (192 – 1 086 m^3/kg PGE) for production activities and the removal of waste products (Glaister and Mudd, 2010; Haggard *et al*., 2015). Although most of this water is reused within the production process, leaks from transport pipelines and seepage from tailing dams can cause the production water to enter aquatic ecosystems. While the effects of these waste discharges to aquatic ecosystems remain unclear, it can cause disturbances in the surface PGE geochemical cycle (Almécija *et al*., 2017).

The Hex River, in South Africa's North-West Province (NWP), is the major river that drains an intensive PGE mining area within the Bushveld Igneous Complex (Rustenburg area) where some of the world's most productive Pt mines are located (Ololade *et al*., 2008; Almécija *et al*., 2017; Johnson-Matthey, 2018; Erasmus *et al*., 2020). Platinum and other metals associated with Pt mining activities can enter the Hex River from untreated mining water, seepage from tailing dams, runoff from waste dumps or via aerial deposition from ore smelters and dust from waste dumps and tailing dams (Salomons, 1995; Hudson-Edwards, 2003; Miller *et al*., 2007; Resongles *et al*., 2015). This river is not only subjected to Pt and Cr mining activities, but also receives urban and industrial effluent from an increasingly growing (± 3.0% per annum) city of Rustenburg, the most populated municipality, with around 626 522 people currently living here, in the NWP (Ololade *et al*., 2008; Stats SA, 2016; Almécija *et al*., 2017). Increasing nutrient inputs enter the Hex River from treated sewage derived from the city of Rustenburg. Raw sewage originating from the rapid growth of informal settlements in close proximity to the river, further contributes to the nutrient inputs (Ololade *et al*., 2008; Stats SA, 2016).

1.2 Assessing ecosystem health of rivers

Increased pollutant concentrations in aquatic ecosystems from anthropogenic activities (*i.e.* mining, industrial, agricultural and urban), make it essential to monitor and assess the ecosystem health of aquatic systems (Zhang *et al*., 2018; Chetty and Pillay, 2019; Marshall and Negus, 2019). Platinum mining, as with all other base metal mining activities, are responsible for an influx of various metals and metalloids (hereafter referred to as metals), as well as several other chemicals used in the mining and production process (Salomons, 1995; Macklin *et al*., 2006; Hadzi *et al*., 2019). These pollutants can have detrimental effects on aquatic biota that occurs within these systems (Hassaan *et al*., 2016; Chetty and Pillay, 2019; Landers

et al., 2019). It is, therefore, critically important to monitor river health to ensure that river systems are used in a sustainable manner so that ecological, social, domestic, and economic demands are satisfied (Zhang *et al*., 2018). To assess river ecosystem health comprehensively, physical, chemical, and biological indicator variables need to be assessed to ensure that a holistic perspective is obtained. Various assessments aid in the collection of these variables, which in turn help to conclude whether an ecosystem is in a healthy or unhealthy state. Conventional ecosystem health assessments include various indices to assess the health status. The present study, however, followed an ecotoxicological approach to assess the ecosystem's health.

1.2.1 Water and sediment quality assessment

To determine water and sediment quality in river systems, it is important to distinguish whether the assessment is related to ecosystem health or human consumption, as these assessments depend on different guidelines to indicate whether the water and sediment quality is good or bad. Water and sediment variables can vary considerably in river systems. This is due to chemical factors such as adsorption to sediments and suspended particles, solution reactions, and precipitation, as well as physical factors such as dilution, sedimentation, advection, dispersion, and flow velocity (Chapman, 1998; Ali *et al*., 2016; Lin *et al*., 2018; Rathoure, 2020). Fluctuations in the hydrology of rivers are essential to be monitored seasonally, to obtain a holistic overview of the health of the system. Water samples provide a short term indication of river health at a specific time interval, where numerous factors should be monitored such as *in situ* parameters (*i.e.* pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), and temperature), as well as nutrient, metal, industrial chemicals (PCBs and furans/dioxins), and hydrocarbon concentrations (Chapman, 1998; Jachimowski, 2017; Park *et al*., 2019). While sediment samples provide a longer term indication of river health (Sierra *et al*., 2017), chemical indicators (*i.e.* metals and hydrocarbons) and physical indicators (*i.e.* particle size and organic carbon content) need to be monitored (Simpson *et al*., 2005; Rozpondek and Rozpondek, 2018). The chemical and physical composition of sediments, as well as the interaction with other environmental factors, determine the species composition, diversity, and density of aquatic biota within a system (Simpson *et al*., 2005). The concentrations of metals and other pollutants in both water and sediment can have detrimental effects on aquatic biota that can bioaccumulate these pollutants if it is biologically available (Simpson *et al*., 2005; Herrmann *et al*., 2016; Kumari, 2018). It is therefore important to also measure pollutant concentrations in aquatic biota that will provide additional information on the ecosystems health.

1.2.2 Aquatic macroinvertebrate assessment

Aquatic macroinvertebrates are key components of any aquatic ecosystem. Due to their ability to inhabit various types of aquatic ecosystems, they are effective nutrient recyclers, essential food sources for numerous vertebrate species, and represent different trophic levels, therefore completing aquatic food chains (Deborde *et al*., 2016; Ojija *et al*., 2017; Pastorino *et al*., 2020).

These organisms are considered as good bioindicators of aquatic systems, since they are indicators of effects of both short and long term pollution. Some species are more intolerant while other species are very sensitive to pollution and can be affected by biological, chemical, and physical conditions, hence their ability to represent the accumulative effects of pollution (Ojija *et al*., 2017; Mangadze *et al*., 2019; Pastorino *et al*., 2020). Using macroinvertebrates as bioindicators, rather than algae and fish species, have an advantage as the former do not experience rapid blooms and mortality in response to nutrient pollution, have lower mobility and thus cannot escape pollution as easily (Deborde *et al*., 2016). Finally, they are simple and cost-effective to sample, and using appropriate guidelines are relatively easy to identify (Mangadze *et al*., 2019).

1.2.3 Fish assessment

Fish have a greater species diversity than all other groups of vertebrates (Lima *et al*., 2017), while fish behaviour and its biological traits are also well studied. Due to its importance as a food source and its recreational value, fish are of high importance to humans (Resh, 2008). Fish have longer life expectancies than macroinvertebrates, can reveal longer term exposure events and accumulation patterns, while residing in different trophic levels and can indicate whether biomagnification of pollutants in the food web occurs (Chovanec *et al*., 2003; Ruaro *et al*., 2016). Typically, predatory fish are at the top of aquatic food webs, provide a food source for other vertebrates (*i.e.* birds, reptiles, mammals, humans) and are important to monitor in order to assess which pollutants can biomagnify and pose a risk to ecosystem and human health (Authman *et al*., 2015).

1.2.4 Environmental parasitology

While fish can be used as bioindicators, their associated parasites can also provide valuable information on metal pollution in aquatic ecosystems (Nachev and Sures, 2016; Sures *et al*., 2017). Endoparasites are not exposed to the ambient environment and are only exposed to pollutant concentrations through the hosts they inhabit. These parasites (Acanthocephala, Cestoda, Nematoda, Digenea) have the ability to accumulate various elements, especially non-essential elements, at extraordinarily high concentrations (Sures *et al*., 2017). Due to the ability to accumulate elements in higher concentrations than their fish hosts, these parasites can be used to reliably detect elements that naturally occur in trace concentrations (Sures, 2004). Some endoparasite taxa also lack a digestive system, thus they indicate which metals are biologically available and can be useful to provide valuable information on the bioavailability of pollutants in aquatic ecosystems (Sures and Siddall, 1999; Sures *et al*., 2017).

1.3 Problem statement, hypotheses, and aims

Platinum group elements naturally only occur in trace concentrations. However, anthropogenic activities have increased PGE concentrations worldwide in the last 30 years and PGE concentrations can even be detected in remote places on Earth, such as Greenland (Rauch and Peucker-Ehrenbrink, 2015). Anthropogenic sources of PGE into the environment have mainly been attributed to automobile catalytic converters, but an increase in metal production, chemical and electrical industries, as well as medical applications also attribute to environmentally detected concentrations (Pawlak *et al*., 2014). The supply of PGE worldwide has increased from 2014 to 2019 by 21, 15, and 27% for Pt, Pd, and Rh, respectively. However, due to its exceptional properties, the use in applications of PGE have increased the demand by 4.1, 4.5, and with 12% from 2014 to 2019 (Johnson-Matthey, 2018). Notwithstanding the demand still surpasses the supply by 38, 59, and 41% for Pt, Pd, and Rh, respectively.

Extensive studies have been completed on PGE emissions into the environment from automobile catalytic converters and the effects thereof on aquatic and terrestrial ecosystems (summarised in Zereini and Wiseman, 2015). However, only limited studies have focused on discharges from PGE mining and production activities. Data on environmental concentrations, especially in biota near these production sites, are still lacking. Almécija *et al*. (2017) found elevated concentrations of Cr, Ni, Cu, and Pt within the Hex River's sediment, while Maboeta *et al*. (2006) reported that concentrations of Ni and Cu in soil samples are associated with Pt mining activities. Rauch and Fatoki (2013) also found an association with the concentrations of Cr and Cu with Pt mining, especially in the Bushveld Igneous Complex, and reported high levels of Pt in soil samples, that were affected by Pt mining activities. This Ph.D. study will focus on Pt exposure from mining activities in the world's most productive Pt mining region, and assess the exposure to the aquatic environment, as well as aquatic biota. It will also focus on the effects of Pt and associated metals on the aquatic biota within the system.

1.3.1 General hypotheses

The hypotheses tested in the present study:

1. Platinum smelters and production activities will be the main contributor to metal (Cr, Ni, Cu, Zn, As, Cd, Pt, Pb) pollution in the Hex River catchment.
2. The behaviour of Pt and associated metals in water and sediments will be influenced by altered physicochemical parameters as a result of changes in seasonal river runoff patterns.
3. Macroinvertebrate community structures will be significantly altered from the reference conditions due to Pt mining related responses and, macroinvertebrate traits will be altered as a result of changes in water and sediment quality.

4. Macroinvertebrate families from the impacted sites will accumulate higher metal concentrations compared to the reference sites, while predator families will accumulate higher metal concentrations than lower trophic level families.
5. Fishes collected from the impacted impoundment (Bospoort Dam) downstream of the Pt mining and urban activities will accumulate higher metal concentrations and have impaired health when compared to fishes from the reference impoundment (Olifantsnek Dam) upstream of all the mining activities, while fish from the impacted system will consequently pose a human health risk to people that consume these fish.
6. Fish endoparasitic helminths will have higher metal concentrations at the Pt mining impacted site compared to the reference site, infected fish will have lower metal concentrations than uninfected fish, and biomarker responses in infected fish will be more pronounced compared to uninfected fish.

1.3.2 Aims

To test the above hypotheses, the main aims of the present study were to:

- Determine the main sources of Pt and associated metal contamination to the Hex River catchment; to assess the interaction between water and sediment metal concentrations associated with Pt mining activities in the Hex River, and compare the metal concentrations to international guideline values.
- Determine the water and sediment physicochemical variables from seasonal surveys (2017 – 2018) at selected sites in the Hex River and assess the interaction between the metal concentrations and these variables.
- Determine whether spatial or temporal changes have occurred within the aquatic macroinvertebrate community structures due to Pt mining.
- Determine the behaviour of metals associated with Pt mining in the Hex River system, as well as the metal concentrations in water, sediment and aquatic macroinvertebrates at different impacted sampling sites along the Hex River, and evaluate the bioaccumulation of metals in various macroinvertebrate families.
- Determine the metal accumulation in three important fish species from impoundments in the Hex River system and to assess the potential human health risk posed by the consumption of these fish species.
- Determine the metal accumulation in helminth parasite species compared to their hosts from a reference and Pt mining impacted impoundment, to assess whether there is a difference between bioaccumulation of metals in infected and uninfected hosts, and to analyse the effects of parasite infection and metal exposure using biomarker responses.

1.4 Layout of Thesis

The present study has been divided into eight chapters:

Chapter 1: General introduction with background information relating to the rationale of the study, as well as the set hypotheses and aims.

Chapter 2: A review of the impacts of platinum mining on the Hex River catchment. Seventeen sites were sampled representing reference conditions, mining, urban, industrial and combined inputs of metals during October 2018. These sites were located in the Hex River and several tributaries.

Chapter 3: A temporal and spatial assessment of the water and sediment quality variables that influence metal behaviour in the Hex River over two years (2017-2018). Four sites in the Hex River and two sites in impoundments were sampled. Three of the sites (two river sites and one impoundment site) were located upstream of mining activities and served as reference sites, while two river sites and one impoundment site were located downstream of the intensive Pt mining activities.

Chapter 4: An assessment of the effects of platinum mining activities on macroinvertebrate community structures within the Hex River. The macroinvertebrate community structures of the six above mentioned sites were compared between reference and impacted conditions. Taxonomy-based, as well as a trait-based approach was used to assess the community structures.

Chapter 5: An assessment of metal accumulation in riverine macroinvertebrates from a platinum mining region. A reference, mining impacted, combined impacted, and a Pt mining settling pond were sampled and metal accumulation in different macroinvertebrate families were assessed. These families represented different functional feeding groups and trophic levels.

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Chapter 6: An assessment of the effects of metal pollution on fish health and human health risks, associated with the consumption of fish in an intensive platinum mining region. Three economic and ecological important fish species were collected (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*) from a reference and Pt mining impacted impoundment.

Chapter 7: An assessment of the role of parasites in monitoring environmental pollution. *Cyprinus carpio* and its associated cestode *Atractolytocestus huronensis*, as well as *Clarias gariepinus* and its associated nematode *Contracaecum* sp. were collected from a reference and impacted impoundment. The metal interaction between parasites and its host were assessed, while the effects of parasites on their hosts were also determined using biomarker responses.

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Chapter 8: An in depth discussion of the findings, as well as the conclusions drawn from the study and recommendations for future studies considering Pt mining regions.

CHAPTER 2: REVIEW OF THE IMPACTS OF PLATINUM MINING ON THE HEX RIVER CATCHMENT, SOUTH AFRICA

2.1 Introduction

Mining activities normally only comprise of relatively small areas of land. It can, however, have extensive impacts on the environment, especially the aquatic environment (Salomons, 1995). The biggest transfer of metals from mining activities to rivers and dams are from leaching or seepage of tailing dams and runoff from waste rock dumps. Leaching and seepage entering the aquatic ecosystem could be in both dissolved and in particulate forms (Salomons, 1995; Hudson-Edwards, 2003; Ochieng *et al*., 2010). Metals can also enter the aquatic environment via aerial deposition resulting from emissions of ore smelters, as well as dust from waste dumps and tailing dams (Rauch and Fatoki, 2015). Once these metals have entered the aquatic ecosystem, it can be transported long distances downstream from the point source pollution (Salomons, 1995). According to Chapman (1998), there are several chemical and physical processes that can reduce the point source contamination of dissolved metals. The chemical processes include precipitation, adsorption onto suspended particles or sediments and solution reactions, while the physical processes include dilution, advection, sedimentation, and dispersion (Chapman, 1998). Although mining activities are a major source of metals into the environment, other sources include natural weathering of minerals and ores, as well as industrial and urban effluent (Bradl, 2005; Roig *et al*., 2016).

Metal analyses of water samples only are inefficient to fully identify metal source pollution to river systems due to the variability of pollutant concentrations and flow (Roig *et al*., 2016). In addition, more than 90% of trace metals in river systems are interrelated to sediments and suspended particles (Wei *et al*., 2016), making sediments an important medium to assess metal contamination in aquatic ecosystems (Silva and Rezende, 2002). It is, therefore, essential to monitor both water and sediment quality to assess and determine the exposure and impact of pollution sources on the aquatic environment. To monitor the water quality variables in the Hex River, it is important to understand the geology and the lithology of the river catchment (Wolmarans *et al*., 2017) as these ultimately, also influence the water chemistry. The Hex River catchment is situated on diverse geology with some of the richest mineral deposits in the world (Cawthorn, 2010). North of the Magaliesberg Mountain Range, the geology consists predominantly of the Bushveld Igneous Complex and contains two platinum-bearing layers - the Merensky Reef and the Upper Group 2 (UG2) chromitite layer (Schouwstra and Kinloch, 2000; Cawthorn, 2010). The Merensky Reef consists of pegmatoidal feldspathic pyroxenite, olivine, and chromitite layers, which contain pyrrhotite, chalcopyrite, and pentlandite base-metal sulphides and are rich in minerals such as Pt, Cr, Cu and Ni (Schouwstra and Kinloch, 2000).

Land-use within the catchments is another essential component to consider when assessing water quality (King *et al*., 2018). In the Hex River, these activities include agricultural activities, industrial activities and associated effluent, intensive mining activities, urban effluent and informal settlements (Ololade *et al*., 2008). Impacts of agricultural activities on water quality include over-abstraction of water for irrigation purposes, runoff of fertilizers and agrochemicals, as well as soil erosion from runoff (du Preez *et al*., 2018). Industrial activities located in the largest city in the region, Rustenburg, which can contribute to chemical waste into the Hex River through storm water runoff and effluent discharges, are metallurgical industries, breweries, cement, ceramics and tiles, batteries, foundry, animal feed and abattoirs (du Plessis, 2006).

Intensive mining activities (especially Pt and Cr) occurring within the Rustenburg area could release metals and other chemicals associated with the mining process into surface waters. This could be directly due to the following leaching from slime and tailing dams, leaking delivery pipelines, runoff from rock and sand dumps. It could also be indirectly via air deposition from the dust of processing plants, waste dumps and tailing dams, and emissions from smelters that enter surface waters (Rauch and Fatoki, 2015). The city of Rustenburg is expanding with a subsequent increase in traffic, sewage (treated and untreated), and impervious surfaces (*i.e.* paving, roads, roofs). Impervious surfaces increase the volume of runoff, which would previously have seeped into the ground, and thus end up in surface waters. This increase in runoff also facilitates the increase of chemicals into surface waters from road dust and traffic emissions (Ruchter *et al*., 2015). In South Africa, informal human settlements generally have little to no basic services (electricity, running water, sewage removal) (DEA, 2012). The lack of these services results in an increase in nutrient inputs into surface waters via untreated sewage. These informal settlements are also generally near surface water systems such as the Hex River, leading to additional litter and general municipal waste within the system. Storm water runoff from informal settlements is thus the major contributor to non-point pollution sources entering the Hex River and its tributaries (du Plessis, 2006).

The aims for this chapter were to determine the main sources of Pt and associated metal contamination to the Hex River catchment; to assess the interaction between water and sediment metal concentrations associated with Pt mining activities in the Hex River, and compare the metal concentrations to international guideline values. The hypothesis was that Pt smelters and production activities will be the main contributor to metal pollution in the Hex River catchment.

2.2 Materials and Methods

2.2.1 Study area

The Hex River catchment is situated in the north eastern part of the North West Province, South Africa, close to the city of Rustenburg. This river forms part of the Limpopo River system and is the main regional arterial drainage for the Rustenburg area, and flows in a northerly direction into the Crocodile River (Ololade *et al*., 2008; Almécija *et al*., 2017). The Hex River catchment forms part of the Crocodile (West) and Marico Water Management Area and drains an area of 1 080 m^2, providing water to three impoundments (Olifantsnek Dam, in the upper reaches; Bospoort Dam, in the middle reaches; Vaalkop Dam, in the lower reaches) (Erasmus *et al*., 2019). The different tributaries of the Hex River include the Rooikloof Spruit, Waterkloof Spruit, Sand Spruit, Waterval Spruit, Klipfontein Spruit, Klipgat Spruit, Dorp Spruit, Paardekraal Spruit and Boschfontein Spruit (Fig. 2.1). The Hex River stretches approximately 31 km from the origin to Olifantsnek Dam (south of the Magaliesberg mountain range, 1 585 – 1 206 m above sea level) with minimal anthropogenic impacts. From Olifantsnek Dam the river flows for approximately 40 km through the mining and urbanised Rustenburg area before flowing into Bospoort Dam (north of the Magaliesberg mountain range, 1 206 – 1 076 m above sea level). Upstream of Olifantsnek Dam the land-use mostly consists of small-scale agricultural activities and game farms. Land-use between Olifantsnek and Bospoort Dams is characterised by large-scale agricultural activities, intensive Pt and Cr mining activities, as well as urban and industrial activities.

Although the average annual rainfall in the Rustenburg area is 661 mm with a mean annual runoff of 18.4 million m^3 in the Hex River at Bospoort Dam (EEM, 2003), evaporation exceeds rainfall within this catchment (Howard *et al*., 2002). The rainy season normally occurs from November to March (summer months), with the peak in January (du Plessis, 2006). Rainfall in this catchment is, however, unreliable and severe drought conditions have occurred for approximately 12% of the time in the last 70 years (EEM, 2003).

Sampling was carried out during a low flow period (October 2018) to assess and establish the influence and baseline of different land-use activities on the water and sediment quality of the Hex River system. Seventeen sampling sites were selected in the Hex River catchment. Site selection was based on the availability of water, accessibility of the site and the proximity to a specific type of upstream land-use activity. These sites included 10 sites within the Hex River (HX), one site in the Waterval Spruit (WV), one site in the Klipfontein Spruit (KF), two sites in the Dorp Spruit (DS) and one site in the Boschfontein Spruit (BF). Two sites were located in Olifantsnek Dam (OL) and Bospoort Dam (BS). The study area was divided into reference sites (HX 1, OL, HX 2, HX 3), mining impacted sites (HX 4, HX 5, KF), industrial impacted sites (WV, DS 1), urban impacted sites (HX 7, DS 2) and sites with a combined impact (HX 6, HX 8, HX 9, HX 10, BF, BS) (Fig 2.1 and Tables 2.1 – 2.17).

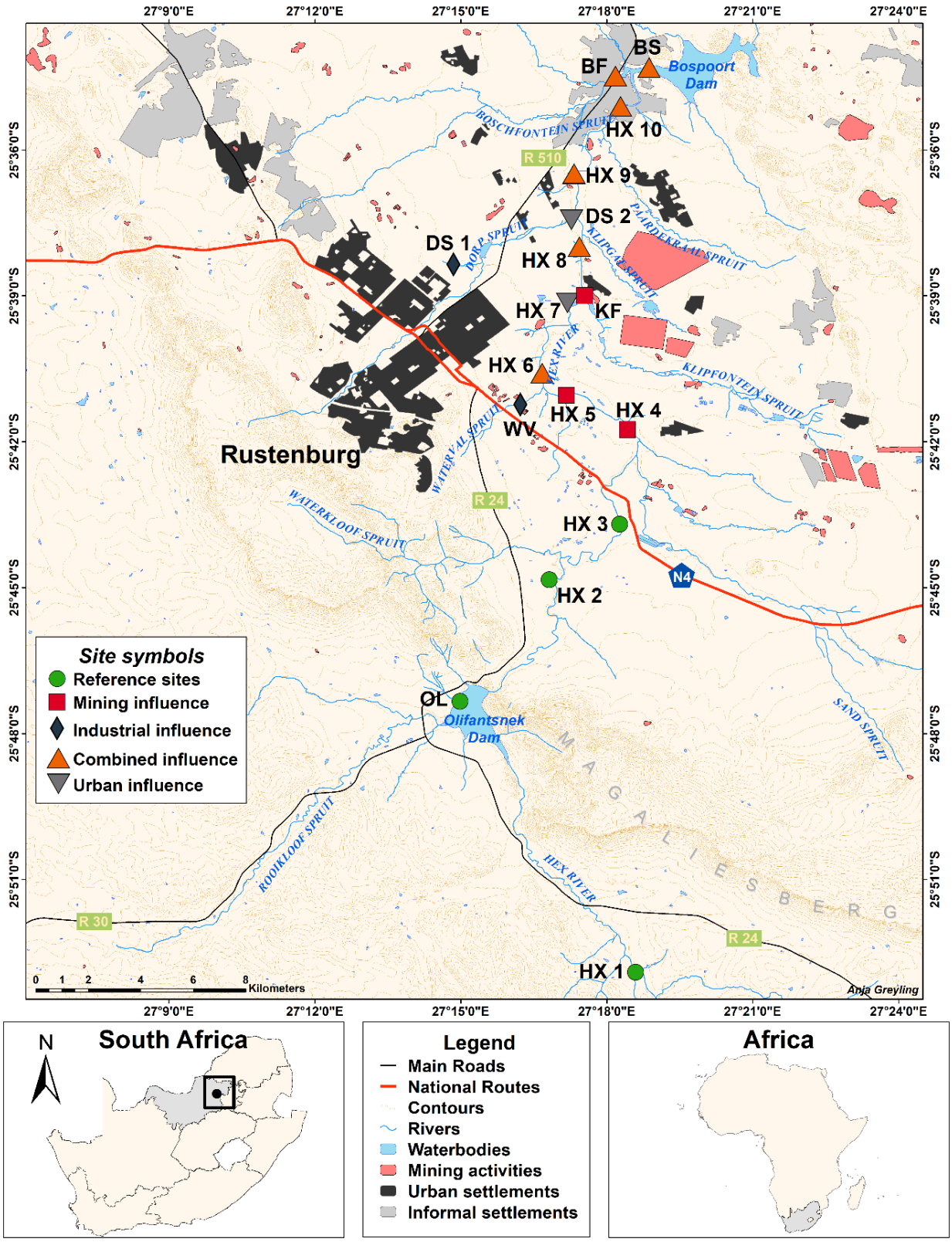


Figure 2.1: Map of the study area, indicating the sampling sites in the Hex River and its tributaries, as well as mining activities, urban and informal settlements in the surrounding area.

Table 2.1: Physical characteristics of HX 1.

HX 1 Hex River near the origin	
	
Coordinates	S 25°52’54.9” E 27°18’36.3”
Altitude	1 288 m
Site description	This site is located within the transition zone of the Magaliesberg Biosphere Reserve approximately 12 km upstream of Olifantsnek Dam. Few anthropogenic impacts are present at this site, with only small-scale agricultural activities and game farms in the surrounding areas.
Biotope description	Headwater zone, gravel to sandy substratum with stones in current, overhanging tree canopy, marginal vegetation with riffle and run biotope.
Primary lithology	Slate, Shale, Hornfels, Diabase (Walraven, 1981)

Table 2.2: Physical characteristics of OL.

OL Olifantsnek Dam	
	
Coordinates	S 25°47’25.3” E 27°15’14.7”
Altitude	1 206 m
Site description	This site is located within the upstream impoundment of the Hex River. The Hex River and Rooikloof Spruit are the major arteries that supply water to this impoundment. The water from this impoundment is used for irrigation purposes and recreational activities (*i.e.* fishing and sailing). Few anthropogenic impacts are present, with only small-scale agricultural activities.
Biotope description	Headwater zone, sandy to muddy substratum, few to no marginal vegetation and pool biotope.
Primary lithology	Slate, Shale, Hornfels, Diabase, Surface deposits (Walraven, 1981)

Table 2.3: Physical characteristics of HX 2.

HX 2 Hex River upstream of Waterkloof Spruit	
	
Coordinates	S 25°44’50.3” E 27°16’48.4”
Altitude	1 169 m
Site description	This site is located approximately 6.5 km downstream of Olifantsnek Dam and approximately 1.3 km upstream of the confluence with the Waterkloof Spruit. More anthropogenic impacts are present, with larger-scale agricultural activities and informal settlements on the banks of the Hex River.
Biotope description	Headwater zone, sandy to muddy substratum with stones in current, overhanging tree canopy, marginal vegetation and run biotope.
Primary lithology	Pyroxenite, Norite (Walraven, 1981)

Table 2.4: Physical characteristics of HX 3.

HX 3 Hex River upstream of Sand Spruit	
	
Coordinates	S 25°43’43.8” E 27°18’20.9”
Altitude	1 154 m
Site description	This site is located approximately 3.7 km downstream of the confluence of the Waterkloof Spruit and approximately 3.4 km upstream of the confluence with the Sand Spruit. This site is downstream of intensive agricultural activities and is situated next to the Platinum Highway (N4).
Biotope description	Middle water zone, gravel to sandy substratum with large boulders in stream, overhanging tree canopy, marginal vegetation, and algae, run and pool biotope.
Primary lithology	Pyroxenite, Norite (Walraven, 1981)

Table 2.5: Physical characteristics of HX 4.

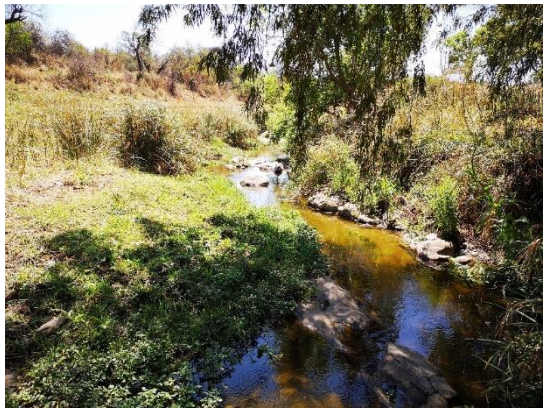
HX 4 Hex River downstream of Sand Spruit	
	
Coordinates	S 25°41’44.4” E 27°18’25.5”
Altitude	1 137 m
Site description	This site is located approximately 1.2 km downstream of the confluence with the Sand Spruit. This site is downstream of platinum and chromium mining activities and tailing dams.
Biotope description	Middle water zone, sandy substratum with boulders in stream, overhanging tree canopy, marginal and aquatic vegetation present with run biotope.
Primary lithology	Norite, Anorthosite (Walraven, 1981)

Table 2.6: Physical characteristics of HX 5.

HX 5 Hex River upstream of Waterval Spruit	
	
Coordinates	S 25°41’04.2” E 27°17’08.9”
Altitude	1 132 m
Site description	This site is located approximately 1.7 km downstream of a platinum mine on the banks of the Hex River, and approximately 0.9 km upstream of the confluence with the Waterval Spruit.
Biotope description	Middle water zone, gravel to sandy substratum, bedrock and boulders in stream, overhanging tree canopy, marginal and aquatic vegetation with run biotope.
Primary lithology	Norite (Walraven, 1981)

Table 2.7: Physical characteristics of WV.

WV Waterval Spruit downstream of Rustenburg eastern industrial zone	
	
Coordinates	S 25°41’15.5” E 27°16’03.9”
Altitude	1 151 m
Site description	This site is located approximately 1.3 km upstream of the confluence with the Hex River. Anthropogenic activities include various industrial activities (electrical, chemical) and logistics companies moving stone and chromium ore.
Biotope description	Lower water zone, muddy to sandy substratum, marginal vegetation with run biotope.
Primary lithology	Norite (Walraven, 1981)

Table 2.8: Physical characteristics of HX 6.

HX 6 Hex River downstream of Waterval Spruit	
	
Coordinates	S 25°40’33.2” E 27°16’38.8”
Altitude	1 127 m
Site description	This site is located approximately 0.8 km downstream of the confluence of the Waterval Spruit.
Biotope description	Middle water zone, sandy to muddy substratum with bedrock and large boulders in stream, overhanging tree canopy, marginal vegetation with riffle and run biotope.
Primary lithology	Norite, Merensky Reef (Walraven, 1981)

Table 2.9: Physical characteristics of HX 7.

HX 7 Hex River downstream of Rustenburg's wastewater treatment plant	
	
Coordinates	S 25°39'02.8" E 27°17'23.7"
Altitude	1 112 m
Site description	This site is located approximately 0.6 km downstream of Rustenburg's wastewater treatment plant and approximately 0.3 km upstream of the confluence with Klipfontein Spruit. This site is covered by the alien invasive water hyacinth (*Eichhornia crassipes*). Anthropogenic influences include wastewater effluent, as well as large-scale informal settlements on the banks of the Hex River.
Biotope description	Middle water zone, sandy to muddy substratum, marginal and aquatic vegetation with pool and run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.10: Physical characteristics of KF.

KF Klipfontein Spruit downstream of platinum smelter and concentrator	
	
Coordinates	S 25°39'01.2" E 27°17'32.8"
Altitude	1 110 m
Site description	This site is located approximately 0.5 km upstream of the confluence with the Hex River. Anthropogenic impacts in the Klipfontein Spruit include the base metal refinery, smelter, and acid plant converter of platinum mining operations, as well as several waste dumps and tailing dams. Seepage and overflow of tailing dams can be ascribed as noteworthy pollution sources.
Biotope description	Lower water zone, muddy substratum with marginal vegetation and run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.11: Physical characteristics of HX 8.

HX 8 Hex River downstream of Klipfontein Spruit	
	
Coordinates	S 25°38’00.7” E 27°17’26.2”
Altitude	1 102 m
Site description	This site is located approximately 1.7 km downstream of the confluence of the Klipfontein Spruit and approximately 1.2 km upstream from the confluence of the Dorp Spruit. Various anthropogenic impacts are present, draining an intensive informal settlement area (Boitekong) next to the Hex River, livestock grazing and drinking from the river.
Biotope description	Lower water zone, gravel to sandy substratum, stones in current, overhanging tree canopy, marginal and aquatic vegetation, as well as algae with run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.12: Physical characteristics of DS 1.

DS 1 Dorp Spruit downstream of Rustenburg northern industrial zone	
	
Coordinates	S 25°38’19.4” E 27°14’41.9”
Altitude	1 134 m
Site description	This site is located approximately 1 km downstream of the Rustenburg northern industrial zone. Storm runoff and effluent from industries (batteries, cement, abattoir, steel, feed mills) enter the Dorp Spruit.
Biotope description	Headwater zone, muddy substratum, marginal vegetation with run biotope.
Primary lithology	Gabbro, Norite, Anorthosite (Walraven, 1981)

Table 2.13: Physical characteristics of DS 2.

DS 2 Dorp Spruit before Hex River confluence	
	
Coordinates	S 25°37’27.4” E 27°17’13.8”
Altitude	1 100 m
Site description	This site is approximately 0.4 km upstream of the confluence with the Hex River. The Dorp Spruit meanders through the city of Rustenburg and receives runoff from the urban effluent. It also drains the northern side of Boitekong, as well as raw sewage that enters the Dorp Spruit just before the confluence with the Hex River.
Biotope description	Lower water zone, gravel to sandy substratum with boulders in stream, overhanging tree canopy, marginal and aquatic vegetation, as well as algae with run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.14: Physical characteristics of HX 9.

HX 9 Hex River downstream of Dorp Spruit	
	
Coordinates	S 25°36’29.4” E 27°17’20.2”
Altitude	1 095 m
Site description	This site is located approximately 1.8 km and 2 km downstream of the confluence with the Dorp Spruit and Klipgat Spruit, respectively. It is downstream of large tailing dams and seepage and runoff enter the Hex River, it is also downstream and surrounded by informal settlements.
Biotope description	Lower water zone, gravel to sandy substratum with stones in current, marginal vegetation and algae, pool, run, and riffle biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.15: Physical characteristics of HX 10.

HX 10 Hex River upstream of Bospoort Dam	
	
Coordinates	S 25°35’08.3” E 27°18’15.5”
Altitude	1 086 m
Site description	This site is approximately 2.4 km upstream of Bospoort Dam. This site is downstream of Thekwane’s wastewater treatment plant, platinum mining rock dumps, and tailing dams and surrounded by informal settlements. Water hyacinth also covers this site.
Biotope description	Lower water zone, sandy substratum, marginal and aquatic vegetation, as well as algae with pool and run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.16: Physical characteristics of BF.

BF Boschfontein Spruit upstream of Bospoort Dam	
	
Coordinates	S 25°34’55.1” E 27°17’50.4”
Altitude	1 088 m
Site description	This site is located approximately 1.8 km upstream of Bospoort Dam. The Boschfontein Spruit drains Kanana informal settlement, platinum mining activities, as well as Meriting wastewater treatment plant. Water hyacinth was also present at this site.
Biotope description	Lower water zone, muddy substratum, marginal and aquatic vegetation with pool and run biotope.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 2.17: Physical characteristics of BS.

BS Bospoort Dam	
	
Coordinates	S 25°34’23.5” E 27°19’01.7”
Altitude	1 082 m
Site description	This site is located within the downstream impoundment of the Hex River. The Hex River and Boschfontein Spruit are the major arteries that supply water to this impoundment. Local subsistence fishers catch fish from this impoundment. Various intensive anthropogenic impacts are present from urban and industrial effluent, platinum and chromium mining activities, as well as informal settlement and raw sewage effluent. Approximately 36.8% of the impoundment was covered by water hyacinth during this survey.
Biotope description	Lower water zone, sandy substratum, marginal and aquatic vegetation, pool biotope.
Primary lithology	Gabbro, Norite, Syenite (Walraven, 1981)

2.2.2 Field sampling

In situ water quality variables were sampled at each site and included pH, electrical conductivity (EC) and temperature (all by ExStik II EC500, Extech Instruments), as well as dissolved oxygen (DO) and oxygen saturation (both by ExStik II DO600, Extech Instruments). Water samples were collected for metal and nutrient analyses in triplicate in pre-cleaned polyethylene containers. Sediment samples for metal analysis were collected manually in triplicate from the upper 10 cm in pre-cleaned polyethylene containers. Samples were frozen at -20 °C until further analyses.

2.2.3 Laboratory analyses

2.2.3.1 Water quality

Unfiltered water samples were analysed for nutrients and other chemical water quality variables using the appropriate test kits and following the standard protocols with a spectrophotometer (Spectroquant® Pharo 300, Merck). Water quality variables and respective test kits were: ammonium (NH_4-N, 114752), chloride (Cl, 114897), chemical oxygen demand (COD, 101796), nitrate (NO_3-N, 109713), nitrite (NO_2-N, 114776), sulphate (SO_4, 114791), ortho-phosphate (PO_4-P, 114848) and total hardness (TH, 100961). Subsequently, separate water sub-samples were filtered through a cellulose nitrate filter (0.45 µm, Sartorius Stedim Biotech) to determine the suspended solid concentrations (mg / 100 mL) and the dissolved metal concentrations. Filtered water samples were acidified with HNO_3 (sub-boiled from 65%; p.a. quality, Merck) to an acid concentration of 1 ‰ and stored at room temperature until metal analysis.

2.2.3.2 Sediment quality

Sediment samples were first freeze-dried (FreeZone® 6, Labconco) and organic content and percentage particle size distribution were determined, according to methods described in Wepener and Vermeulen (2005). Organic content was determined using the loss on ignition method (Mataba *et al*., 2016), where 30 g sediment sample was heated in a crucible to 600 °C for five hours in a muffle furnace (L 40/11, Nabertherm) and reweighed to calculate the percentage organic carbon (OC) content. An Endecott dry sieving system was used to determine the particle size distribution of 200 g sediment samples from each site. Sediment samples were sieved for 10 minutes on a sieve shaker (VB200/300, KingTest) using a set of sieves with a mesh sizes of 4000 µm, 2000 µm, 1000 µm, 500 µm, 212 µm, and 65 µm. The following particle size categories were applied: gravel (> 4000 µm), very coarse sand (4000 – 2000 µm), coarse sand (2000 – 500 µm), medium sand (500 – 212 µm), fine sand (212 – 65 µm) and mud (< 65 µm). Sieves were cleaned with 10% HCl solution and oven dried between samples and replicates to avoid contamination. Subsequently, approximately 0.2 g (dry weight) of only the < 65 µm grain size sediment were digested in a mixture of 3 mL HCl (37%, supra pure quality, Merck) and 0.5 mL HNO_3 (sub-boiled from 65%; p.a. quality, Merck) adapted from Djingova *et al*. (2003) using a Mars 5 microwave digestion system (CEM Corporation, Kamp-Lintfort). Following digestion, samples were diluted to a volume of 5 mL with MilliQ® water.

2.2.3.3 Metal analyses

The concentrations of vanadium (V), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), silver (Ag), cadmium (Cd), platinum (Pt), gold (Au), and lead (Pb) were determined using quadrupole inductively coupled plasma-mass spectroscopy (ICP-MS) (Elan 6000, PerkinElmer) equipped with an auto-sampler system (AS-90, PerkinElmer). For the quantification, the following mass lines were evaluated: ^{51}V, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{107}Ag, ^{111}Cd, ^{195}Pt, ^{197}Au and ^{206}Pb. Prior to measurements, the digested solutions of the sediments were diluted 1:5 and the water samples 1:2 with 1% HNO_3 (sub-boiled from 65%; p.a. quality, Merck). Yttrium and Tm (both Certipur®, Merck) were added as an internal standard at a concentration of 10 µg/L.

The ICP-MS operated at 1000 W plasma power, 14 L/min plasma gas flow, 0.95 L/min nebulizer gas flow and a sample flow-rate of 1 mL/min regulated by a peristaltic pump. Calibration of the ICP-MS was performed using a series of 11 dilutions of a multi-element standard solution. With this calibration, the concentrations of the elements were calculated in the samples using corresponding regression lines with a correlation factor of ≥ 0.999. Due to several interferences on the ICP-MS, concentrations of Cr were analysed by means of atomic absorption spectrometry (AAS) using a PerkinElmer AAnalyst 600 equipped with Zeeman-effect background correction, following the method of Nawrocka and Szkoda (2012). The detection limits for both water and sediment samples were determined as three times the standard deviation of the blank measurements, respectively (Table 2.18).

Quality assurance for the sediment analysis was ensured by analysing BCR723 reference material (road dust standard reference material, Institute for Reference Materials and Measurements, European Commission) and NCS DC 73310 reference material (stream sediment reference material, National Analysis Centre for Iron and Steel, China), that provided certified values for Pt and indicative values for various trace metals (see Table 2.18). All metals considered in the present study showed recovery rates within 20% of the certified range (Table 2.18). As concentrations of Pt determined by ICP-MS can be affected by interferences, the potential mass interferences during the detection of Pt were assessed. Following the measurement series, a matrix-adapted addition with hafnium (Hf) was performed as described by Ek *et al*. (2004). However, obtained interference rates were always less than 2% and therefore no Hf-correction was applied.

Table 2.18: Recovery rates (%) and the detection limits of the metals of interest obtained for different certified reference materials, as well as water and sediment, respectively. Detection limits for the water and sediment samples were determined as three times the standard deviation of the blank measurements.

Elements	Recovery rates (%)		Detection limits	
	BCR-723	NCS DC 73310	Water (µg/L)	Sediment (µg/g)
Ag	n.c.	102	0.0021	0.002
As	n.c.	92	0.830	0.261
Au	n.c.	116	0.039	0.0012
Cd	88	99	0.0051	0.003
Co	86	92	0.041	0.008
Cr	80	88	0.047	0.686
Cu	n.c.	85	1.103	1.210
Mn	85	89	1.193	1.246
Ni	94	101	0.082	0.135
Pb	118	86	0.127	0.231
Pt	104	n.c.	0.0032	0.0002
V	99	106	0.174	0.706
Zn	94	86	2.450	1.823

n.c. – not certified.

2.2.4 Statistical analyses

Data were tested for normality and homogeneity of variance using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively (Pyrczak, 2016). One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used to test for significant differences in nutrient and metal concentrations between sites (GraphPad Prism v7). The level of significance was set at $p < 0.05$. Principal Component Analyses (PCA) (Canoco v5.12) were constructed to assess the spatial patterns associated with the water and sediment quality. Data for the PCAs were log-transformed $y = \log(x + 1)$, as well as standardised and centred (Šmilauer and Lepš, 2014).

2.3 Results

The Hex River is subjected to various anthropogenic impacts and each impact contributes different chemicals into surface water that influence the water and sediment quality. For the present study, the focus was on metals derived from Pt mining activities in the Hex River catchment. Although concentrations of V, Mn, Co, Cr, Ni, Cu, Zn, As, Ag, Cd, Pt, Au, and Pb were determined (see Appendix, Tables A1 and A2), only Cr, Ni, Cu, Zn, As, Cd, Pt, and Pb originates from activities that are associated with Pt mining, and was therefore the main focus for the purpose of the present study. However, it is notable that concentrations of V ($p < 0.0001$) and Au ($p = 0.010$) in the water were significantly higher at sites influenced by mining activities, while concentrations of Mn ($p = 0.0024$) were significantly higher at sites influenced by combined effects. Concentrations of Co ($p < 0.0001$) and Ag ($p = 0.0005$) were significantly higher at sites influenced by industry and urban effluent, respectively. Concentrations of V in the sediment were significantly higher ($p < 0.0001$) at the reference sites, while concentrations of Mn ($p = 0.026$) in the sediment were significantly higher at sites influenced by mining activities. Concentrations of Ag ($p = 0.013$) and Au ($p = 0.0002$) in the sediment were significantly higher at sites influenced by urban effluents.

2.3.1 Water quality variables

2.3.1.1 General water characteristics and nutrients

It is evident that various activities in the Hex River catchment contribute to different concentrations of pollutants to the system and influence the water chemistry. Mining impacted sites had significantly higher pH values ($p = 0.0084$) than the combined sites. Urban effluent had the highest contribution to EC values, while mining activities had higher concentrations of DO, although not significant. Mining impacted sites had significantly to higher concentrations of Cl ($p = 0.0008$), SO_4 ($p = 0.0002$), Mg and Ca (total hardness; $p = 0.0055$) and NO_3 ($p = 0.0207$) at sites HX 4, HX 5 and KF, while industrial impacted sites had significantly higher concentrations of NH_4 ($p = 0.0201$) and COD ($p = 0.0029$) at sites WV and DS 1 (Table 2.19). Urban impacted sites had significantly higher concentrations of Cl ($p = 0.0027$), TH ($p < 0.0001$), NO_3 ($p = 0.0199$), NO_2 ($p = 0.0329$) and PO_4 ($p < 0.0001$) at sites HX 7 and DS 2, while the sites with combined-influences (HX 9, HX 10 and BF) had significantly higher concentrations of Cl ($p = 0.0006$), SO_4 ($p = 0.0028$), TH ($p = 0.0028$), NH_4 ($p = 0.0031$), NO_2 ($p = 0.0245$), PO_4 ($p = 0.0013$) and COD ($p = 0.0201$).

Table 2.19: Water and sediment quality variables analysed for samples collected in the Hex River and tributaries. Sites are grouped into reference sites (HX 1 – HX 3), sites with mining-influence (HX 4, HX 5, KF), sites with industrial-influence (WV, DS 1), sites with urban-influence (HX 7, DS 2) and sites with a combined-influence (HX 6, HX 8, HX 9, HX 10, BF, BS). Only the mean of n = 3 is presented.

		Reference				Mining		Indus	Comb	Urban	Mining	Comb	Indus	Urban	Combined			
	Code	HX 1	OL	HX 2	HX 3	HX 4	HX 5	WV	HX 6	HX 7	KF	HX 8	DS 1	DS 2	HX 9	HX 10	BF	BS
Water quality variables																		
pH	pH	7.1	7.9	7.8	7.5	7.9	8.6	7.5	7.6	7.6	8.3	7.2	8.3	7.6	7.4	7.3	7.5	7.2
Temperature (°C)	TEMP	28.1	23.3	17.0	22.1	20.7	21.1	17.0	19.5	23.9	27.2	21.5	27.5	23.0	19.5	19.7	19.5	18.1
Electrical conductivity (µS/cm)	EC	106	189	583	529	1 733	944	347	803	864	560	2 090	1 061	3 950	2 880	2 370	1 312	1 466
Dissolved oxygen (mg/L)	DO	3.7	10.5	7.0	5.2	9.0	13.7	6.9	9.4	7.2	13.0	3.1	3.6	3.8	0.7	2.6	2.9	9.7
Suspended solids (mg/100mL)	SS	19.0	19.0	1.2	1.5	8.5	11.0	1.5	4.1	6.1	16.5	1.2	77.8	1.7	2.6	2.1	3.4	6.2
Turbidity (FAU)	TURB	39	23	9	20	19	46	10	20	25	28	11	41	17	32	19	25	12
Ammonium (mg/L)	NH4	0.039	0.037	0.093	0.19	1.4	0.027	1.2	0.19	1.1	0.019	0.12	5.2	3.8	6.7	7.2	10.5	0.066
Nitrate (mg/L)	NO3	0.47	0.70	0.83	1.6	46.3	2.1	0.17	4.9	1.9	3.0	2.1	2.0	36.9	14.4	3.7	1.4	1.7
Nitrite (mg/L)	NO2	0.008	0.017	0.050	0.061	1.2	0.063	0.13	0.26	0.25	0.060	0.043	0.57	1.5	3.7	0.31	0.073	0.017
Sulphate (mg/L)	SO4	67.0	87.7	109.7	116.0	239.0	196.3	62.0	139.0	141.0	876.0	387.0	181.7	292.7	484.7	485.3	200.0	321.3
Ortho-phosphate (mg/L)	PO4	0.030	0.030	0.018	0.067	0.077	0.070	0.21	0.060	3.4	0.11	1.9	0.70	1.4	0.83	1.9	3.7	0.77
Chloride (mg/L)	Cl	6.3	18.4	25.3	19.7	216.0	106.0	15.7	95.7	123.0	708.0	263.0	144.0	585.0	545.3	412.7	165.3	310.7
Chemical oxygen demand (mg/L)	COD	36.4	13.1	11.1	15.7	22.4	29.5	18.4	24.9	30.2	38.9	26.9	84.9	25.7	54.1	32.1	66.9	29.5
Total hardness (mg/L)	TH	44.7	52.7	186.0	156.7	393.3	222.3	96.0	164.3	170.0	455.3	258.0	158.0	905.3	398.7	314.7	162.7	242.7
Sediment quality variables																		
Organic content (%)	OC	4.4	2.0	3.6	5.9	4.0	4.7	2.3	6.0	1.7	3.9	1.0	3.6	1.5	1.5	0.7	5.8	2.7
Gravel (%)	GR	20.4	3.3	1.6	30.2	2.9	42.7	2.9	21.5	15.5	6.7	20.8	2.4	6.7	30.6	12.7	2.5	7.3
Very coarse sand (%)	VCS	13.8	2.5	1.1	2.7	3.0	10.4	2.7	4.9	9.9	2.8	21.8	2.4	27.4	10,4	10.0	3.7	8.1
Coarse sand (%)	CS	39.3	13.1	10.0	18.5	38.9	25.3	28.0	27.8	31.3	17.1	40.4	24.2	52.8	23.3	45.5	13.9	28.1
Medium sand (%)	MS	11.2	16.7	41.1	21.8	37.0	14.5	47.4	24.2	30.4	29.6	14.0	20.4	9.7	25.1	26.9	30.6	29.0
Fine sand (%)	FS	10.1	56.4	39.1	19.0	13.3	5.7	15.8	15.7	11.1	35.3	2.7	20.7	3.0	9.2	4.3	42.0	24.8
Mud (%)	M	5.1	8.0	7.1	8.0	4.8	1.4	3.2	5.9	1.8	8.6	0.4	29.9	0.4	1.5	0.5	7.3	2.7

2.3.1.2 Metal concentrations in water

Reference site metal concentrations in the water of the Hex River catchment ranged from 0.37 – 0.82 µg/L for Cr, 3.9 – 14.7 µg/L for Ni, 3.0 – 10.6 µg/L for Cu, 9.2 – 22.8 µg/L for Zn, 1.1 – 3.7 µg/L for As, 0.017 – 0.030 µg/L for Cd, 0.0040 – 0.0055 µg/L for Pt and 0.22 – 0.47 µg/L for Pb (Fig. 2.2). Between the reference sites (HX 1 – HX 3), site HX 1 had the highest concentrations of Ni, Cu, Zn and Cd, while site OL and site HX 2 had the highest concentrations of Pt and Cr, respectively and site HX 3 had the highest concentrations of As and Pb. Only the concentrations of Zn at HX 1 were significantly higher ($p = 0.0012$) than the other reference sites (see Table 2.20).

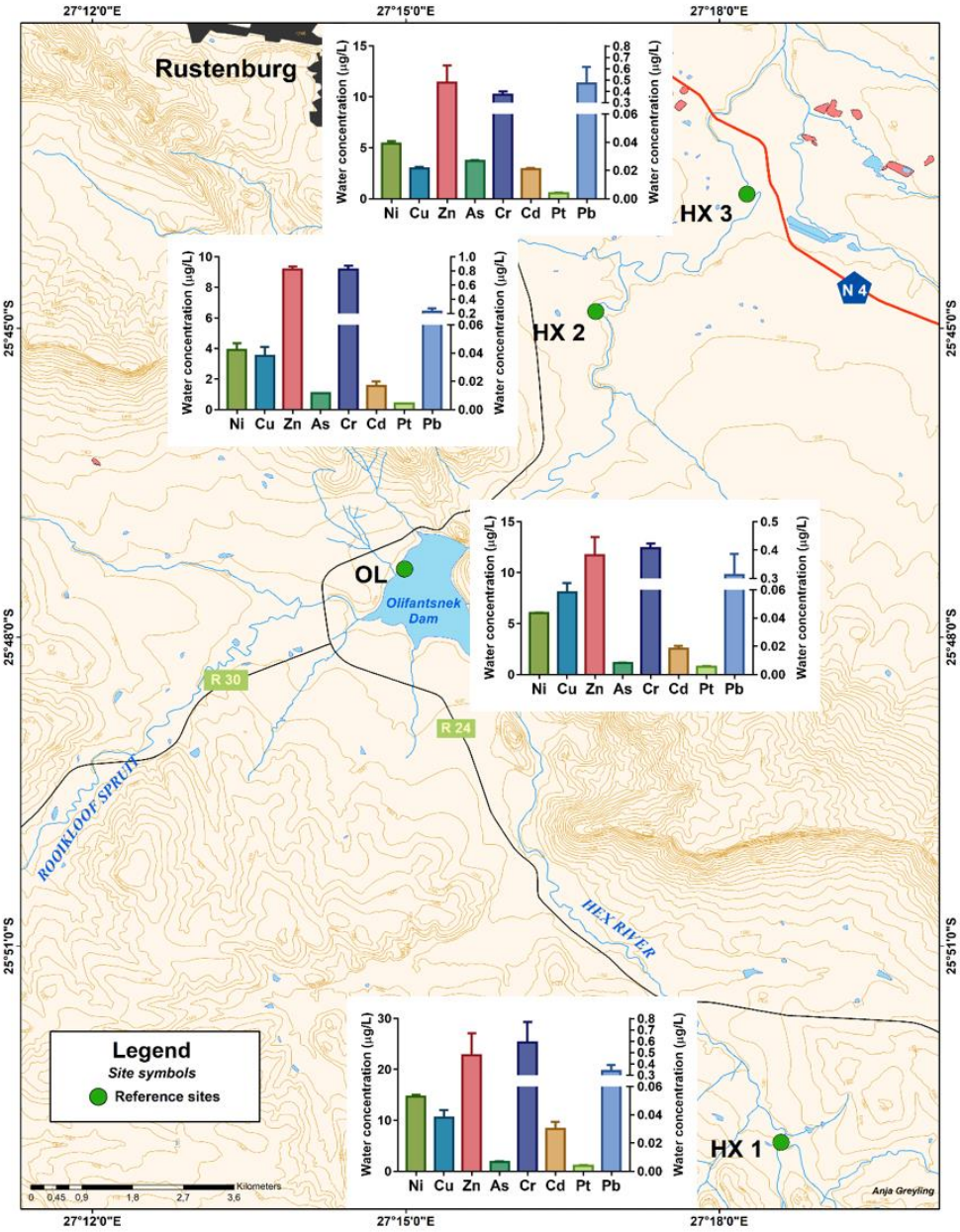


Figure 2.2: Map of sites HX 1 – HX 3, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/L) in water samples (n = 3). Concentrations of Ni, Cu, Zn and As are displayed on the left y-axis, while concentrations of Cr, Cd, Pt, and Pb are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.20.

The metal concentrations in the water at the impacted sites HX 4 and KF, ranged between 0.29 – 0.42 µg/L for Cr, 14.7 – 726.3 µg/L for Ni, 4.6 – 38.5 µg/L for Cu, 9.6 – 21.4 µg/L for Zn, 1.4 – 12.1 µg/L for As, 0.015 – 0.12 µg/L for Cd, 0.0071 – 0.20 µg/L for Pt and 0.16 – 0.43 µg/L for Pb (Fig. 2.3). The mining-influenced sites had significantly higher concentrations of Ni ($p < 0.0001$), Cu ($p < 0.0001$), and As ($p = 0.0144$) at site KF, as well as Zn ($p = 0.0060$) and Pt ($p < 0.0001$) at site HX 4 (see Table 2.20). Only the concentrations of Cd were significantly higher ($p < 0.0001$) at the urban-influenced site (HX 7).

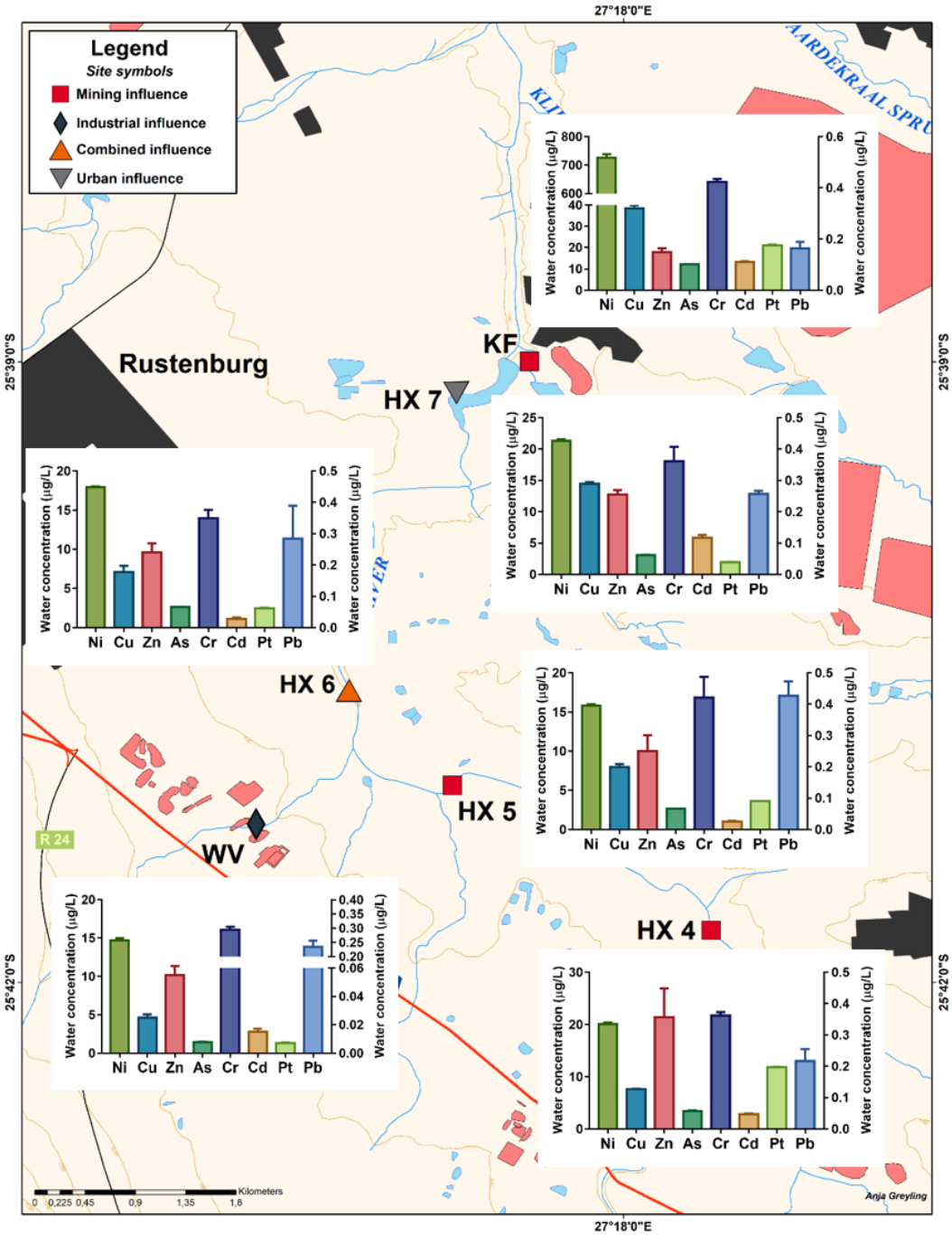


Figure 2.3: Map of sites HX 4 – KF, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/L) in water samples (n = 3). Concentrations of Ni, Cu, Zn and As are displayed on the left y-axis, while concentrations of Cr, Cd, Pt, and Pb are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.20.

At the sites within the lower reaches of the Hex River catchment, the metal concentrations in the water ranged from 0.29 – 0.52 for Cr, 15.9 – 131.8 µg/L for Ni, 2.2 – 14.0 µg/L for Cu, 5.8 – 21.0 µg/L for Zn, 2.4 – 18.5 µg/L for As, 0.013 – 0.071 µg/L for Cd, 0.011 – 0.088 µg/L for Pt and 0.16 – 0.42 µg/L for Pb (Fig. 2.4). Between the lower reaches sites, the site with industrial-influence (DS 1) had significantly higher concentrations of Zn ($p = 0.0103$) and Cd ($p = 0.0020$), while the urban-influenced site (DS 2) had the highest concentrations of As ($p < 0.0001$) and Pt ($p < 0.0001$). Sites with a combined-influence had the highest concentrations of Ni ($p = 0.0010$; $p < 0.000.1$) and Zn ($p = 0.0007$; $p = 0.0080$) at sites HX 8 and HX 9, respectively (see Table 2.20).

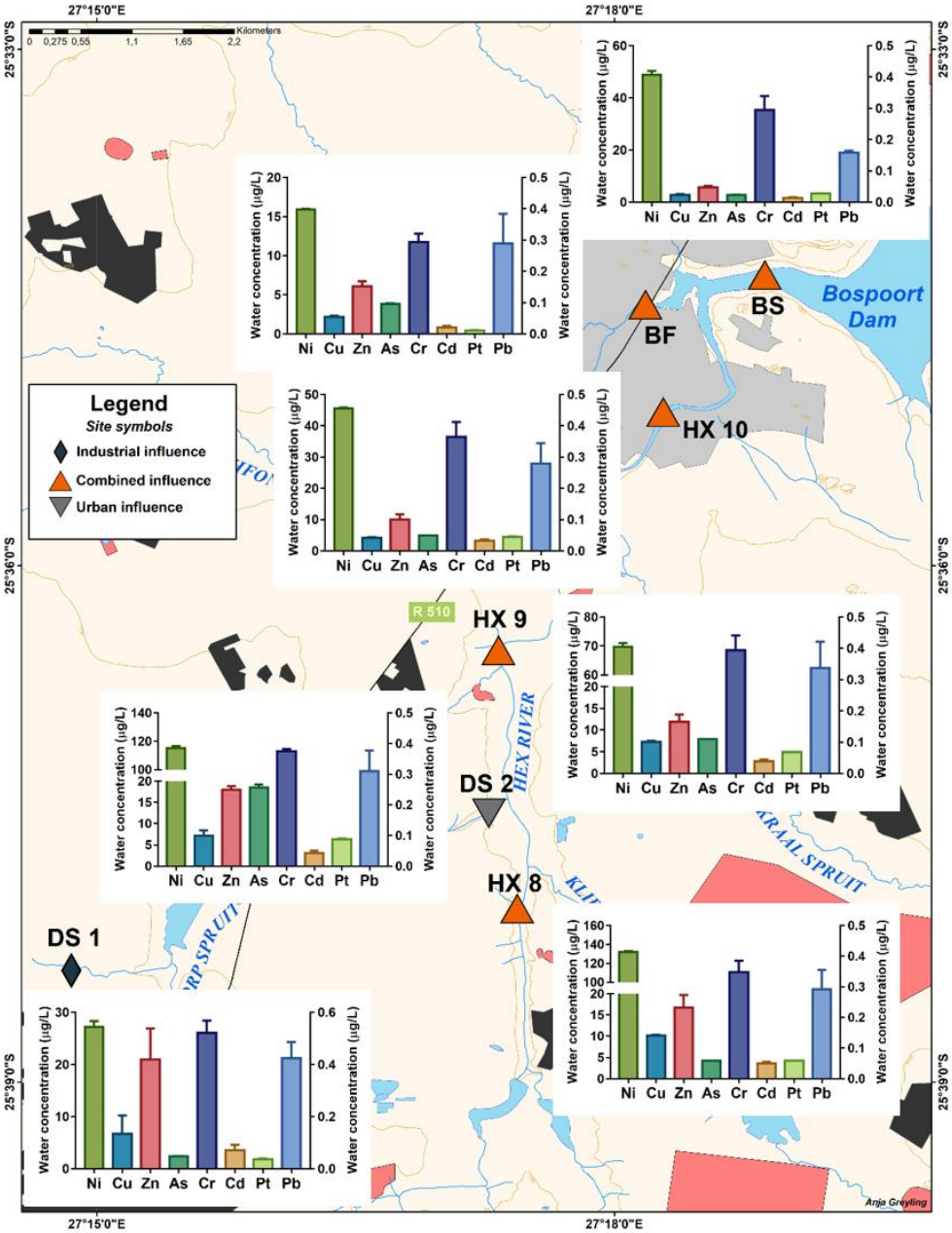


Figure 2.4: Map of sites HX 8 – BS, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/L) in water samples (n = 3). Concentrations of Ni, Cu, Zn and As are displayed on the left y-axis, while concentrations of Cr, Cd, Pt, and Pb are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.20.

Concentrations of Cr and Zn were highest at the reference sites, where after it gradually decreased downstream towards site BS, while increases in concentrations occurred at sites HX 2, HX 5, KF, DS 1 and HX 4, KF, DS 1 for Cr and Zn, respectively. Concentrations of Cu, Cd, and Pb were relatively constant throughout the river system, however, spikes in concentrations occurred at KF and HX 9 for Cu, at HX 4, HX 7 and DS 1 for Cd, as well as at HX 3, HX 5 and DS 1 for Pb. Concentrations of Ni, As and Pt gradually increased from the reference sites towards the downstream sites, with spikes in concentrations that occurred at KF and DS 1 for Ni, at HX 3, KF and DS 2 for As, as well as at HX 4, KF and DS 2 for Pt. Most of the spikes in metal concentrations in the Hex River system, derived from the tributaries Klipfontein Spruit and Dorp Spruit. However, for all of the metals, the concentrations in the water steadily decreased downstream of these spikes. Throughout the Hex River, the highest concentrations of Cr, Zn, and Pb were recorded at the reference sites HX 2, HX 1 and HX 3, respectively, while the highest concentrations of Ni, Cu and Pt were found at sites influenced by mining activities site KF (Ni, Cu) and HX 4 (Pt). The highest concentrations of As and Cd were recorded at sites influenced by urban effluent, As at site DS 2 and Cd at site HX 7.

Table 2.20: Significant differences in dissolved metal concentrations (Cr, Ni, Cu, Zn, As, Cd, Pt, Pb) between sites in the Hex River and its tributaries. Dissolved concentrations of Cr and Pb had no significant differences between sites.

	HX 1	OL	HX 2	HX 3	HX 4	HX 5	WV	HX 6	HX 7	KF	HX 8	DS 1	DS 2	HX 9	HX 10	BF	BS
HX 1	-	Zn	Zn	Zn		Zn	Zn	Zn	Zn					Zn	Zn	Cu, Zn	Zn
OL		-															
HX 2			-														
HX 3				-													
HX 4	Pt	Zn, Pt	Zn, Pt	Zn, Pt	-	Zn, Pt	Zn, Cd, Pt	Zn, Pt	Zn, Pt		Pt	Pt	Pt	Zn, Pt	Zn, Pt	Zn, Pt	Zn, Cd, Pt
HX 5	Pt	Pt	Pt	Pt		-	Pt		Pt		Pt	Pt			Pt	Pt	Pt
WV							-										
HX 6	Pt	Pt	Pt	Pt			Pt	-								Pt	Pt
HX 7	Cd, Pt	Cd, Pt	Cu, Cd, Pt	Cu, Cd, Pt	Cd	Cd	Cu, Cd, Pt	Cd	-		Cd	Cd	Cd	Cd	Cu, Cd	Cu, Cd	Cu, Cd
KF	Ni, Cu, As, Cd, Pt	Ni, Cu, As, Cd, Pt	Ni, Cu, Zn, As, Cd, Pt	Ni, Cu, As, Cd, Pt	Ni, Cu, As, Cd	Ni, Cu, As, Cd, Pt	Ni, Cu, As, Cd, Pt	Ni, Cu, Zn, As, Cd, Pt	Ni, Cu, As, Pt	-	Ni, Cu, Cd, Pt	Ni, Cu, As, Cd, Pt	Ni, Cu, Cd, Pt	Ni, Cu, Cd, Pt	Ni, Cu, Cd, Pt	Ni, Cu, Zn, As, Cd, Pt	Ni, Cu, Zn, As, Cd, Pt
HX 8	Ni, Pt	Ni, Cd, Pt	Ni, Cd, Pt	Ni, Pt	Ni	Ni	Ni, Cd, Pt	Ni	Ni		-	Ni			Ni	Ni, Zn, Pt	Zn, Cd, Pt
DS 1	Cd, Pt	Zn, Cd	Zn, Cd, Pt	Zn, Cd, Pt		Zn, Cd	Zn, Cd	Zn, Cd	Zn			-		Zn, Cd	Zn, Cd	Zn, Cd	Zn, Cd
DS 2	Ni, As, Pt	Ni, As, Pt	Ni, Zn, As, Pt	Ni, As, Pt	Ni, As	Ni, As	Ni, As, Pt	Ni, Zn, As	Ni, As, Pt		As	Ni, As, Pt	-	As	As, Pt	Ni, Zn, As, Pt	Zn, As, Pt
HX 9	Pt	Pt	Cu, Pt	Cu, Pt			Cu, Pt					Pt		-	Cu	Cu, Pt	Cu, Pt
HX 10	Pt	Pt	Pt	Pt			Pt								-	Pt	
BF																-	
BS																	-

2.3.2 Metal concentrations in sediment

Reference site metal concentrations in the sediment in the Hex River catchment, ranged between 432.2 – 968.1 µg/g for Cr, 56.6 – 185.8 µg/g for Ni, 22.5 – 41.3 µg/g for Cu, 42.1 – 79.1 µg/g for Zn, 14.6 – 20.3 µg/g for As, 0.040 – 0.076 µg/g for Cd, 0.0091 – 0.018 µg/g for Pt and 6.5 – 10.7 µg/g for Pb (Fig. 2.5). Site HX 1 had the highest concentrations of Cu, Zn, As and Cd, whereas site HX 2 had the highest concentrations of Cr, Ni, and Pt, while site HX 3 had the highest concentrations of Pb.

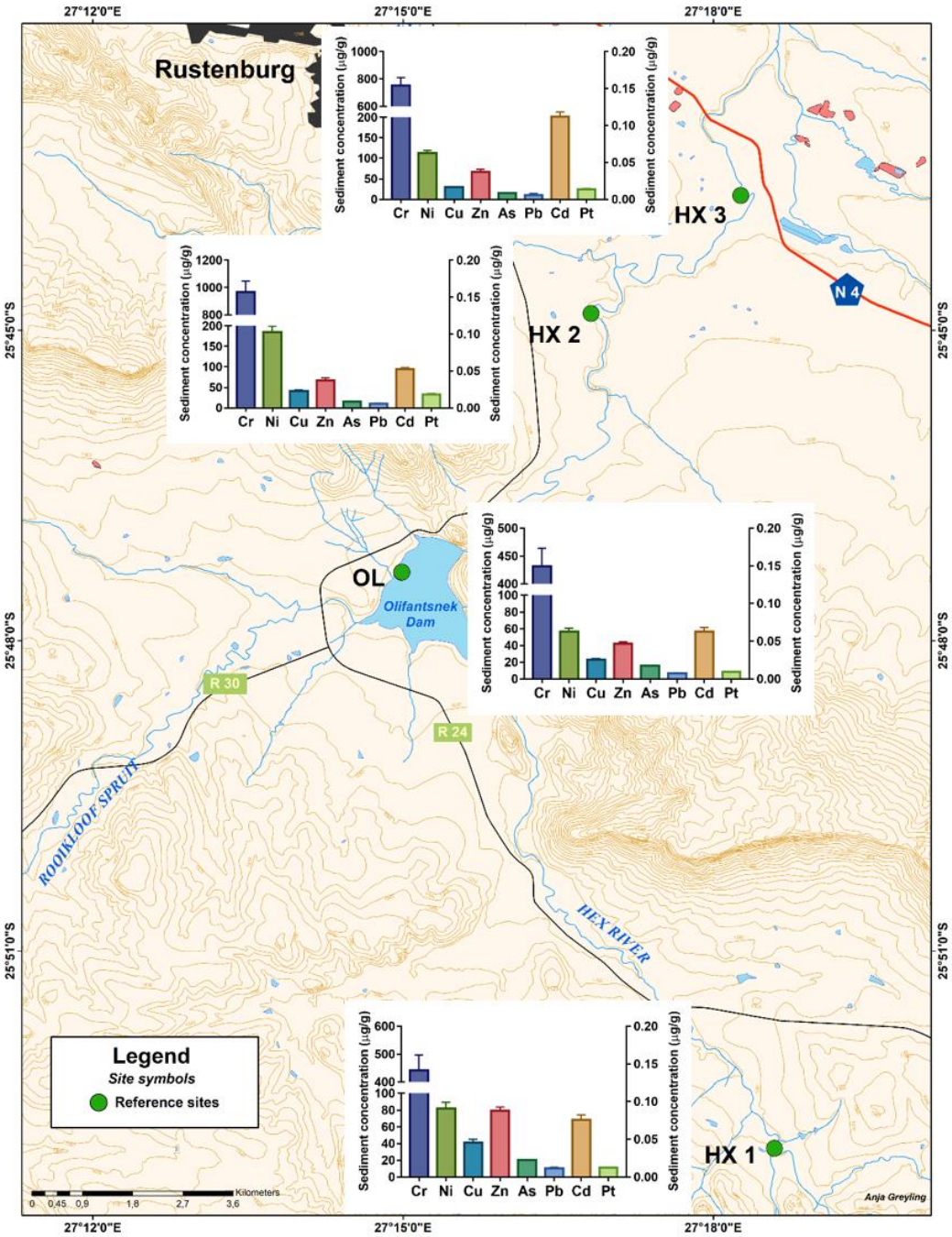


Figure 2.5: Map of sites HX 1 – HX 3, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/g) in sediment samples (n = 3). Concentrations of Cr, Ni, Cu, Zn, As and Pb are displayed on the left y-axis, while concentrations of Cd and Pt are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.21.

Between the sites located in the middle reaches (HX 4 – KF), the metal concentrations in the sediment ranged from 495.0 – 1 418.1 µg/g for Cr, 152.9 – 362.0 µg/g for Ni, 32.7 – 218.2 µg/g for Cu, 37.0 – 130.3 µg/g for Zn, 14.6 – 21.2 µg/g for As, 0.062 – 0.57 µg/g for Cd, 0.015 – 0.36 µg/g for Pt and 6.1 – 28.0 µg/g for Pb (Fig. 2.6). The mine impacted sites had the highest concentrations of Cr and As, and Ni at sites HX 5 and KF, respectively. The urban-influenced site (HX 7), had significantly higher concentrations of Cu ($p < 0.0001$), Zn ($p = 0.0158$), Cd ($p < 0.0001$), Pt ($p < 0.0001$), and Pb ($p = 0.0013$) (see Table 2.21).

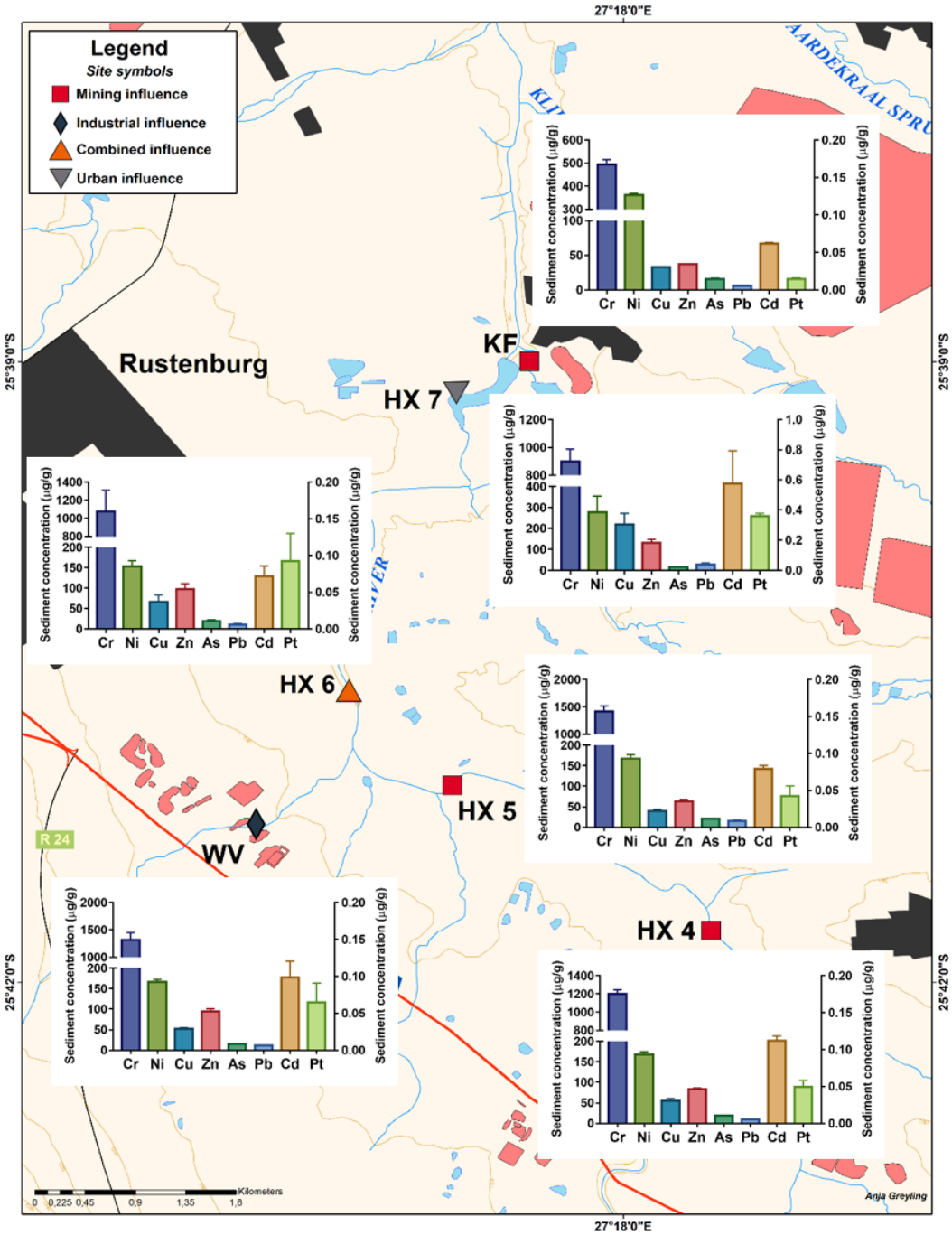


Figure 2.6: Map of sites HX 4 – KF, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/g) in sediment samples (n = 3). Concentrations of Cr, Ni, Cu, Zn, As and Pb are displayed on the left y-axis, while concentrations of Cd and Pt are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.21.

Amongst the lower reaches of the Hex River catchment, the metal concentrations in the sediment ranged between 112.4 – 1 118.8 µg/g for Cr, 46.5 – 179.6 µg/g for Ni, 12.5 – 108.2 µg/g for Cu, 20.2 – 227.6 µg/g for Zn, 12.6 – 19.0 µg/g for As, 0.030 – 0.31 µg/g for Cd, 0.0059 – 0.29 µg/g for Pt and 3.6 – 22.0 µg/g for Pb (Fig. 2.7). The industrial-influenced site (DS 1) had significantly higher concentrations of Cr ($p = 0.0049$) and Pt ($p = 0.0045$), while the urban-influenced site (DS 2) had significantly higher concentrations of Zn ($p = 0.0020$). The sites with combined-influences, had the highest concentrations of Ni ($p = 0.0072$), Cu ($p = 0.0180$), Cd ($p = 0.0061$), and Pb ($p = 0.0092$) at sites HX 8 (see Table 2.21).

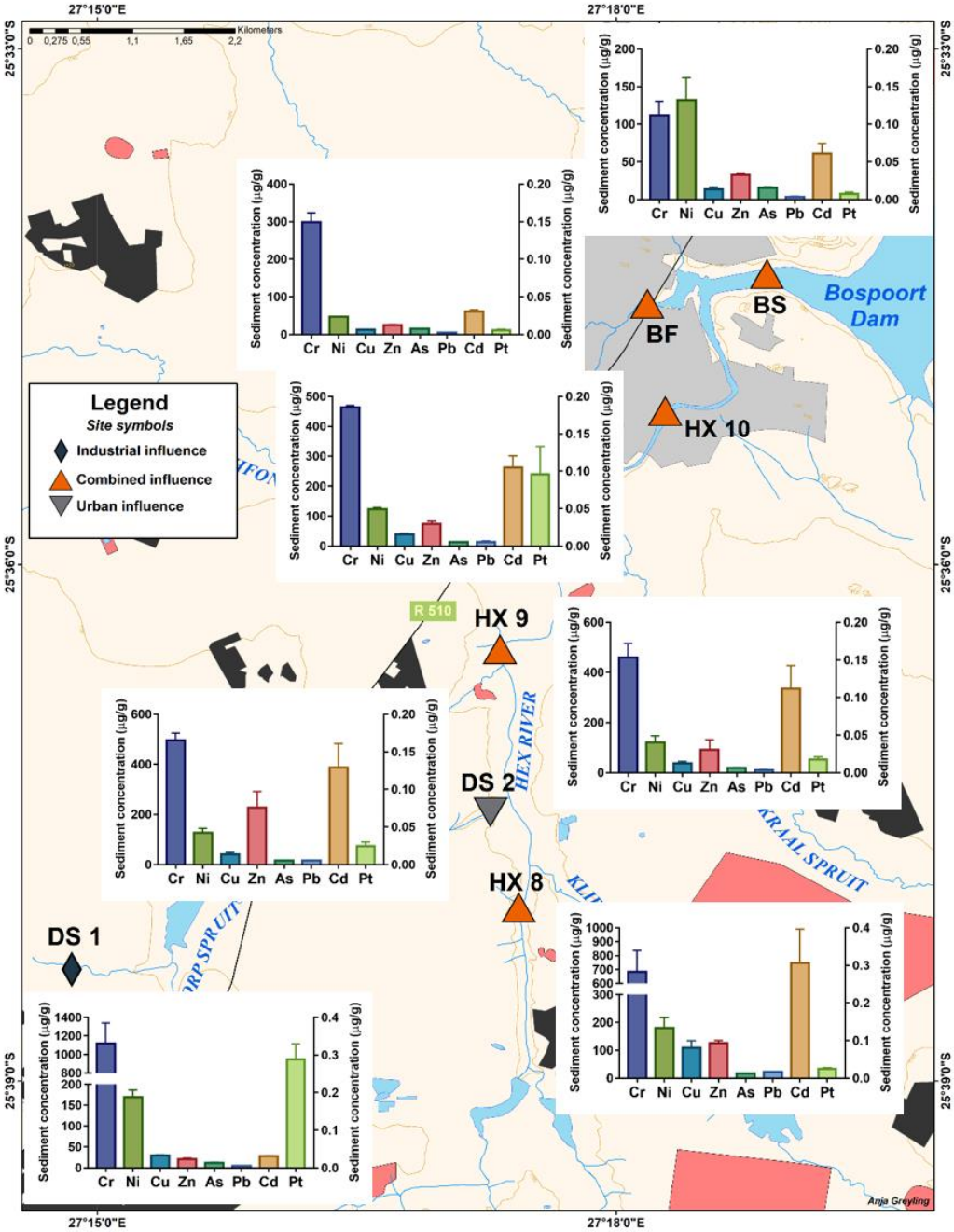


Figure 2.7: Map of sites HX 8 – BS, with metal concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt and Pb (µg/g) in sediment samples (n = 3). Concentrations of Cr, Ni, Cu, Zn, As and Pb are displayed on the left y-axis, while concentrations of Cd and Pt are displayed on the right y-axis. Significant differences in metal concentrations between sites are summarised in Table 2.21.

Concentrations of Cr and Pt increased significantly from the reference sites towards the middle reaches, where after it decreased towards the lower reaches of the Hex River. Spikes in concentrations occurred at sites HX 2, HX 5 and DS 1 for Cr, as well as at sites HX 4, HX 6, HX 7, DS 1 and HX 10 for Pt. Concentrations of Ni, Cu, Zn, Cd, and Pb were relatively constant throughout the river system, however, spikes in concentrations occurred at sites HX 2, HX 7 and KF (Ni), at site HX 7 (Cu), at sites HX 7 and DS 2 (Zn), at sites HX 4, HX 7 and DS 2 (Cd), as well as at sites HX 5, HX 7, DS 2 and HX 10 (Pb). Concentrations of As gradually decreased from the upper reaches towards the lower reaches of the Hex River, while spikes in concentrations occurred at sites HX 4, HX 5 and HX 9. Most of the spikes in metal concentrations in the Hex River originated from the tributaries Dorp Spruit (Cr, Zn, Cd, Pt, Pb) and Klipfontein Spruit (Ni). These metal concentrations in the sediment decreased further downstream of the spikes. Throughout the whole Hex River catchment, the highest concentrations of Cr and As were recorded at site HX 5, whereas site KF had the highest concentration of Ni, site DS 2 had the highest concentrations of Zn, while the highest concentrations of Cu, Cd, Pt, and Pb were recorded at site HX 7.

Table 2.21: Significant differences in sediment metal concentrations (Cr, Ni, Cu, Zn, As, Cd, Pt, Pb) between sites in the Hex River and its tributaries. Concentrations of As in the sediment had no significant differences between sites.

	HX 1	OL	HX 2	HX 3	HX 4	HX 5	WV	HX 6	HX 7	KF	HX 8	DS 1	DS 2	HX 9	HX 10	BF	BS
HX 1	-																
OL		-															
HX 2	Ni	Ni	-													Cr, Ni	Cr
HX 3				-													Cr
HX 4	Cr	Cr, Ni			-					Cr			Cr	Cr	Cr	Cr, Ni	Cr
HX 5	Cr	Cr, Ni		Cr		-				Cr	Cr		Cr	Cr	Cr	Cr, Ni	Cr
WV	Cr	Cr, Ni					-			Cr			Cr	Cr	Cr	Cr, Ni	Cr
HX 6		Cr						-								Cr, Ni	Cr
HX 7	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	-	Cu, Zn, Cd, Pt, Pb	Ni, Cu, Cd, Pt	Ni, Cu, Zn, Cd, Pb	Ni, Cu, Cd, Pt	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Cd, Pt, Pb	Ni, Cu, Zn, Cd, Pt, Pb	Cr, Ni, Cu, Zn, Cd, Pt, Pb
KF	Ni	Ni	Ni	Ni	Ni	Ni	Ni	Ni		-	Ni	Ni	Ni	Ni	Ni	Ni	Ni
HX 8	Ni, Cd	Ni, Cd, Pb	Cd	Cd	Cd, Pb	Cd	Cd	Cd		Cd, Pb	-	Zn, Cd, Pb		Cd	Cd	Ni, Cu, Zn, Cd, Pb	Cu, Zn, Cd, Pb
DS 1	Pt	Ni, Pt	Pt	Pt	Pt	Pt	Pt	Pt		Pt	Pt	-	Pt	Pt	Pt	Cr, Ni, Pt	Cr, Pt
DS 2	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	Zn	-	Zn	Zn	Zn	Zn
HX 9														-			
HX 10															-		
BF																-	
BS																	-

2.3.3 Multivariate spatial analysis

Between the sites, there were clear spatial variances in water and sediment quality variables (Fig. 2.8). Sites influenced by different anthropogenic stressors grouped together and were characterised by the same variables. The reference sites were all characterised by finer sediment particles with higher organic content and higher dissolved oxygen concentrations in the water. Sites that are impacted by mining activities, were characterised by higher metal concentrations in the water (Cu, Zn, Cd, Pt) and sediment (Cr, Ni, Pt), as well as higher chemical oxygen demand, temperature, and turbidity. Industrial effluent at Site DS 1 associated with higher pH, suspended solids and concentrations of Cr and Pb in the water. Urban effluent associated with higher nutrient concentrations (NO_3, NO_2), salts (Cl, SO_4, TH), as well as metal concentrations in the water (Ni, As) and sediment (Cu, Cd). Sites that are influenced by combined anthropogenic impacts were characterised by coarser sediment particles, high electrical conductivity, concentrations of nutrients (NH_4, PO_4) and metals in the sediment (Zn, As, Pb). There was a strong correlation between water and sediment concentrations of Cr, Ni, As, and Pt, while the Pb showed no correlation between water and sediment concentrations. For Cu, Zn, and Cd there was a weak positive correlation between water and sediment concentrations.

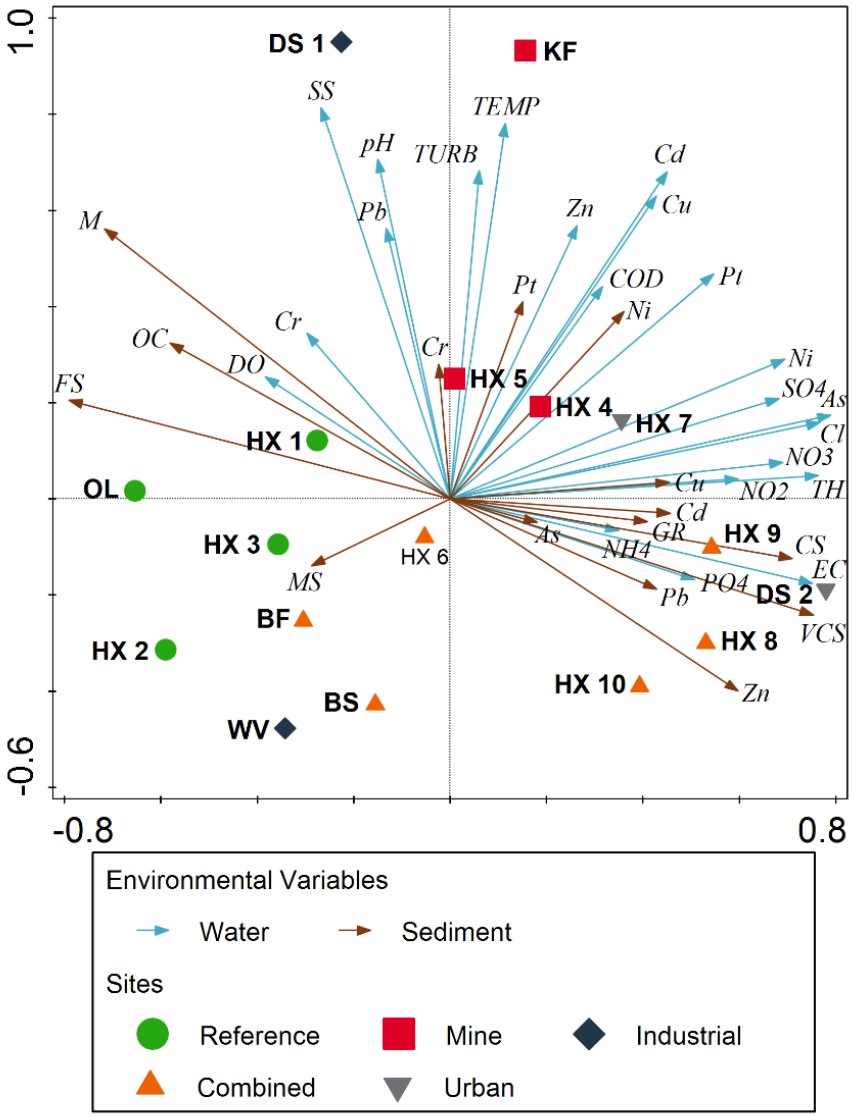


Figure 2.8: PCA biplot of the water and sediment quality variables measured at sites in the Hex River and tributaries. The biplot describes 41.7% of the variation, with 26.1% on the first axis and 15.6% on the second axis. Water quality variables include dissolved metal concentrations, nutrients and in situ parameters. Sediment quality variables include metal concentrations, particle size: gravel (GR), very coarse sand (VCR), coarse sand (CS), medium sand (MS), fine sand (FS), and mud (M), as well as organic content (OC).

When only the water quality variables were considered, slight changes in the spatial variation are evident (Fig. 2.9). The reference sites completely differed from the other sites and all associated with higher dissolved oxygen, whereas sites that are impacted by mining activities (KF), and industrial effluent (DS 1) were associated with higher metal concentrations (Cr, Ni, Cu, Zn, Cd, Pt, Pb), chemical oxygen demand, temperature, pH and turbidity. The sites with combined impacts and urban effluent (DS 2) were characterised by higher nutrient and As concentrations and electrical conductivity. The tributaries, DS 1 and KF grouped together, while DS 2 and site HX 9 in the Hex River downstream of DS 2, grouped together. This indicates that the tributaries are the sources of dissolved metals (DS 1, KF) and nutrients (DS 2) into the Hex River system.

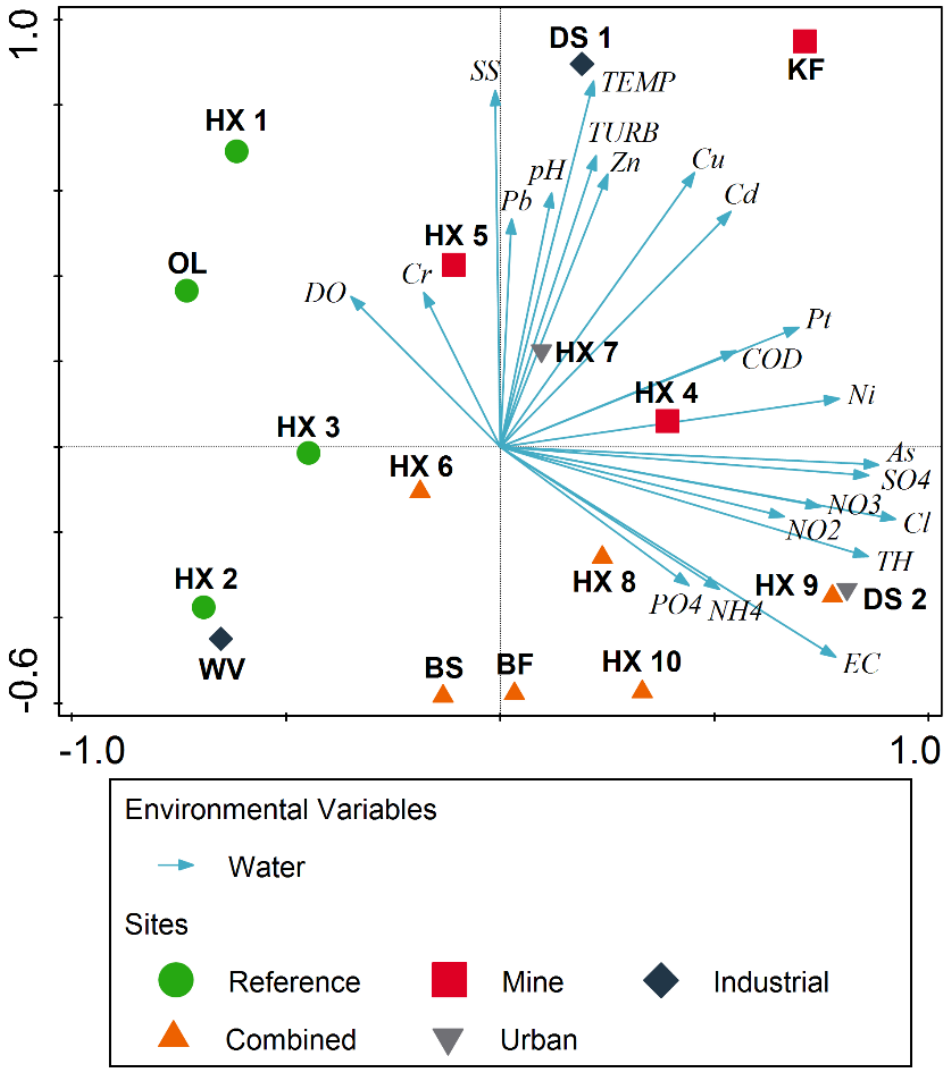


Figure 2.9: PCA biplot of the water quality variables [metal (Cr, Ni, Cu, Zn, As, Cd, Pt, Pb) and nutrient (NH_4, NO_3, NO_2, SO_4, PO_4) concentrations, physicochemical (pH, EC, temperature, DO, turbidity, Cl, TH, COD, SS)] of sites in the Hex River and tributaries. The biplot explains 54.9% of the variation, with 33.7% on the first axis and 21.2% on the second axis.

2.4 Discussion

2.4.1 Water quality variables

Water quality variables are important factors to monitor in river systems, as water chemistry is the main driver and predictor of biota present in aquatic ecosystems (Dalu *et al*., 2017a; 2017b). Water chemistry does not only influence aquatic biota but also determines if the water is fit for human consumption and usage. Several countries have developed guideline values for nutrient and metal concentrations to protect river ecosystems and to ensure that the water is safe for human consumption. The water quality criteria used to evaluate the data obtained from the present study against were: the Australian and New Zealand's guidelines for fresh water quality (ANZECC) (ANZECC, 2000), European Union's environmental quality standards (EU-EQS) (EU-EQS, 2008), South Africa's target water quality range for aquatic ecosystems (TWQR) (DWAF, 1996), as well as the United States Environmental Protection Agency national recommended water quality criteria for aquatic life (USEPA) (USEPA, 2009) (see Appendix A, Table A3). The EU-EQS have no guideline values for Cr, Cu, Zn and As, the TWQR has no values for Ni and the USEPA has no values for Cu. There are no national or international water quality guidelines for Pt in aquatic ecosystems.

2.4.1.1 General water characteristics and nutrients

The sources of nutrients into the Hex River were evident and mining activities contributed to high concentrations of Cl, SO_4, TH, and higher pH values. These salts and ions are added and used in several processes within the Pt mining sector making it common pollutants in tailing waters (Slatter *et al*., 2009; Glaister and Mudd, 2010). Industrial effluent contributed to high concentrations of NH_4, a salt typically used in several cleaning operations within industrial activities (DWAF, 1996). High concentrations of EC, Cl, TH, NO_3, NO_2, and PO_4 were found in urban effluent, while sites with combined-influences had high concentrations of all the nutrients tested. This can be contributed to treated and untreated sewage effluent. Fluctuations in pH can increase the solubility of nutrients (*e.g.* NH_4, NO_3, NO_2 and PO_4) (Matthews and Bernard, 2015). When assessing the total nitrogen (NH_4 + NO_3 + NO_2), sites influenced by mining (HX 4), urban (DS 2), and combined activities (HX 9, HX 10, BF) can be classified as hypertrophic (> 10 mg/L TWQR) Sites that are influenced by urban (HX 7, DS 2), industrial (DS 1) and combined activities (HX 8, HX 9, HX 10, BF) can be classified as hypertrophic, in view of total phosphorus (> 0.25 mg/L TWQR). However, if the ratio of N:P is assessed, sites that are influenced by industrial (WV), urban (HX 7) and combined effluents (HX 8, HX 10, BF) are classified as hypertrophic (< 10:1, TWQR; DWAF 1996). Hypertrophic conditions can induce toxic algal blooms, which can cause oxygen depletion, loss of biodiversity and posing a health risk to animals and humans (DWAF, 1996; Matthews and Bernard, 2015).

2.4.1.2 Metal concentrations

Metal concentrations in water are indicative of recent or short-term metal contamination in surface waters, and dissolved metal concentrations are more readily available to biota (Sierra *et al*., 2017). Metals can enter the Hex River catchment naturally (*e.g.* weathering of the geology) and from anthropogenic activities (*e.g.* mining activities, industrial and urban effluent). Dissolved metals with a potential natural origin in the present study, include Zn, Cr, and Pb, as these metals were the highest during the present study at the reference sites HX 1 – HX 3, respectively and occur in shale and base-metal sulphide ores (Cawthorn, 2010). Dissolved metals contributing by Pt mining activities include Ni, Cu, and Pt, as these metals were the highest at sites KF (Ni, Cu) and HX 4 (Pt), while sites with urban effluent had the highest concentrations of As (DS 2), Cu and Cd (HX 7).

Concentrations of Ni at most of the sites, except sites OL, HX 2 and HX 3 exceeded the ANZECC value, while the concentrations at sites HX 4, HX 7 – HX 10, KF, DS 1 – DS 2 and BS exceeded the EU-EQS value (see Appendix A, Table A3). Concentrations of Ni at sites KF, HX 8, DS 2 and HX 9 also exceeded the USEPA's chronic effect value (CEV), whereas the concentrations at site KF exceeded the acute effect value (AEV). The concentrations of Cu at all the sites exceeded the ANZECC, as well as the TWQR and CEV, while concentrations at sites HX 7, KF and HX 9 exceeded the AEV values for very hard water. These values are indicative of naturally high concentrations of Cu in the environment, while anthropogenic activities contribute to the already elevated concentrations. The concentrations of Zn at all of the sites exceeded the TWQR and CEV, whereas most of the sites, except sites BF and BS, also exceeded the ANZECC value; while at sites KF and DS 2, concentrations of As exceeded the TWQR.

Comparing the metal concentrations obtained during the present study with results from a previous study completed on the Hex River at relatively the same sites (Somerset *et al*., 2015), the concentrations of Ni, Cu, Cd, Pt, and Pb were in the same ranges (see Appendix A, Table A4). However, during the present study, the highest concentrations of Ni and Pt were 33- and 12-fold higher than concentrations obtained by Somerset *et al*. (2015). Comparing the present results with a study completed in a relatively pristine South African river, the Marico River (Kemp *et al*. 2017), the concentrations of Ni (73), Cu (28), Zn (three), As (10) and Pb (12) were several fold higher in the Hex River, while concentrations of Cr were higher in the Marico River. Comparing the results of the Hex River with other studies completed in South African river systems impacted by other mining activities (*e.g.* coal and gold), coal mining effluent in the Olifants River had higher concentrations of Zn and Cd (Dabrowski and de Klerk, 2013), while gold mining effluent in the Blesbok Spruit had higher concentrations of Cr, Zn, Cd and Pb (Roychoudhury and Starke, 2006). Hoppstock and Alt (2000) reported concentrations of Pt in the Elbe River, Germany of 0.0008 µg/L. Monticelli *et al*. (2010) reported concentrations of Pt in the Costa River, Italy of 0.0026 µg/L. Both these rivers are impacted by heavy traffic and would reflect

rivers contaminated by catalyst derived Pt. Comparing the concentrations of Pt in the water of an intensive Pt mining area (present study) with concentrations obtained from catalyst derived Pt in urban rivers (aforementioned rivers in Germany and Italy), the concentrations in the Hex River were 250- and 77-fold higher, respectively. This indicates that Pt from mining and production activities are larger contributors to Pt pollution to river systems compared to auto catalysts and more studies are needed to assess the impacts of Pt mining activities on aquatic systems. The present study also provides valuable information on the actual Pt concentrations in aquatic systems associated with Pt mining activities and that these concentrations are of major concern and potentially pose a major risk to aquatic biota and the aquatic ecosystem.

2.4.2 Sediment quality variables

Metal concentrations in sediment indicate past or longer-term metal contamination and sediments can act as a sink, as well as a source for dissolved metal contamination that can affect the surface water quality (Roig *et al*., 2016; Wei *et al*., 2016). Metal concentrations in sediments can be bioavailable for benthic biota and consequently enter the aquatic food web (Simpson *et al*., 2013; Roig *et al*., 2016; Wei *et al*., 2016). Metals in the sediment derived from Pt mining activities included Cr and As (HX 5), as well as Ni (KF), where these concentrations were the highest at the mining impacted sites. Urban effluent contributed to Cu, Cd, Pt and Pb (HX 7), as well as Zn (DS 2), where these metals were the highest at the urban impacted sites. Although these metal concentrations in the sediments were the highest at these sites, they are not necessarily entering the Hex River at these sites, as dissolved metals can enter surface waters upstream and precipitate further downstream (Veses *et al*., 2011). However, concentrations of Ni and Cd were the highest in the sediment and the water at sites KF and HX 7, respectively, indicating potential source pollution at these sites from mining activities and urban effluent.

The Australian and New Zealand's sediment quality guidelines values for low and high possible effects (ANZECC $SQGV_{low/high}$) (Simpson *et al*., 2013), the Netherlands' sediment quality objective (SQO) (Hin *et al*., 2010) and the United States Environmental Protection Agency sediment quality guideline (USEPA SQG) (USEPA, 2002) were used to assess metal concentrations in sediment. These international guidelines were used (see Appendix A, Table A3), as South Africa has no sediment quality guidelines. There are no SQO for Cr and Ni, while there are no international sediment quality guidelines for Pt in aquatic ecosystems. Concentrations of Cr at all the sites exceeded the ANZECC $SQGV_{low}$ and USEPA SQG values, while the concentrations of Cr at most of the sites, except sites BF and BS, exceeded the ANZECC $SQGV_{high}$ values (see Appendix A, Table A3). Concentrations of Ni at all the sites also exceeded the ANZECC $SQGV_{low}$ and USEPA SQG. The concentrations at most of the sites, except BF exceeded the ANZECC $SQGV_{high}$ value. Concentrations of Cu at most of the sites, except OL, HX 3, KF, DS 1, BF and BS, exceeded the SQO value. The concentrations of Cu at site HX 6 exceeded the ANZECC $SQGV_{low}$ value and at sites HX 7 and HX 8 exceeded the USEPA SQG value. Concentrations of Zn at sites HX 7, HX 8

and DS 2 exceeded the USEPA SQG value, while concentrations at site DS 2 exceeded the SQO and ANZECC $SQGV_{low}$ values. The concentrations of As at all of the sites exceeded the SQO, whereas the concentrations at sites HX 1 and HX 5 exceeded the ANZECC $SQGV_{low}$.

Comparing metal concentrations found in the sediment during the present study with previous studies completed in the Hex River at relatively the same sites (Somerset *et al*., 2015; Almécija *et al*., 2017), the concentrations of Ni, Cu, Cd, Pt, and Pb, were in the same ranges (see Appendix A, Table A4). Almécija *et al*. (2017) also reported on concentrations for Cr, Zn and As in the Hex River. The highest concentrations obtained during the present study were four-fold higher for Ni, three-fold higher for Cu, two-fold higher for Cd, 514-fold higher for Pt and seven-fold higher for Pb compared to the concentrations reported by Somerset *et al*. (2015). The highest concentrations of the following metals measured in the present study: Cu (three-fold), Zn (two-fold), As (four-fold), Cd (six-fold) and Pt (nine-fold) were higher than those reported by the Almécija *et al*. (2017) study. Comparing the sediment concentrations of the present study with a relatively pristine South African river, the Marico River (Kemp *et al*., 2017), the concentrations of Cr (two), Cu (two), As (two), Cd (three) and Pb (four) were several fold higher in the Hex River, while the concentrations of Ni and Zn were in the same range as the Marico River. Comparing the sediment concentrations of the Hex River with other South African rivers impacted by coal and gold mining activities, the Olifants River (Gerber *et al*., 2015) had lower metal sediment concentrations than the Hex River, while the Blesbok Spruit (Roychoudhury and Starke, 2006) had higher concentrations of Ni, Cu, Zn, As, Cd and Pb.

The highest concentrations reported for Pt in sediments derived from catalysts and road runoff were 0.085 µg/g (summarised in Ruchter *et al*., 2015; Appendix A, Table A4). Sediment concentrations higher than 0.02 µg/g is considered highly polluted (Haus *et al*., 2010). The highest concentration of Pt reported in the present study was 0.36 µg/g and is four-fold higher than sediment concentrations in urban river systems, while it is 18-fold higher than the polluted concentrations reported by Haus *et al*. (2010). Rauch and Fatoki (2009) analysed road dust collected from streets in Rustenburg and found concentrations of Pt as high as 0.39 µg/g. These high concentrations were, however, ascribed to a combination of catalyst and mining derived Pt, but mostly to mining derived Pt.

2.4.3 Interaction between water and sediment metal concentrations

Concentrations of Cr in water and sediment both had a spike at sites HX 2 and DS 1. However, in the middle reaches of the river when a decrease in dissolved Cr concentrations was evident, an increase in sediment concentrations were found. Lower down the river at sites HX 9 to BS, both the dissolved and sediment concentrations decreased. Concentrations of Ni in the water and sediment were stable throughout the river system until an increase in dissolved concentrations at KF and an increase in sediment concentrations at HX 7 and KF. Concentrations of Cu in the water and sediment were also stable throughout the river system, until an increase in sediment

concentrations occurred at site HX 7, where after it increased in water but decreased in sediments. Concentrations of Cu in both the water and sediment decreased in the lower reaches of the Hex River. Concentrations of Zn in the sediment decreased at sites KF and DS 1, while it increased in the water at these sites. Downstream of these sites, the concentrations in the water decreased and increased in the sediment. The concentrations of Zn in both water and sediment decreased towards the lower reaches of the river. Where an increase in sediment concentrations occurred, a decrease of concentrations of As was evident in water concentrations, and *vice versa*. In the upper and middle reaches of the river, an increase in concentrations of Cd in both water and sediment was evident, while at site DS 1 the concentrations increased in the water and decreased in the sediment. The concentrations in both water and sediment decreased at the lower reaches of the catchment. Regarding concentrations of Pb, with an increase in water concentrations, a decrease in sediment concentrations was found, except at site HX 5, where both water and sediment concentrations increased. The concentrations of Pb in both water and sediment decreased towards the lower reaches of the Hex River.

From the multivariate statistics clear spatial differences were evident, where reference sites separated from impacted sites across axis 1, while mining-influenced sites separated from combined-influenced sites on axis 2. The reference sites had higher concentrations of DO and low concentrations of COD indicating that these sites were less polluted. The mining-influenced sites had high concentrations of COD, and metal that are associated with Pt mining activities, while the combined-influenced sites had high concentrations of nutrients associated with sewage effluent.

Platinum deriving from mining and refining activities pose a far greater threat to the Hex River's water and sediment, compared to sources such as catalyst derived or medical derived Pt. The high dissolved concentrations of Pt at site HX 4, are indicative of a point source of Pt contamination from the mines in the vicinity. These contamination sources can potentially be derived from runoff from the rock or sand dumps, leakage or seepage of the tailing dams, spillage from pipelines or air deposition from the Pt smelter, approximately 3 km from this site. These dissolved concentrations of Pt potentially pose a risk to biota that occur in the river and humans that utilise the water, as this Pt is bioavailable. The high Pt concentrations at site HX 7 potentially originate from road runoff that enters the sewage water but can also be from the significantly higher dissolved fraction that enters the river at site HX 4, steadily decreasing to site HX 7 while increasing in the sediment fraction.

The phenomenon, of metal concentrations decreasing in the water and increasing in sediment concentrations, can be ascribed to the fact that the dissolved metals bind, absorbs and precipitate downstream. This increased concentration of metals in the sediment can, however, still be bioavailable and pose a risk to benthic biota, or even to pelagic biota, if the metals are resuspended in the water column when physical, chemical or hydrological disturbances occur

(Arain *et al*., 2008; Wei *et al*., 2016). The decrease in both water and sediment concentrations of Cr, Cu, Zn, Cd, and Pb in the lower reaches of the Hex River can potentially be ascribed to the water hyacinth or other vegetation present at sites HX 10, BF and BS that accumulate the dissolved metals from the water, preventing precipitation into the sediment (Farago and Parsons, 1994; Bednarova *et al*., 2014).

2.4.4 Sources of metals in the Hex River catchment

Metal concentrations in the Bushveld Igneous Complex are naturally high in the parental rock and are elevated compared to normal upper continental crust values (Barnes and Maier, 2002; Almécija *et al*., 2017). Concentrations of Cr and Ni are 22-fold higher, Cu and Pt are 12- and 10-fold higher, Cd and As are six and four-fold higher, while concentrations of Zn and Pb are three and two-fold higher in the Bushveld Igneous Complex compared to normal upper continental crust values (Barnes and Maier, 2002; Almécija *et al*., 2017). Although the metal concentrations are naturally high, intensive Pt and Cr mining activities, urban and industrial effluent contribute to an anthropogenic increase in metal concentrations in addition to the already elevated background concentrations. (Almécija *et al*., 2017; Erasmus *et al*., 2020).

Chromium can enter the aquatic environment naturally by weathering and erosion of chromite ores (DWAF, 1996; Wang *et al*., 2009). Nickel, Cu, and Zn can originate naturally by means of weathering of minerals such as olivine, which weathers easily (Bradl, 2005). Arsenic, Cd, Pt, and Pb originate naturally from weathering of various base-metal sulphide ores (DWAF, 1996; Bradl, 2005; Wang *et al*., 2009). These ores and minerals are commonly found throughout the Bushveld Igneous Complex. Shale is also a natural source of Cr, Ni, Cu, Zn, As, Cd and Pb and occurs abundantly at the reference sites (HX 1 and OL).

These metals can also enter the Hex River by means of several anthropogenic sources, including mining activities (HX 4, HX 5 and KF), industrial effluent (WV and DS 1), urban effluent (HX 7 and DS 2), as well as a combined effect from above-mentioned sources (HX 6, HX 8, HX 9, HX 10, BF and BS). Anthropogenic sources of Cr, Ni, Cu, As, Cd, Pt, and Pb are, amongst others, from mining, smelting and refining industries and can enter the surface water via runoff, spillage, seepage or deposition (DWAF, 1996; Bradl, 2005; Cempel and Nikel, 2006; Wang *et al*., 2009; Rauch and Fatoki, 2015). Anthropogenic sources from sewage and industrial effluent include: Cr, Ni, As and Pb from ceramic manufacturing, Cu from corrosion of pipes and roofing, Zn from fertilizers and insecticides, As from additives to animal feed, metallurgy industries, as well as several pesticides and herbicides, Ni and Cd from Ni/Cd battery and anti-corrosive metal coating industries and Pb from lead-acid battery industries (DWAF, 1996; Bradl, 2005; Wang *et al*., 2009). All of these industries and activities are present in Rustenburg, meaning that metals can enter the Hex River via sewage and industrial effluent. Other sources of Pt and Pb include traffic related road runoff from mechanical and chemical abrasion of automotive catalytic converters and emissions from combustion of gasoline (Rauch and Fatoki, 2009; Hwang

et al., 2016). Additional sources of Pt into the environment include medical and dental applications (Pt-based anticancer drugs), chemical and electrical industries, as well as Pt jewellery applications (Rauch and Peucker-Ehrenbrink, 2015).

2.5 Conclusion

The Hex River catchment is impacted by a variety of anthropogenic influences, including agricultural, industrial and mining activities, as well as urban and informal settlement effluents. During the present study, we determined which of these activities were the main source of Pt pollution, as well as assessed the interaction between the water and sediment metal concentrations in the Hex River. From the results, it was evident that Pt mining and refining activities were the main sources of Pt, Ni, and Cu, as well as Cr, Ni and As contamination in the water and sediment, respectively. Urban effluent contributed to elevated concentrations of As and Cd, as well as Cu, Zn, Cd, Pt and Pb contamination in the water and sediment, respectively. Most of these metal concentrations exceeded the various water and sediment quality guideline values, but it should be noted that the background concentrations in the Bushveld Igneous Complex are already several times higher than normal upper crust levels and that these anthropogenic influences contribute to already elevated levels. The high concentrations of Pt in this river system pose a potential threat to aquatic biota living in the river, as well as humans utilising the water. From the comparison to previous studies completed in the Hex River, it is evident that Pt contamination and associated metals increase over time and raise concern for the environmental management of mining regions. The data allow us to support the hypothesis, that Pt smelters and production activities will be the main contributor to metal pollution in the Hex River, as these activities contributed to significantly higher concentrations of Pt in the Hex River catchment.

CHAPTER 3: WATER AND SEDIMENT QUALITY VARIABLES INFLUENCING METAL BEHAVIOUR IN THE HEX RIVER

3.1 Introduction

River systems that drain intensive mining regions are usually contaminated with metals and other pollutants (Resongles *et al*., 2015). The metals and pollutants potentially enter the river from untreated mining water, tailing dams, smelting slags, and waste dumps resulting in both point and diffuse source effects (Salomons, 1995; Miller *et al*., 2007; Resongles *et al*., 2015). Once metals entered the river system, the behaviour is determined by the water chemistry, the suspended sediment composition, and the substrate sediment composition (Mohiuddin *et al*., 2012; Islam *et al*., 2015). The physicochemical characteristics of the water and sediment cause the metals to undergo frequent changes due to sorption, dissolution and precipitation processes (Ali *et al*., 2016). Metal concentrations in river systems can vary considerably between seasons due to variation in flow and input sources (Roig *et al*., 2016). River systems like the Hex River, however, are prone to drought and the drought-rewetting process can also have an effect on metal concentrations (Lin *et al*., 2018). During rainfall events, surface runoff transports soil, organic matter, clay, along with silt minerals into the river that can influence the sorption and chemical transformation of metals (Bouskill *et al*., 2010; Lin *et al*., 2018; Rathoure, 2020). It is, therefore, essential to monitor water and sediment quality seasonally in river systems due to these fluctuations in the hydrology of rivers.

Flow changes within river systems, especially droughts, negatively influence water quality by affecting the water temperature, major ions, eutrophication and metals (Wilbers *et al*., 2009; Nosrati, 2015; Nedjai *et al*., 2016). An increase in water temperatures and decrease in flow-rates may result in higher concentrations of pollutants within the system, as released by point sources, increased sediment deposition and decreased concentrations of dissolved oxygen (Zwolsman and van Bokhoven, 2007; van Vliet and Zwolsman, 2008). The increase in major ions and nutrients influences the transformation and interaction of metals (Zimmermann *et al*., 2004; Ghosh *et al*., 2011; Crea *et al*., 2013; Jarošíková *et al*., 2017; Buxton *et al*., 2019).

Monitoring of metal concentrations in water and sediments of the aquatic environment is a necessity, as these matrices are the exposure medium to aquatic organisms (Farag *et al*., 2007; Hassaan *et al*., 2016; Liu *et al*., 2019). Metals can enter organisms via diffusion, ingestion or adsorption (O'Mara *et al*., 2019) and can affect organisms from the lowest trophic levels (*e.g.* periphyton) to the highest trophic levels (*e.g.* fish; humans).

The hypothesis tested for this chapter is that the behaviour of Pt and associated metals in water and sediments will be influenced by altered physicochemical parameters as a result of changes in seasonal river runoff patterns. The aims were to determine the water and sediment physicochemical quality variables from seasonal surveys (2017 – 2018) at selected sites in the Hex River and assess the interaction between the metal concentrations and these variables.

3.2 Materials and methods

3.2.1 Study area

From the general survey completed in Chapter 2 it was evident that the Hex River, where specific "hot spots" were identified, is subjected to various anthropogenic influences. For this chapter and the remaining chapters of the thesis, the focus will be on four sites located in the Hex River and two sites in the impoundments (Fig. 3.1; Tables 3.1 – 3.6). These sites include three reference sites (Site 1, Site 2, Olifantsnek Dam), as well as three impacted sites associated with Pt mining activities (Site 4, Site 5, Bospoort Dam). These sites were selected based on the impacts described in Chapter 2. Site 1 was a new site, approximately 8.6 km further upstream than site HX 1, while Site 2 is the same as site HX 1 in Chapter 2. Site 4 was located downstream of the Pt mining-influences and was approximately 1 km upstream from site HX 7 in Chapter 2, in order to consider the various impacts described. This site served as a monitoring point for mining effluent before the influence of the city of Rustenburg. Site 5 was located further downstream and received both urban and industrial effluents. Informal settlements have the same influence on Site 5 and is the same as site HX 9 in Chapter 2. The impoundment sites were the same as the impoundment sites described in Chapter 2. During the surveys completed between 2017 and 2018, South Africa experienced an extreme drought caused by a strong El Niño event (Baudoin *et al*., 2017). The effects thereof could be seen during the November 2017 survey, where Sites 1 and 2 were completely dry. Similarly, the water levels of Olifantsnek Dam and Bospoort Dam, decreased from 74% and 102% in April 2017 to 29% and 82%, respectively, in November 2018.

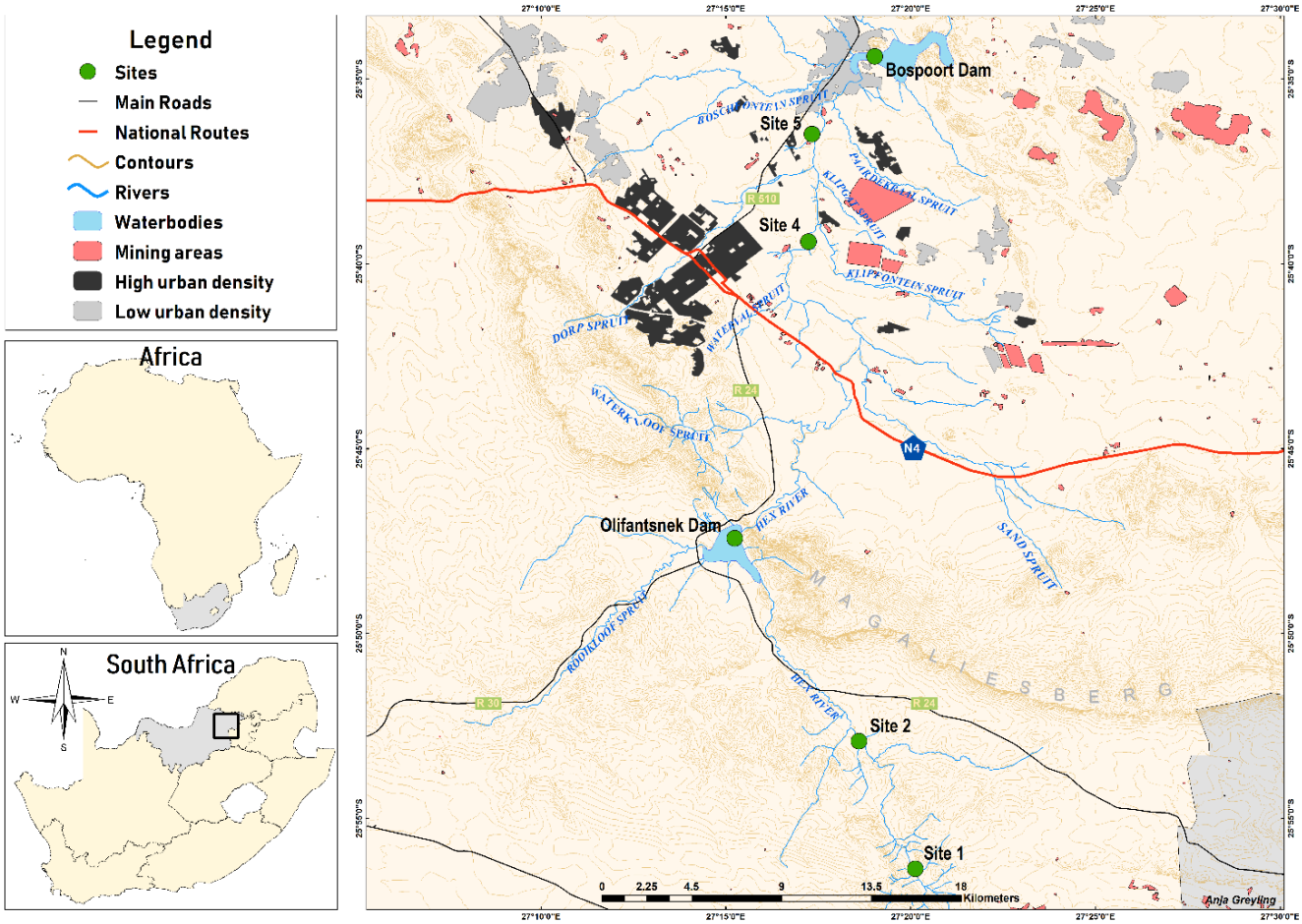


Figure 3.1: Map of the study area, indicating the sampling sites in the Hex River and the two impoundments, as well as mining activities, urban and informal settlements in the surrounding area.

Table 3.1: Physical characteristics of Site 1.

Site 1 Near the origin of the Hex River	
	
Coordinates	S 25°56’21.8” E 27°20’07.5”
Altitude	1 384 m
Site description	This site is located approximately 7 km downstream of the origin of the Hex River. The land cover surrounding this site is relatively natural with only a few game farms in the vicinity. This site was dry in the November 2017 survey and could not be sampled.
Biotope description	Headwater zone, gravel to sandy substratum with stones in current, overhanging tree canopy, marginal vegetation with pool, riffle and run biotopes.
Primary lithology	Slate, Shale, Hornfels, Diabase (Walraven, 1981)

Table 3.2: Physical characteristics of Site 2.

Site 2 Upstream of Olifantsnek Dam	
	
Coordinates	S 25°52’54.9” E 27°18’36.3”
Altitude	1 288 m
Site description	This site is located approximately 8.5 km downstream of Site 1 and 12 km upstream of Olifantsnek Dam. Few anthropogenic impacts are present at this site, with only small-scale agricultural activities and game farms in the surrounding areas. This site was also dry in the November 2017 survey and could not be sampled, during the March 2018 survey the site had water but was mostly rainwater that pooled up and only water and sediment samples were collected.
Biotope description	Headwater zone, gravel to sandy substratum with stones in current, overhanging tree canopy, marginal vegetation with riffle and run biotope.
Primary lithology	Slate, Shale, Hornfels, Diabase (Walraven, 1981)

Table 3.3: Physical characteristics of OL.

OL Olifantsnek Dam	
	
Coordinates	S 25°47’25.3” E 27°15’14.7”
Altitude	1 206 m
Site description	This site is located within the upstream impoundment of the Hex River. The water from this impoundment is used for irrigation purposes and recreational activities (*i.e.* fishing and sailing). Few anthropogenic impacts are present, with only small-scale agricultural activities. This impoundment was 73.6% full during the April 2017 survey, 69.9% during the June 2017 survey, 54.4% during the November 2017 survey, 44.0% during the March 2018 survey and, during the November 2018 survey, this impoundment only had 28.9% water.
Biotope description	Headwater zone, sandy to muddy substratum, few to no marginal vegetation and pool biotope.
Primary lithology	Slate, Shale, Hornfels, Diabase, Surface deposits (Walraven, 1981)

Table 3.4: Physical characteristics of Site 4.

Site 4 Downstream of Pt and Cr mining activities	
	
Coordinates	S 25°39’24.2” E 27°17’14.3”
Altitude	1 115 m
Site description	This site is located approximately 2 km downstream of the Pt and Cr mining activities and Pt ore transport rail line located in the Hex River catchment and approximately 4 km away from the Pt smelter. This site is also located upstream of Rustenburg’s wastewater treatment works and industrial effluent to monitor the influence of mining activities on the Hex River.
Biotope description	Middle water zone, gravel to sandy substratum with stones in current, marginal vegetation and algae, pool, run and riffle biotopes.
Primary lithology	Gabbro, Norite, Anorthosite (Walraven, 1981)

Table 3.5: Physical characteristics of Site 5.

Site 5 Downstream of the city of Rustenburg	
	
Coordinates	S 25°36’29.4” E 27°17’20.2”
Altitude	1 095 m
Site description	This site is located downstream of the city of Rustenburg. It receives urban and industrial effluent, treated and untreated sewage runoff, and is surrounded by informal settlements. It is situated approximately 5 km upstream of Bospoort Dam and can be used to monitor the influence of urban and industrial effluent on the Hex River.
Biotope description	Lower water zone, gravel to sandy substratum with stones in current, marginal vegetation and algae, pool, run and riffle biotopes.
Primary lithology	Gabbro, Norite (Walraven, 1981)

Table 3.6: Physical characteristics of BS.

BS Bospoort Dam	
	
Coordinates	S 25°34’23.5” E 27°19’01.7”
Altitude	1 082 m
Site description	This site is located within the downstream impoundment of the Hex River. Various intensive anthropogenic impacts are present from urban and industrial effluent, Pt and Cr mining activities, as well as informal settlement effluent. This impoundment was 102.1% full during the April 2017 survey, 102.0% during the June 2017 survey, 99.7% during the November 2017 survey, 101.9% during the March 2018 survey. During the November 2018 survey, this impoundment only had 82.4% water. During all the surveys, approximately 8.9 – 50.3% of the impoundment was covered by water hyacinth, with the lowest density in November 2017 and the highest in November 2018.
Biotope description	Lower water zone, sandy substratum, marginal and aquatic vegetation, pool biotope.
Primary lithology	Gabbro, Norite, Syenite (Walraven, 1981)

3.2.2 Field sampling

Triplicate water and sediment samples were collected from the six selected sites during the April 2017, June 2017, November 2017 and March 2018 surveys. Additional sampling was undertaken in the two impoundment sites during November 2018. During each survey, *in situ* water quality variables (pH, EC, temperature, DO), and sediment samples were collected using the methods described in Chapter 2. To assess the influence of runoff on the physicochemical characteristics and metals in water and sediments, the survey periods were selected to represent different hydro-periods, *i.e.* high runoff and flow (April 2017, March 2018) and low runoff and flow (June 2017, November 2017, 2018).

3.2.3 Laboratory analyses

Water samples (n = 3) were analysed for nutrient and metal concentrations. Sediment samples (n = 3) were analysed for metal concentrations, as well as particle size distribution and organic content, using the methods described in Chapter 2. Concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt, and Pb were measured in both media. Metals selected are associated with Pt mining and production activities, as well as urban effluent that enters the Hex River system (Chapter 2).

3.2.4 Statistical analyses

Normality and homogeneity of variance of the data were tested using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively, using GraphPad Prism v7. When the data were parametric, Two-way ANOVA with Tukey's multiple comparison test was performed. In the case of non-parametric data, Kruskal-Wallis tests with Dunn's multiple comparison-tests were performed to test for significant differences between sites and surveys (Pyrczak, 2016). One-way ANOVA with Tukey's multiple comparison test was performed to test for significant differences of *in situ* parameters between sites. Pearson correlation coefficients between water and sediment quality variables, and metal concentrations were analysed. Statistical significance of the spatial and temporal variation of water and sediment quality variables, and metal concentrations in water and sediment was determined at $p < 0.05$. Additionally, a Principle Component Analysis (PCA) (Canoco v5.12) was created to assess the temporal and spatial patterns associated with the water and sediment quality. All the data used for PCA analysis were log-transformed $x = \log(y + 1)$, as well as centred and standardised (Šmilauer and Lepš, 2014).

3.3 Results

3.3.1 Water quality

The pH values recorded throughout the surveys at a specific site were relatively constant. Between the sites it varied from 7.2 to 8.8, while the pH values at Site 4 were on average higher than the other sites (Tables 3.7 and 3.8). Electrical conductivity increased from the reference sites (Site 1, 2 and Olifantsnek Dam) towards Bospoort Dam. The EC values at Site 5 were significantly higher ($p = 0.0008$) than Sites 1, 2, 4, and Olifantsnek Dam. Bospoort Dam had significantly higher ($p = 0.0035$) EC values than all the reference sites, and Site 4 had significantly higher ($p = 0.0232$) EC values than Olifantsnek Dam. The DO also decreased from the reference sites towards Bospoort Dam and Site 1 had significantly higher ($p = 0.033$) concentrations of DO than Bospoort Dam. The total nitrogen was significantly higher at Site 4 and Site 5 ($p < 0.0001$) compared to the reference sites and was consistently the highest concentration at all the sites during the June 2017 survey. The PO_4 at Site 5 was significantly higher ($p < 0.0001$) than all the other sites and the highest concentrations of PO_4 were found during the April 2017 survey at Site 1 and during the June 2017 survey, at Olifantsnek Dam and Site 5. The March 2018 survey had the highest concentrations of PO_4 at Sites 2, 4, and Bospoort Dam. Compared to the reference sites, concentrations of SO_4 were significantly higher at Site 4 ($p = 0.0034$), Site 5 ($p < 0.0001$), and Bospoort Dam ($p < 0.0001$). The highest concentrations of SO_4 were found during the June 2017 survey at Site 2, during the November 2017 survey at Sites 4 and 5, during the March 2018 survey at Site 1, and during the November 2018 survey at the two impoundments. Concentrations of Cl were significantly higher at Site 4 ($p = 0.0010$), Site 5 ($p < 0.0001$) and Bospoort Dam ($p < 0.0001$) than the reference sites. These concentrations were the highest during the June 2017 survey at Site 2 and Olifantsnek Dam, during the November 2017 survey at Sites 4 and 5, during the March 2018 survey at Site 1, and during the November 2018 survey at Bospoort Dam.

3.3.2 Sediment quality variables

The organic content of the sediment varied between sites and surveys. Site 1 ($p = 0.0198$) and Olifantsnek Dam ($p = 0.0187$) had significantly higher organic content than the impacted sites (Tables 3.7 and 3.8). The highest organic content occurred at Sites 1, 5, and Olifantsnek Dam during the April 2017 survey, whereas the highest percentages were found at Sites 2, 4, and Bospoort Dam during the March 2018 survey. The sediment particle size distribution varied considerably within a site between surveys. However, during the March 2018 survey an increase in finer substrate (fine sand and mud) occurred at all of the sites, while coarser particles (gravel and very coarse sand) decreased.

Table 3.7: Mean (n = 3) water and sediment quality variables analysed at the reference sites (Site 1, Site 2, Olifantsnek Dam) during the four surveys for the river sites (04/2017; 06/2017;11/2017; 03/2018) and five surveys for the impoundment site (04/2017;06/2017; 11/2017; 03/2018; 11/2018).

		Site 1				Site 2				Olifantsnek Dam				
	Code	04/17	06/17	11/17	03/18	04/17	06/17	11/17	03/18	04/17	06/17	11/17	03/18	11/18
Water quality variables														
pH	pH	7.4	8.2	-	8.2	7.8	8.4	-	7.4	7.4	8.7	8.8	8.2	7.9
Temperature (°C)	TEMP	14.5	11.1	-	23.8	15.6	10.8	-	24.4	21.4	15.6	25.6	24.3	23.3
Electrical conductivity (µS/cm)	EC	271	301	-	375	279	335	-	71	162	155	183	210	189
Dissolved oxygen (mg/L)	DO	12.5	11.2	-	7.3	10.4	10.4	-	2.9	5.6	9.2	7.1	5.5	10.5
Suspended solids (mg/100mL)	SS	1.0	2.0	-	1.8	3.3	0.7	-	44.4	7.0	10.7	9.0	5.1	3.8
Turbidity (FAU)	TURB	13	14	-	12	18	11	-	71	22	36	48	15	38
Ammonium (mg/L)	NH4	0.06	0.04	-	0.12	0.05	0.03	-	0.15	0.07	0.07	0.11	0.09	0.02
Nitrate (mg/L)	NO3	0.6	3.0	-	0.06	1.0	2.0	-	0.09	4.2	4.9	1.4	0.2	0.3
Nitrite (mg/L)	NO2	0.008	0.002	-	0.004	0.006	0.003	-	0.022	0.012	0.013	0.010	0.008	0.007
Sulphate (mg/L)	SO4	34.7	48.0	-	54.0	39.7	51.7	-	42.7	30.3	34.0	41.7	57.0	86.3
Ortho-phosphate (mg/L)	PO4	0.09	0.07	-	0.05	0.04	0.07	-	0.26	0.09	0.14	0.18	0.10	0.04
Chloride (mg/L)	Cl	10.7	9.2	-	11.1	7.3	12.2	-	8.5	10.3	13.4	9.0	10.6	11.8
Chemical oxygen demand (mg/L)	COD	5.6	6.1	-	10.3	5.6	2.7	-	13.6	11.7	13.7	16.0	13.3	12.6
Total hardness (mg/L)	TH	81.3	92.0	-	70.0	85.0	103.3	-	32.0	51.0	56.3	44.0	53.7	61.0
Sediment quality variables														
Organic content (%)	OC	8.7	5.6	-	7.7	4.1	3.6	-	4.5	7.3	6.3	6.2	3.8	2.0
Gravel (%)	GR	0.9	16.4	-	16.0	33.9	12.7	-	7.6	1.0	1.3	1.7	0.3	3.3
Very coarse sand (%)	VCS	1.9	9.0	-	6.7	26.6	20.1	-	9.3	1.2	1.2	3.6	0.5	2.5
Coarse sand (%)	CS	23.9	33.7	-	22.6	27.5	54.5	-	44.7	23.7	17.9	20.2	2.6	13.1
Medium sand (%)	MS	43.2	23.8	-	19.0	6.4	8.0	-	23.6	20.5	24.9	25.1	9.6	16.7
Fine sand (%)	FS	21.9	13.4	-	16.9	4.0	3.7	-	10.8	40.0	44.9	43.7	73.1	56.4
Mud (%)	M	8.2	3.7	-	18.8	1.6	0.9	-	4.0	13.6	9.8	5.8	13.9	8.0

- site was dry during the survey and could not be sampled

Table 3.8: Mean (n = 3) water and sediment quality variables analysed at the impacted sites (Site 4, Site 5, Bospoort Dam) during the four surveys for the river sites (04/2017; 06/2017;11/2017; 03/2018) and five surveys for the impoundment site (04/2017;06/2017; 11/2017; 03/2018; 11/2018).

		Site 4				Site 5				Bospoort Dam				
	Code	04/17	06/17	11/17	03/18	04/17	06/17	11/17	03/18	04/17	06/17	11/17	03/18	11/18
Water quality variables														
pH	pH	8.0	8.4	8.8	8.3	7.5	8.0	8.7	7.8	8.7	7.7	7.8	7.7	7.2
Temperature (°C)	TEMP	19.8	13.4	27.0	24.9	21.1	13.2	26.6	23.8	21.6	14.0	23.9	28.3	23.1
Electrical conductivity (µS/cm)	EC	780	799	899	548	1 048	1 392	2 100	1 646	808	963	1 180	1 160	1 552
Dissolved oxygen (mg/L)	DO	9.4	12.3	9.6	5.5	7.6	6.7	5.3	1.5	3.8	2.9	4.0	2.9	8.1
Suspended solids (mg/100mL)	SS	2.7	6.0	2.5	1.5	1.7	2.0	4.6	3.6	2.7	2.0	1.4	4.0	1.9
Turbidity (FAU)	TURB	22	19	18	13	21	12	24	14	12	6	21	12	9
Ammonium (mg/L)	NH4	0.04	0.04	0.07	0.12	1.08	2.85	0.18	3.94	0.14	0.49	0.09	0.27	0.13
Nitrate (mg/L)	NO3	13.3	25.6	1.8	4.1	13.6	15.0	19.0	5.7	5.0	5.8	1.3	2.0	0.4
Nitrite (mg/L)	NO2	0.23	0.10	0.03	0.03	0.37	0.79	0.39	0.46	0.06	0.09	0.06	0.02	0.04
Sulphate (mg/L)	SO4	43.7	122.0	143.7	83.7	170.7	179.0	280.0	239.3	188.7	207.0	195.7	200.0	233.3
Ortho-phosphate (mg/L)	PO4	0.07	0.07	0.08	0.17	0.52	0.65	0.98	0.89	0.09	0.08	0.19	0.27	0.20
Chloride (mg/L)	Cl	64.0	71.3	116.3	52.0	109.0	204.7	474.7	224.0	94.7	17.9	163.7	201.7	240.0
Chemical oxygen demand (mg/L)	COD	8.3	7.0	20.5	19.8	12.1	13.7	29.8	25.8	10.4	10.1	37.8	19.9	21.0
Total hardness (mg/L)	TH	172.3	195.7	205.0	126.7	176.7	232.0	362.7	277.3	126.7	155.0	129.7	154.3	200.7
Sediment quality variables														
Organic content (%)	OC	1.6	1.2	2.5	5.7	3.5	1.1	1.7	3.2	5.4	1.8	4.6	7.9	2.3
Gravel (%)	GR	1.5	21.8	17.4	13.4	14.6	18.1	6.8	3.4	6.7	2.5	9.9	5.9	1.1
Very coarse sand (%)	VCS	4.8	16.2	18.1	11.4	10.8	6.3	5.8	2.5	8.2	4.2	7.3	6.4	1.7
Coarse sand (%)	CS	38.2	37.6	40.8	35.1	24.3	28.7	31.9	20.3	25.9	14.2	20.9	26.1	11.0
Medium sand (%)	MS	33.5	16.7	14.9	18.5	23.2	33.0	38.3	43.8	33.2	41.2	33.8	34.5	27.5
Fine sand (%)	FS	19.9	7.1	7.6	14.7	22.2	12.9	15.5	25.1	23.2	36.3	25.1	22.7	55.0
Mud (%)	M	2.1	0.7	1.3	7.0	4.8	1.1	1.8	5.0	2.9	1.6	3.0	4.3	3.7

3.3.3 Dissolved metal concentrations

Concentrations of Pt ($p < 0.0001$; $F = 201.3$) at Site 4, were significantly higher than all of the sites during all the surveys, while concentrations of Cr ($p = 0.013$; $F = 120.6$) was also significantly higher during the April 2017 survey (Table 3.9; Appendix B, Tables B 1 and B 2). Site 5 had significantly higher concentrations of Ni ($p < 0.0001$; $F = 346.2$) than the other sites, except for Bospoort Dam during the April 2017 and June 2017 surveys. Site 5 had significantly higher concentrations of Ni ($p = 0.005$; $F = 70.7$) and As ($p = 0.0003$; $F = 177.6$) than all of the sites during the November 2017 and March 2018 surveys. Olifantsnek Dam had significantly higher concentrations of Cr ($p < 0.0001$; $F = 125.0$) than Bospoort Dam, while Bospoort Dam had significantly higher concentrations of Ni ($p < 0.0001$; $F = 346.2$), As ($p < 0.0001$; $F = 42.6$), and Pt ($p = 0.0016$; $F = 483.3$) than Olifantsnek Dam during the November 2018 survey. The concentrations of Cr ($p < 0.0001$; $F = 64.7$), Ni ($p < 0.0001$; $F = 23.9$), Cu ($p < 0.0001$; $F = 7.5$), Zn ($p = 0.0009$; $F = 2.9$), As ($p < 0.0001$; $F = 42.6$), Cd ($p < 0.0001$; $F = 7.9$), Pt ($p < 0.0001$; $F = 49.4$), and Pb ($p < 0.0001$; $F = 7.1$) had significant interactions between sites and surveys.

Only those water quality variables that had significant correlations with dissolved metal concentrations associated with Pt mining are presented in Table 3.9. Electrical conductivity ($p < 0.0001$), TH ($p = 0.0007$), SO_4 ($p < 0.0001$), Cl ($p = 0.0013$), PO_4 ($p = 0.0033$), NH_4 ($p = 0.0284$), NO_3 ($p = 0.0349$), and NO_2 ($p = 0.0003$) had significantly positive correlations with concentrations of Ni. There was a significant ($p = 0.0453$) negative correlation between pH and Cu and a significant positive correlation between Cu and turbidity ($p = 0.0114$). Chemical oxygen demand ($p = 0.0025$) was positively correlated with Zn. Arsenic was positively correlated to EC ($p < 0.0001$), TH ($p < 0.0001$), SO_4 ($p = 0.0008$), Cl ($p < 0.0001$), PO_4 ($p < 0.0001$), COD ($p < 0.0001$), NH_4 ($p = 0.0450$), and NO_2 ($p = 0.0046$). Similarly, Cd was positively correlated with EC ($p = 0.0076$), TH ($p = 0.0031$), SO_4 ($p = 0.0307$), Cl ($p < 0.0001$), PO_4 ($p = 0.0006$), and COD ($p = 0.0082$). The EC ($p = 0.0139$), TH ($p = 0.0004$), NO_3 ($p = 0.0003$), and NO_2 ($p = 0.0410$) had a significantly positive correlation with concentrations of Pt. Only SS ($p = 0.0333$) showed a significant positive correlation with concentrations of Pb.

Table 3.9: Correlation matrices (R^2 and p-value) of the significant correlations between dissolved metal concentrations and water quality variables.

	Ni	Cu	Zn	As	Cd	Pt	Pb
pH	-	0.1700 0.0453	-	-	-	-	-
EC	0.5698 < 0.0001	-	-	0.5663 < 0.0001	0.2816 0.0076	0.2452 0.0139	-
SS	-	-	-	-	-	-	0.1899 0.0333
Turb	-	0.2575 0.0114	-	-	-	-	-
NH_4	0.2002 0.0284	-	-	0.1703 0.0450	-	-	-
NO_3	0.1869 0.0349	-	-	-	-	0.4631 0.0003	-
NO_2	0.4551 0.0003	-	-	0.3110 0.0046	-	0.1764 0.0410	-
SO_4	0.6633 < 0.0001	-	-	0.4060 0.0008	0.1951 0.0307	-	-
PO_4	0.3309 0.0033	-	-	0.5475 < 0.0001	0.4195 0.0006	-	-
Cl	0.3803 0.0013	-	-	0.6331 < 0.0001	0.5400 < 0.0001	-	-
COD	-	-	0.3465 0.0025	0.5330 < 0.0001	0.2770 0.0082	-	-
TH	0.4126 0.0007	-	-	0.5475 < 0.0001	0.3340 0.0031	0.4381 0.0004	-

3.3.4 Sediment metal concentrations

Comparing the sites, Site 1 had significantly higher concentrations of As ($p = 0.0012$; $F = 20.6$) than all of the other sites, while Site 4 had significantly higher concentrations of Pt ($p = 0.0219$; $F = 46.5$) than the other sites, with the exception of Site 1 during the April 2017 survey (Table 3.10; Appendix B, Tables B 3 and B 4). Site 4 had significantly higher concentrations of Cu ($p = 0.0004$; $F = 36.8$), Zn ($p < 0.0001$; $F = 49.9$), Cd ($p < 0.0001$; $F = 17.5$), Pt ($p < 0.0001$; $F = 46.5$), and Pb ($p = 0.0114$; $F = 21.3$) compared to all of the other sites, whereas Site 5 had significantly higher concentrations of Ni ($p = 0.0056$; $F = 25.0$) than the other sites, with the exception of Site 4 during the June 2017 survey. During the November 2017 survey, Site 4 had significantly higher concentrations of Cu ($p = 0.0008$; $F = 46.6$), Zn ($p < 0.0001$; $F = 81.8$), and Pt ($p < 0.0001$; $F = 9.6$) than Olifantsnek Dam, Site 5 and Bospoort Dam. Site 2 had significantly higher concentrations of As ($p = 0.0002$; $F = 165.9$) and Site 4 significantly higher concentrations of Cr ($p < 0.0001$; $F = 47.3$), Ni ($p = 0.0001$; $F = 50.4$), Cu ($p < 0.0001$; $F = 46.6$), Zn ($p < 0.0001$; $F = 81.8$), and Pt ($p < 0.0001$; $F = 9.6$) compared to all of the other sites during the March 2018 survey. Olifantsnek Dam had significantly higher concentrations of Cr ($p = 0.0011$; $F = 47.3$) and Zn ($p = 0.0235$; $F = 81.8$) than Bospoort Dam during the November 2018 survey. The concentrations of Cr ($p < 0.0001$; $F = 8.7$), Ni ($p < 0.0001$; $F = 9.1$), Cu ($p < 0.0001$; $F = 9.3$), Zn

($p < 0.0001$; $F = 19.6$), As ($p < 0.0001$; $F = 63.4$), Cd ($p < 0.0001$; $F = 4.1$), Pt ($p < 0.0001$; $F = 4.6$), and Pb ($p < 0.0001$; $F = 9.5$) had significant interactions between sites and surveys.

3.3.5 Spatial and temporal variations in water and sediment quality variables

Spatial variation in the water and sediment quality variables had a greater influence on the ordination of sites than temporal variation (Fig. 3.2). The reference sites (Site 1, 2, Olifantsnek Dam) grouped together and associated with higher concentrations of DO, turbidity, OC, finer substrate, and higher concentrations of As in the sediment. Site 4 associated with higher concentrations of Cr and Pb in the water, coarser substratum, increased pH, and higher concentrations of metals in the sediment (Cr, Ni, Cu, Zn, Cd, Pt, Pb). Site 5 associated with higher medium sized substrate, nutrients (ortho-phosphates and sum of all N). Cl, SO_4, EC, and dissolved concentrations of Ni, Zn, and As. There were no relationships between Zn and As in water and sediments, however, for Pt and Pb there was a strong correlation between the water and sediment concentrations. There was a weak positive correlation between Cr, Ni, Cu and Cd in water and sediment samples.

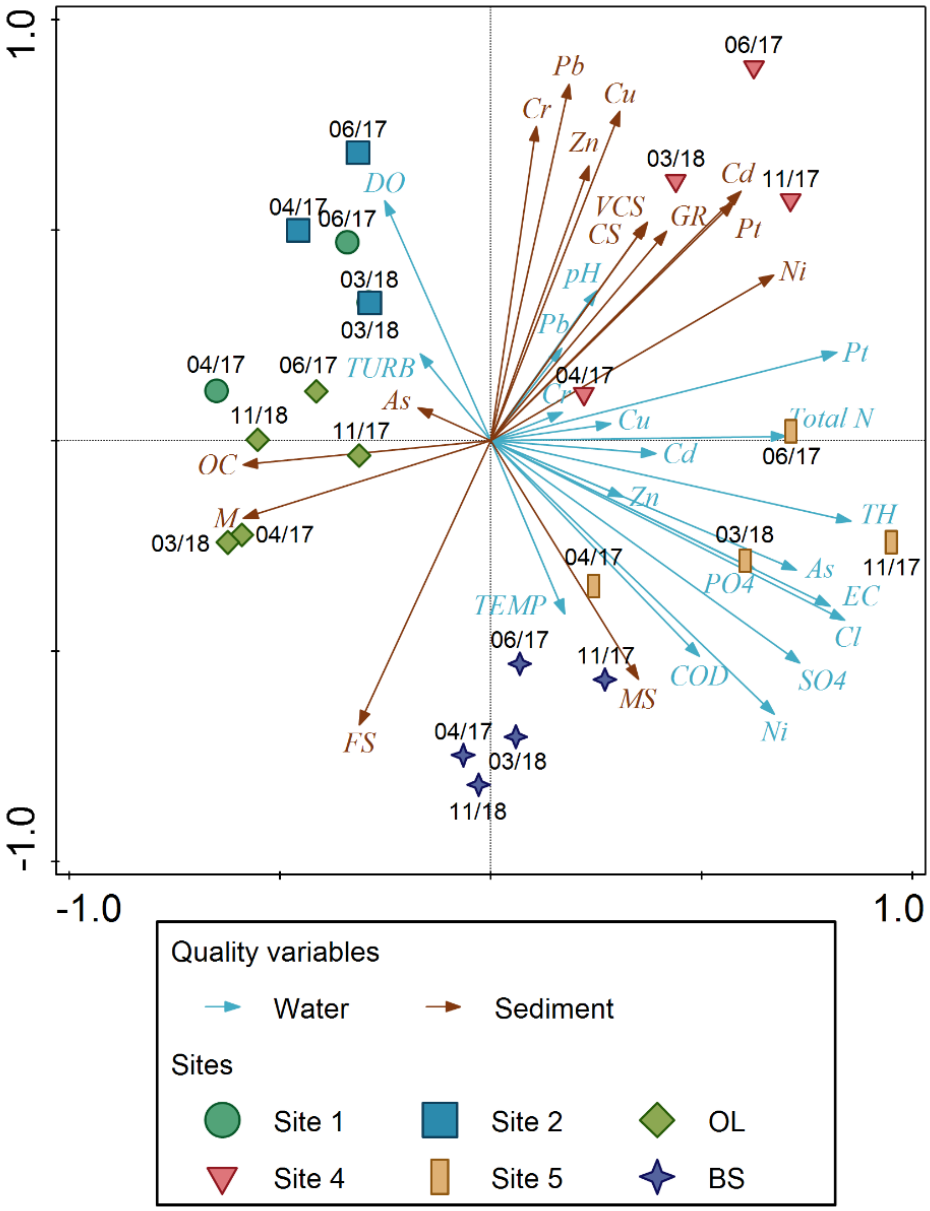


Figure 3.2: PCA biplot of the water and sediment quality variables measured at sites in the Hex River catchment. The biplot describes 46.2% of the variation, with 25.5% on the first axis and 20.7% on the second axis. Water quality variables include dissolved metal concentrations, nutrients and in situ parameters. Sediment quality variables include concentrations of metal, particle size: gravel (GR), very coarse sand (VCR), coarse sand (CS), medium sand (MS), fine sand (FS), and mud (M), as well as organic content (OC). See Tables 3.7 and 3.8 for codes.

Considering only the water quality variables, the variation explained increased to 62% with a larger contribution of temporal variation to the ordination (Fig. 3.3). Sites 1 and 2 associated with higher DO, while Olifantsnek Dam associated with higher turbidity. The November 2017 and March 2018 surveys at Site 4, associated with higher concentrations of Cr, Cu, Zn, Cd, and Pb in the water. The April and June 2017 surveys at Site 5 associated with higher EC values, as well as higher TH and total nitrogen. The November 2017 survey associated with higher As and PO_4 and the March 2018 survey associated with higher Ni, Pt, Cl, and SO_4.

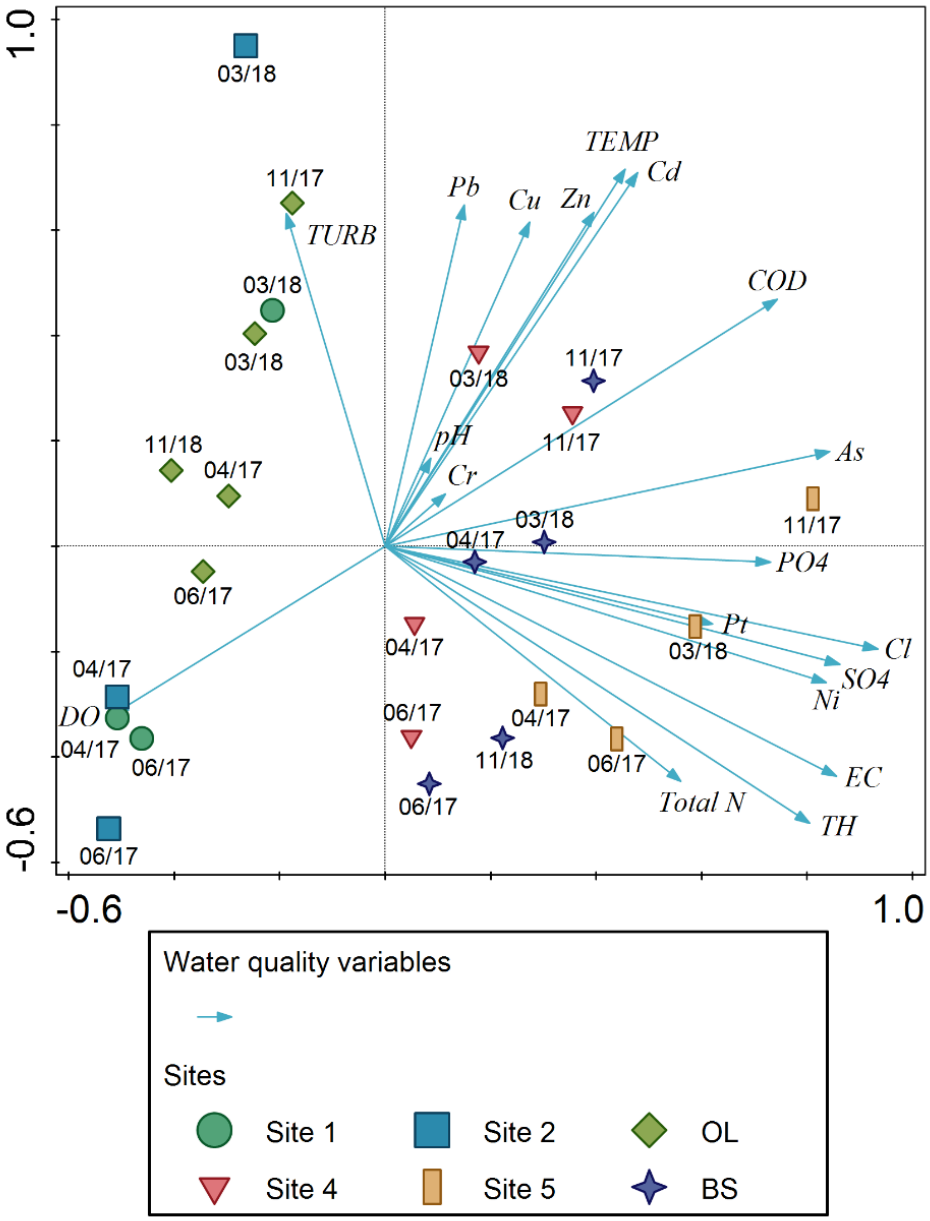


Figure 3.3: PCA biplot of the water quality variables [metal (Cr, Ni, Cu, Zn, As, Cd, Pt, Pb) and nutrient (NH_4, NO_3, NO_2, SO_4, PO_4) concentrations, physicochemical (pH, EC, temperature, DO, turbidity, Cl, TH, COD, SS)] of sites in the Hex River catchment. The biplot explains 58.2% of the variation, with 37.9% on the first axis and 20.3% on the second axis.

3.4 Discussion

Monitoring water and sediment quality in river systems are essential to establish the ecosystem health of these aquatic systems. Pollutant concentrations in river systems can vary considerably between seasons due to flow, and pollutant concentrations that enter the river (Roig *et al*., 2016). These pollutants can enter river systems via runoff from rain, which transport pollutants from the surrounding catchment into the river. Rain can also increase the flow-rate, transport pollutants and sediments from upstream sources downstream (dos Reis *et al*., 2019; Rathoure, 2020). For a holistic assessment of the behaviour of metals in the water and sediments of a river, it is essential to understand the influence of different hydro-periods (*i.e.* high and low flow conditions) on the physicochemical characteristics that have an effect on metals.

3.4.1 Water quality

During the four surveys, all the dissolved metal concentrations peaked in the November 2017 survey. This was during a low flow hydro-period where the sites in the upper reaches were dry and below normal at the sites in the lower reaches of the river system. This increase in metal concentrations can be attributed to metals accumulating due to lower water volumes resulting in a concentration effect (Islam *et al*., 2015; Ali *et al*., 2016). After the rainy season, all the dissolved metal concentrations were the highest in the upper reaches. The increase in metal concentrations at these sites can be ascribed to rain runoff that accumulates metals from the surrounding land-use and transports it into the river (Bouskill *et al*., 2010; Gu *et al*., 2016; Lin *et al*., 2018). The rewetting process of sediments following the dry season can also resuspend loosely bound metals from the sediments into the water column (Lin *et al*., 2018), resulting in increased dissolved metal concentrations.

3.4.2 Interactions between dissolved metal concentrations and water quality variables

In the PCA with water and sediment physicochemical variables, clear spatial differences were evident. The reference sites all grouped with higher OC and DO, indicating non-impacted sites, while mining-influenced site associated with higher metal concentrations in water and sediment and the urban-influenced site and impoundment associated with higher COD, nutrients and salts. In the PCA with only the water physicochemical variables, clear temporal variation is evident, where the November 2017 and March 2018 surveys grouped together and the April and June 2017 surveys grouped together. Clear spatial variation was also evident, where the reference sites associated with high DO, while the mining-influenced sites associated with high metal concentrations and COD and the urban-influenced sites associated with high nutrient concentrations. Concentrations of Cu, Zn, Cd, and Pb increased with a decrease in EC, while concentrations of Ni and Pt increased with an increase in EC, TH, Cl, SO_4 and nutrients.

Nickel enters the Hex River as Ni compounds that are used in various industrial and commercial applications of which Ni soluble salts with the highest commercial use include $NiCl_2$, $NiSO_4$,

$Ni(NO_3)_2$, and $NiCO_3$ (Cempel and Nikel, 2006). These soluble Ni salts are also more bioavailable, however, significantly positive correlations with different salts and nutrients (as reflected in increased EC and TH) probably results in the complexation of Ni thereby reducing its bioavailability (Buxton *et al*., 2019). Several Cu species and compounds are pH dependent. Copper species are more soluble in more acidic waters, and precipitate out of the water as the pH increases (Jarošíková *et al*., 2017). Briggs *et al*. (2003) found that Cu positively correlates with turbidity, which explain the findings of the present study. Ghosh *et al*. (2011) reported that contaminated water normally contains high COD values due to the high metal concentrations which include Zn. Similarly, the present study showed Zn had a significantly positive correlation with COD. As for other metals, As can undergo various complex transformations in water and is influenced by factors such as salinity, pH, sulphide ion concentrations and redox potential (Kumari *et al*., 2017). The significant positive relationship of As with salts and nutrients most likely decreases the bioavailability and therefore the toxicity of As (Esbaugh *et al*., 2013; Kumari *et al*., 2017). According to Crea *et al*. (2013), Cd has various interactions with inorganic components (Cl, OH, CO_3, and SO_4) and nutrients (NO_3 and PO_4) in natural waters. In the present study the bioavailability and therefore the toxicity of Cd is probably negated by water hardness and chemical speciation (Crea *et al*., 2013). Similarly, the Pt that potentially enters the river as Pt salts, would also have a lower bioavailability due to the presence of complexing salts (as reflected in the positive correlations with EC and TH) and thereby decreasing metal uptake by biota (Zimmermann *et al*., 2004). Platinum also act as a nitrate reduction catalyst (da Cunha *et al*., 2000; de Groot and Koper, 2004) and could be the reason for the changes in nutrients observed in the present study, notably the higher NO_2. The significant positive relationship observed between suspended solids and concentrations of Pb, is probably related to catchment derived Pb bound sediments entering the river during increased runoff events (Herngren *et al*., 2005; Song *et al*., 2010).

3.4.3 Sediment quality variables

During the low flow periods metals deriving from mining activities have time to bind and precipitate out of the water column and accumulate in the sediments (Islam *et al*., 2015; Ali *et al*., 2016; Lin *et al*., 2018). However, during the March 2018 survey, while the water levels have increased, the metal concentrations were the highest in the river sites. This can be due to runoff from rain that washes metals and other pollutants into the river, transporting soil from the river banks into the river, while also transporting sediment downstream from upstream sources (Bouskill *et al*., 2010; Gu *et al*., 2016; Rathoure, 2020).

3.4.4 Interactions between sediment metal concentrations and sediment quality variables

Sediments in aquatic systems play an important role for metals, as they act as a sink and store metals, or can act as a source for metals for resuspension back into the water column (Lin *et al*., 2003; Islam *et al*., 2015). Sediment particle size and organic content of sediments play an important part in determining the extent of sorption of metals (Schorer, 1997). Various studies concluded that metals are readily adsorbed to finer sediment particles, compared to the coarser fraction, due to the surface to weight ratio (Schorer, 1997; Liebens, 2001; Lin *et al*., 2003; Islam *et al*., 2015; Unda-Calvo *et al*., 2019). However, in the present study the inverse was found with Cu, Cd, Pt, and Pb all positively correlated with CS (2000 – 500 µm) and significantly negative correlations with FS (212 – 65 µm). The reason for this finding is probably related to the nature of the sediment substrate at the sites where the greatest metal inputs are found, *i.e.* the sediment of the Hex River flowing through the mining and urban areas is dominated by coarse particle size fractions.

3.5 Conclusion

The Hex River is susceptible to varying water levels due to evaporation that exceeds rainfall, and its proneness to droughts, especially in the upper reaches of the catchment. Seasonal monitoring is essential to acquire a holistic overview of metal behaviour during different hydro-periods. Seasonal rainfall and runoff can transport pollutants into the river from the surrounding catchment, while also transporting pollutants and sediments from upper reaches to the lower reaches of the river. The transformation and interaction of metals with water and sediment physicochemical variables, influence the bioavailability, uptake and toxicity of metals to aquatic biota. Although the dissolved and sediment metal concentrations in the Hex River are elevated, it is potentially not in a bioavailable form to aquatic organisms. Since most of the toxicity of the metals, which are associated with Pt mining activities, are determined by water hardness, the high water hardness in the Hex River can potentially alleviate metal toxicity. The pH within the Hex River is also a regulatory factor. With a relatively constant pH between 7.2 and 8.8, most of the metals form carbonate complexes rather than the toxic free metal ion. The various positive correlations with EC and salts (Cl and SO_4) indicate that the metals entering the Hex River might be in metal compounds and not in the free ionic state. The data, therefore, support the hypothesis that the behaviour of Pt and associated metals in water and sediment will be influenced by altered physicochemical parameters as a result of changes in seasonal river runoff patterns.

CHAPTER 4: EFFECTS OF PLATINUM MINING ACTIVITIES ON MACROINVERTEBRATE COMMUNITY STRUCTURES IN THE BUSHVELD IGNEOUS COMPLEX, SOUTH AFRICA

4.1 Introduction

Biological assessment in rivers is internationally recognised as a method for determining ecosystem health (Malherbe *et al*., 2010; Dalu *et al*., 2017a; 2017b; Mangadze *et al*., 2019). Aquatic macroinvertebrates are regarded as effective bioindicators of ecosystem health due to their different sensitivities to habitat alteration and pollution (Dickens and Graham, 2002; Dallas, 2007; Thirion, 2007; Caro-Borrero *et al*., 2016). Both organic and chemical pollution can alter community structures, resulting in a reduction in species diversity (Dalu *et al*., 2017a; 2017b). The use of macroinvertebrates as indicators has various advantages such as their ability to populate a variety of aquatic biotopes, low mobility and the fact that they are easy to sample (Rosenberg and Resh, 1993; Dickens and Graham, 2002; Thirion, 2007; Malherbe *et al*., 2010; Mangadze *et al*., 2019). Macroinvertebrates further perform various important functions within the freshwater ecosystem, such as the retention and breakdown of organic materials, recycling of nutrients and minerals, while also contributing to energy processing at different trophic levels (Rosenberg and Resh, 1993; Allan, 1995, Malherbe *et al*., 2010).

Macroinvertebrate trait-based approaches are new perspectives that are being introduced more frequently into the bioassessment of aquatic ecosystems (Baird *et al*., 2008; Menezes *et al*., 2010). These approaches include biological traits (*e.g.* behavioural and physiological characteristics, and life cycle) and ecological traits (*e.g.* habitat preference, tolerance towards organic enrichment, abiotic factors, and biogeographic distribution), that enable the macroinvertebrates to adapt and overcome certain ecological difficulties (Menezes *et al*., 2010). Trait-based approaches are not meant to replace taxonomy-based approaches. On the contrary, these approaches rather improve and add to the knowledge while using an alternative perspective (Rubach *et al*., 2011).

As such, macroinvertebrates can be used to assess aquatic ecosystem health. In view thereof, for this chapter of the study, this trait-based approach was applied in the Hex River. The water used during Pt mining processes is polluted with, among others, magnesium (Mg), calcium (Ca), chloride (Cl), sulphate (SO_4) and metals (Slatter *et al*., 2009; Glaister and Mudd, 2010, as seen in Chapter 2). The increase in Mg and Ca concentrations influence the water hardness, whereas water hardness generally has a reducing effect on metal toxicity (Sprague, 1995; Yim *et al*., 2006; Ebrahimpour *et al*., 2010; Oliveira-Filho *et al*., 2014). Chloride and SO_4 ions, on the other hand, are detrimental to aquatic biota. According to Mount *et al*. (1997) and Sala *et al*. (2016), Cl is more toxic compared to SO_4 towards aquatic fauna, however, the toxicity can change when both these ions are present and interact with each other. Some of the other major physical and chemical

properties of water that can affect the abundance and distribution of aquatic organisms include, amongst others, temperature, oxygen, electrical conductivity and dissolved solids, salinity, turbidity, light intensity, acidity, as well as nutrient enrichment (Chapman, 1998; Davies and Day, 1998; Davis *et al*., 2018). The pH level of the water is of paramount importance to most aquatic organisms that cannot survive at pH levels lower than 6.5 and higher than 8.5, partly due to the fact that metabolic processes are slowed down (Petrin *et al*., 2007; Peters *et al*., 2013). More importantly, the increase in dissolved metal concentrations caused by a decrease in pH, make the metals more bioavailable within the environment (Peters *et al*., 2013). Elevated nutrient levels in ecosystems can lead to eutrophication, which can have several indirect influences on macroinvertebrate communities (*e.g.* degradation of habitat structures or reduced oxygen levels). Furthermore, very high concentrations of nutrients can have direct toxic effects on macroinvertebrates (Davis *et al*., 2018). Water of suitable quality is essential to sustain a healthy population, while different taxa exhibit different tolerances to specific water quality variables (Phiri, 2000; Dalu *et al*., 2017b).

According to Dalu *et al*. (2017b), water chemistry variables are more predictive than sediment chemistry variables of changes in macroinvertebrate community structures, within highly polluted river systems. However, it is well known that suspended particles may have detrimental effects on macroinvertebrates by inhibiting respiration through clogging of their gills, as well as reduce the visibility of predators and prey to detect each other (Jones *et al*., 2012; Davis *et al*., 2018). River sediments have a great adsorption capacity towards pollutants (Smolders *et al*., 2003) with the result that it acts as nutrient and metal reservoirs, that can subsequently be released into the water column and can be accumulated by aquatic biota (Pallottini *et al*., 2015; Dalu *et al*., 2017b). The changes in water and sediment quality from the upper reaches of the Hex River to the lower reaches, due to various impacts as seen in Chapters 2 and 3, are expected to result in changes in macroinvertebrate community structures.

The hypotheses for this chapter were that macroinvertebrate community structures will be altered from the reference conditions due to Pt mining related responses and, macroinvertebrate traits will be altered as a result of changes in water and sediment quality. The aim of this chapter was, therefore, to determine whether spatial or temporal changes have occurred within the aquatic macroinvertebrate community structures due to Pt mining.

4.2 Materials and Methods

4.2.1 Field sampling

Four surveys (April 2017, June 2017, November 2017 and March 2018) were completed at four preselected river sites (Hex River), while three surveys (April 2017, November 2017 and March 2018) were completed in two impoundment sites (Olifantsnek Dam and Bospoort Dam) (see Figure 3.1 in Chapter 3). *In situ* variables, water and sediment samples were collected and analysed as described in Chapter 2. The results of the water and sediment samples used for this chapter can be seen in Tables 3.7 and 3.8, as well as in Appendix B, Tables B1 and B3. Aquatic macroinvertebrate samples were collected at all of the sites using a sweep net (30 cm square frame with a sturdy handle and a Perlon® gauze net with 1 mm mesh size). Three biotopes were sampled for 15 minutes in total, specifically – stones (in and out of current), gravel, sand, and mud (GSM), and vegetation (aquatic and marginal). The stones biotopes were sampled by disturbing the stones with the feet, while continuously sweeping the net downstream over the disturbed area. The same method was used for the macroinvertebrates in the GSM biotope. Aquatic and marginal vegetation were sampled by pushing the net vigorously into the vegetation and moving it through the vegetation. The contents of the net, after sampling each biotope, were transferred into a white tray filled with site water. Most of the coarse plant material was carefully removed by hand without discarding any macroinvertebrates along with it. Excess water was removed from the sample before it was transferred into a plastic container with a tight-fitting lid and an adequate amount of 70% ethanol was added to fix and preserve the samples. Each plastic container was labelled with the relevant site information and the samples were transported back to the laboratory for identification and further analysis.

4.2.2 Macroinvertebrate identification

The macroinvertebrate samples of each site were transferred into a sorting tray. The organisms were sorted into morpho-types using a stereomicroscope (Zeiss, Stemi 305). Where possible, the morpho-types were identified up to species level; where not, to genus or family level (see Appendix C, Tables C1 and C2). Identification was done with the aid of the Guides to Freshwater Invertebrates of Southern Africa and additional literature (Davies and Day, 1998; Day *et al*., 2001; Day and de Moor, 2002a; 2002b; Day *et al*., 2002; Gerber and Gabriel, 2002a; 2002b; de Moor *et al*., 2003a; 2003b; Stals and de Moor, 2007; Griffiths *et al*., 2015). All specimens identified were enumerated for further statistical analysis.

4.2.3 Traits

After identification of the macroinvertebrates, they were classified according to different traits (*e.g.* sensitivity towards organic enrichment, functional feeding groups, aquatic life stage, mode of respiration, orders and habitat preference) by using the recently compiled South African Macroinvertebrate Trait Database (Odume *et al*., 2018) (Table 4.1). These traits were selected to

assess whether the community structures changed within seasons at a specific site, as well as between sites. These traits can be altered with an increase or decrease of pollutants in the system, or by environmental factors (*e.g.* increase in water velocity, drought, and habitat availability).

Table 4.1: Macroinvertebrate traits used during the present study (sensitivity towards organic enrichment, functional feeding groups, aquatic life stage, mode of respiration, orders and habitat preference) with the associated trait modalities (after Odume *et al*., 2018).

Macroinvertebrate Traits					
Sensitivity towards organic enrichment					
Highly Tolerant (HT)	Moderately Tolerant (MT)	Moderately Sensitive (MS)	Highly Sensitive (HS)	Not Determined (ND)	
Functional Feeding Groups (FFGs)					
Scrapers 1 (Sc 1)	Scrapers 2 (Sc 2)	Grazers 1 (Gr 1)	Grazers 2 (Gr 2)	Shredders (Sh)	Deposit feeders 1 (DF 1)
	Deposit feeders 2 (DF2)	Filter feeders (FF)	Predators 1 (Pr 1)	Predators 2 (Pr 2)	
Aquatic life cycle stage					
	Egg, larva, pupa	Egg, nymph	Only adult	All (egg to adult)	
Mode of respiration					
Tegument/ cutaneous (T/C)	Plastron (Pl)	Gills (G)	Aerial: spiracle (A:S)	Aerial/vegetation: breathing tube, straps/ other apparatus (A/V)	
Orders					
Decapoda	Oligochaeta	Hirudinea	Trombidiformes	Mollusca	Ephemeroptera
Odonata	Hemiptera	Trichoptera	Lepidoptera	Diptera	Coleoptera
Habitat preference					
	Gravel, sand, and mud (GSM)	Stones	Vegetation	Free living	

4.2.3.1 Sensitivity values

Sensitivity values were allocated to each family according to the South African Scoring System (SASS5) sensitivity values (Dickens and Graham, 2002), as well as to lower taxonomic taxa by using Odume *et al*. (2018). These values are classified into four classes: highly tolerant (1 – 3), moderately tolerant (4 – 6), moderately sensitive (7 – 11) and highly sensitive (12 – 15) towards organic enrichment (Table 4.1). No sensitivity value was allocated to the collected taxa that did not have SASS5 sensitivity values. These taxa were still included in the percentage composition but were indicated as not determined (ND).

4.2.3.2 Functional feeding groups

The macroinvertebrates were grouped into functional feeding groups (Table 4.1) per site and per survey using Scheal (2006) and Odume *et al*. (2018). These FFGs consist of:

- Scrapers 1 (Sc 1): Invertebrates that scrape a thin film of micro-organisms off the substrata that comprises mostly of algae (*e.g.* diatoms, epiphytes, epilithon, and filamentous algae).
- Scrapers 2 (Sc 2): Invertebrates that scrape a thin film of both micro-organisms and detritus off the substrata this includes algae and a mix of mainly organic and some inorganic particles that are remains of plant and animal materials.
- Grazers 1 (Gr 1): Invertebrates that feed on whole living plants, leaves, and stems this includes macrophytes, leaves from riparian trees, as well as algae.
- Grazers 2 (Gr 2): Invertebrates that feed on algae only.
- Shredders (Sh): Invertebrates that feed by means of fragmenting leaves and large pieces of plant material including macrophytes, leaves from riparian trees and moss.
- Deposit feeders 1 (DF 1): Invertebrates that gather and collect detritus (very fine particulate organic matter and fine particulate organic matter) off deposited organic material within and off the substrata.
- Deposit feeders 2 (DF 2): Invertebrates that gather and collect algae and detritus within and off the substrata in comparative amounts, depending on availability.
- Filter feeders (FF): Invertebrates that collect small food particles suspended within the water column including various micro-organisms (*e.g.* bacteria, algae and zooplankton).
- Predators 1 (Pr 1): Invertebrates that actively feed on micro-organisms (*e.g.* zooplankton and zoobenthos).
- Predators 2 (Pr 2): Invertebrates that feed on insects to small vertebrates via active predation.

4.2.3.3 Aquatic life cycle stage

The different taxa were classified depending on which part of their life cycle is aquatic (Table 4.1). The groups included: egg, larva and pupa; egg and nymph; adult only; and all (egg to adult).

4.2.3.4 Mode of respiration

The macroinvertebrates were also classified based on the mode of respiration (Table 4.1). This indicates whether the taxa are air breathers, water breathers or have some specialised respiratory apparatus enabling them to survive within a certain environment. These groups comprised of water breathers that breath through their tegument or skin (tegument/cutaneous – T/C), as well as their gills (G), and air breathers that uses modified air storage chambers (plastron – Pl), series of external openings (aerial: spiracle – A:S) and specialised respiratory apparatus (aerial/vegetation: breathing tube, straps/ other apparatus – A/V).

4.2.3.5 Orders

The orders Ephemeroptera, Odonata, Trichoptera, and Lepidoptera are considered to be sensitive towards pollution (Kietzka *et al*., 2019). Therefore, the abundance within these orders should potentially increase with an increase in water quality. The orders Oligochaeta, Hirudinea and Diptera, on the contrary, mostly contain organisms that are tolerant towards pollution and would increase as the water quality decrease (Thirion, 2007). Other orders that were studied were: Decapoda, Trombidiformes, Mollusca, Hemiptera and Coleoptera (Table 4.1).

4.2.3.6 Habitat preference

Macroinvertebrates have certain habitat preferences and are adapted to occur within these habitats (Dallas, 2007; Thirion, 2007). The four major habitats include gravel, sand, and mud (GSM); stones; vegetation; and free living – the macroinvertebrates that normally occur in open water or swim on the water surface (Table 4.1).

4.2.4 Data analysis

Statistical analyses, including the relevant biodiversity indices, as well as K-dominance plots, were applied using PRIMER v6. The species richness was used to determine the total number of species present in a community, while the Shannon-Wiener diversity index (H') was used to characterise species diversity within the community (Heip *et al*., 1998). The higher the value of H', the greater the biodiversity at a specific site. This value was calculated using the following equation:

$$H' = \sum_{i=1}^{s} p_i \ln p_i$$

Pielou's evenness index (J') describes the even distribution between species at a specific site and is defined as H' divided by the natural logarithm of the species richness (Heip *et al*., 1998). In a healthy ecosystem, an even distribution between species within a given community is expected and subsequently, community stability can be related. Values of J' can vary between 0 and 1, whereas 1 indicates an even distribution and 0 an uneven distribution. To calculate J' the following equation was used:

$$J' = -\frac{H'}{\ln S}$$

Statistical significance of the spatial and temporal variation of the selected biodiversity indices was determined at $p < 0.05$ using GraphPad Prism v7. Normality and homogeneity of variance were tested using D'Agostino and Pearson omnibus normality test and Kolmogorov-Smirnov test (with Dallal-Wilkonson-Lilliefor P-value), respectively. For parametric data, means were compared using a One-way ANOVA and Tukey's multiple comparison tests were performed. In the case of non-parametric data, Kruskal-Wallis tests with Dunn's multiple comparison tests were performed to test for significant differences between sites and surveys.

Multivariate statistical analyses were completed using Canoco v5.12. Redundancy analyses were used to summarise the variation in macroinvertebrate species composition, as well as their traits that can be explained by environmental variables (environmental variables that were measured in Chapter 3; see Tables 3.7 and 3.8, as well as Appendix B, Tables B1 and B3). Forward selection with Monte Carlo permutation was performed to determine the significant environmental variables (Black *et al*., 2004). Response curve plots were constructed to assess the response of macroinvertebrate species, as well as their traits to various environmental variables and fitted with a generalised linear model for each of the environmental variables. Only significant responses ($p < 0.05$) were included in the results.

4.3 Results

During the study, a total of 11 167 macroinvertebrate individuals from 61 families totalling 119 taxa were collected and identified. In the river sites, 108 taxa were present (Appendix C, Table C1), while in the impoundment sites 51 taxa were collected (Appendix C, Table C2). Of the 108 taxa collected from the river sites, 35 taxa only occurred at Sites 1 and 2, while 18 taxa only occurred at Sites 4 and 5, whereas 21 taxa were present at all the river sites. As for the 51 taxa collected from the impoundments, 13 taxa only occurred at Olifantsnek Dam, while 25 taxa only occurred at Bospoort Dam, and 13 taxa were present at both of the impoundments.

4.3.1 Macroinvertebrate community structures in the lotic sites

For statistical purposes, the mean diversity index scores of the four surveys were calculated per site and the temporal variation within sites is indicated by the standard deviation. The mean species richness over the four surveys at the river sites declined significantly from 39 at Sites 1 and 2 (reference sites) to a mean of 24 at Site 4, as the anthropogenic activities increased downstream (Fig. 4.1A). Although the species richness declined, the number of individuals increased from a mean of 546 individuals at Site 1, to 980 individuals at Site 4 (Fig. 4.1B and Fig 4.2), while the mean Shannon-Wiener diversity index decreased significantly from Site 1 (mean of 2.71) towards Site 4 (mean of 1.78). The Pielou's evenness index (Fig. 4.1D) also indicated that the reference sites (Sites 1 and 2) had a more even distribution between taxa (mean of 0.74 and 0.71, respectively), whereas the impacted site (Site 4) had one or two dominant taxa in the community (0.57). The dominant taxa (Chironominae) at Site 4 during the March 2018 survey are evident in the K-dominance plot, where it had a 70% dominance, while the taxa at the other sites and surveys had a more even distribution (Fig. 4.2).

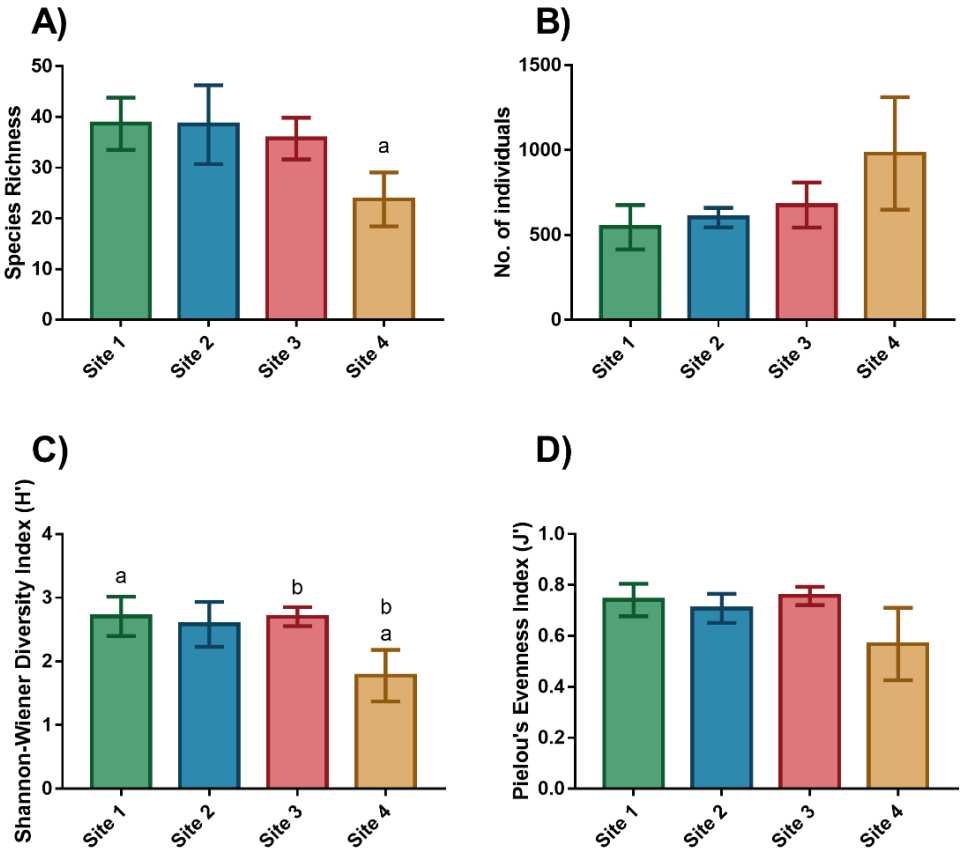


Figure 4.1: The mean ± standard deviation of the calculated biodiversity indices between four sites in the Hex River during four surveys (04/2017; 06/2017; 11/2017; 03/2018). The indices include A) Species Richness, B) Number of individuals, C) Shannon-Wiener Diversity Index and D) Pielou's Evenness Index. Common alphabetical superscripts indicate significant differences (p < 0.05).

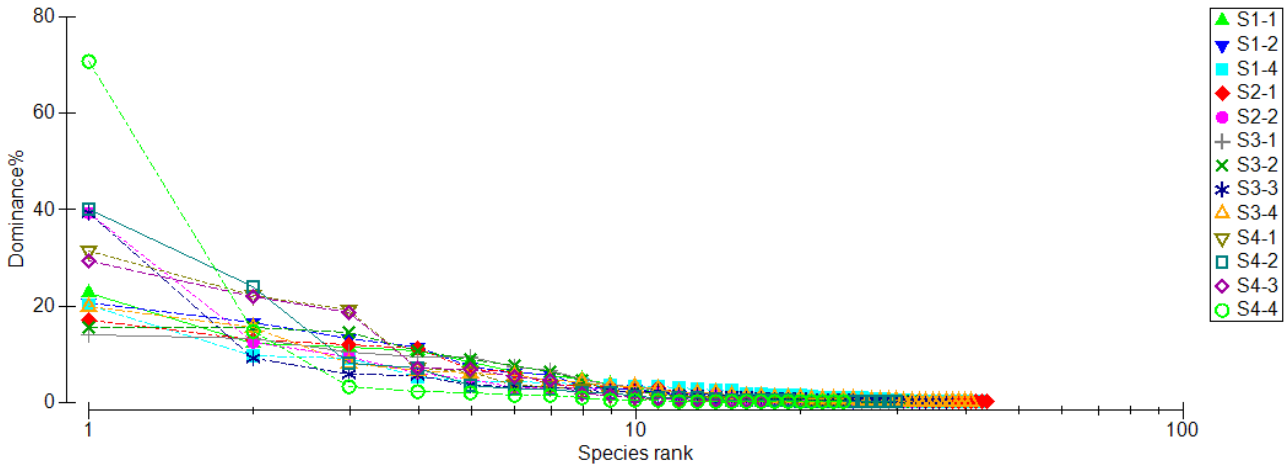


Figure 4.2: K-dominance plot of the macroinvertebrate species collected at four sites in the Hex River during four surveys (04/2017; 06/2017; 11/2017; 03/2018). Sites and surveys are indicated as Site – Survey (*e.g.* S1-1).

4.3.2 Traits in the lotic systems

4.3.2.1 Sensitivity values

The sensitivity of macroinvertebrate taxa varied between surveys within the river sites, but also between the four different sites. Highly tolerant taxa occurred predominantly at all the river sites during all the surveys (Fig. 4.3). However, the highly tolerant taxa increased from the reference sites (Sites 1 and 2) to the impacted sites (Sites 3 and 4), from an average of 36.5% at Site 1 to an average of 63.4% at Site 4. Moderately tolerant, as well as moderately sensitive taxa decreased from an average of 40.8% and 21.7% at Site 1 to an average of 31.3% and 3.3% at Site 4, respectively (Fig. 4.3). Highly sensitive taxa were present at the four sites but only at very low percentages (0.02 – 0.5%). Highly tolerant taxa increased during the March 2018 survey at Site 1 after the site had no water during the November 2017 survey. During the November 2017 survey, highly tolerant taxa increased at Site 3 but recovered during the March 2018 survey. On the other hand, the highly tolerant taxa at Site 4 increased during the November 2017 survey, however, it increased even more during the March 2018 survey and outcompeted all other taxa.

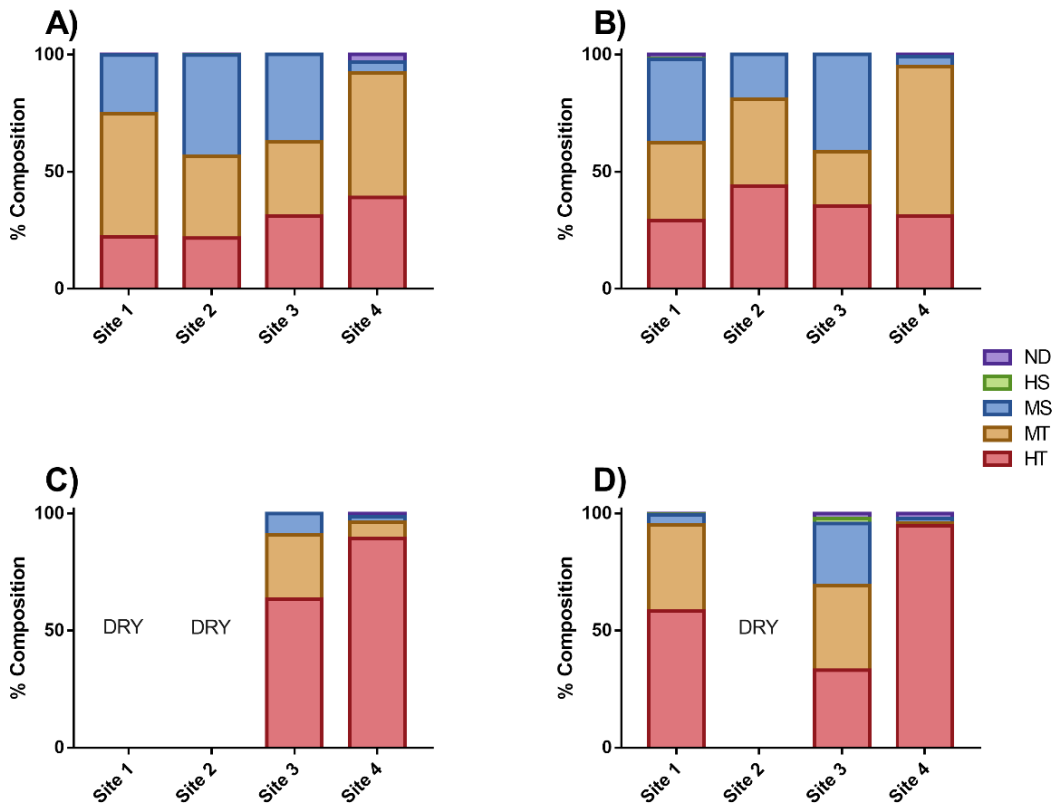


Figure 4.3: Percentage composition of highly sensitive (HS), moderately sensitive (MS), moderately tolerant (MT) and highly tolerant (HT) taxa collected at four sites in the Hex River (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018). Taxa with no sensitivity values allocated were also included as not determined (ND).

4.3.2.2 Functional feeding groups

The functional feeding groups varied between surveys within a site and between the river sites (Fig. 4.4). For most of the river sites, deposit feeders 1 (DF 1) was the dominant feeding group, except for Site 1, where the filter feeders (FF) feeding group was dominant. The second dominant feeding groups ranged between deposit feeders 1 (DF 1) (Site 1, 26.9%), predators 2 (Pr 2) (Site 2, 18.9%), deposit feeders 2 (DF 2) (Site 3, 24.4%) and predators 1 (Pr 1) (Site 4, 19.6%). The functional feeding group composition was altered at Site 1 from June 2017 to March 2018, after the site was dry in the November 2017 survey. At Sites 3 and 4, changes in the functional feeding group composition during November 2017 were evident, where during March 2018 the community recovered again at Site 3 to the same as the previous surveys. Site 4, however, did not recover and deposit feeders 1 (DF 1) dominated the composition.

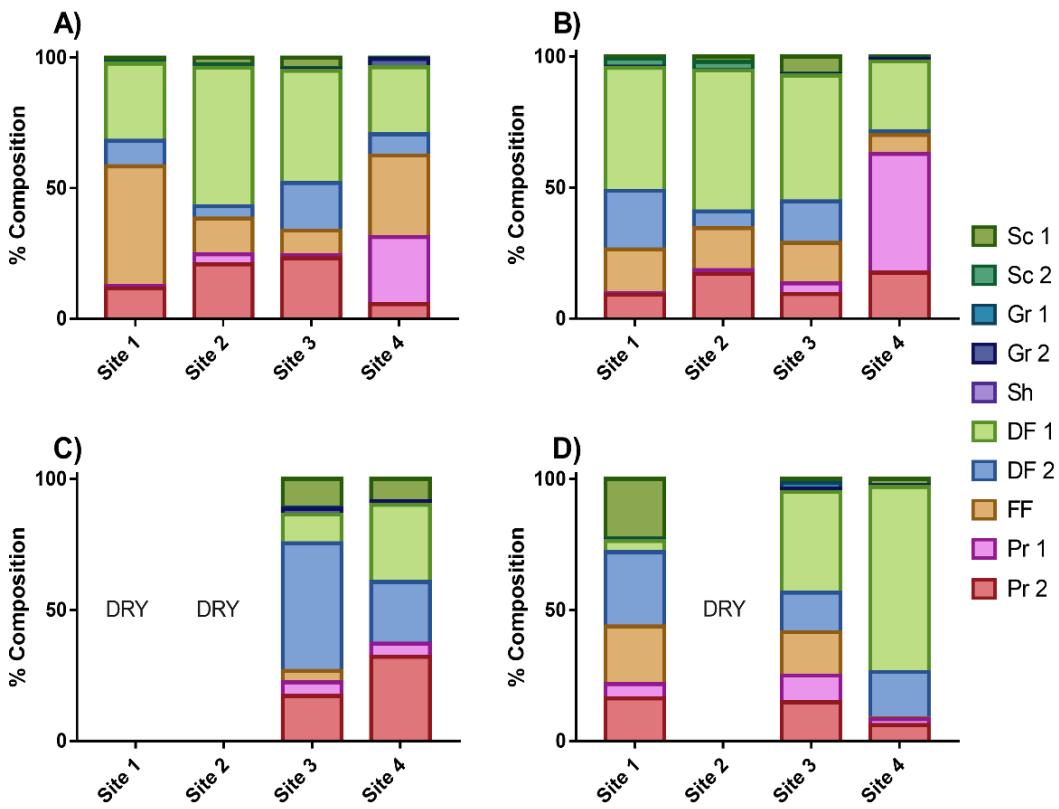


Figure 4.4: Percentage composition of different functional feeding groups [scrapers 1 (Sc 1), scrapers 2 (Sc 2), grazers 1 (Gr 1), grazers 2 (Gr 2), shredders (Sh), deposit feeders 1 (DF 1), deposit feeders 2 (DF 2), filter feeders (FF), predators 1 (Pr 1) and predators 2 (Pr 2)] collected at four sites in the Hex River (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018).

4.3.2.3 Aquatic life cycle stage

The dominant aquatic life stage within the Hex River at Sites 1 and 4 was the egg, larva and pupa stage (42.7% and 69.3%), while at Sites 2 and 3 it was the egg and nymph stage (45.5% and 37.6%) (Fig. 4.5). The second most abundant life stage at Site 1 was egg and nymph (29.2%), at Sites 2 and 3 was the egg, larva and pupa stage (45% and 34.8%), while at Site 4 it was all life stages (14.3%). The same trend, as with the functional feeding groups, is evident at Site 1 where the community structure changed after the river dried up in November 2017. Changes occurred in the community structures at Sites 3 and 4, during the November 2017 survey, where after the community composition recovered during the March 2018 survey to the same composition of the April and June 2017 surveys.

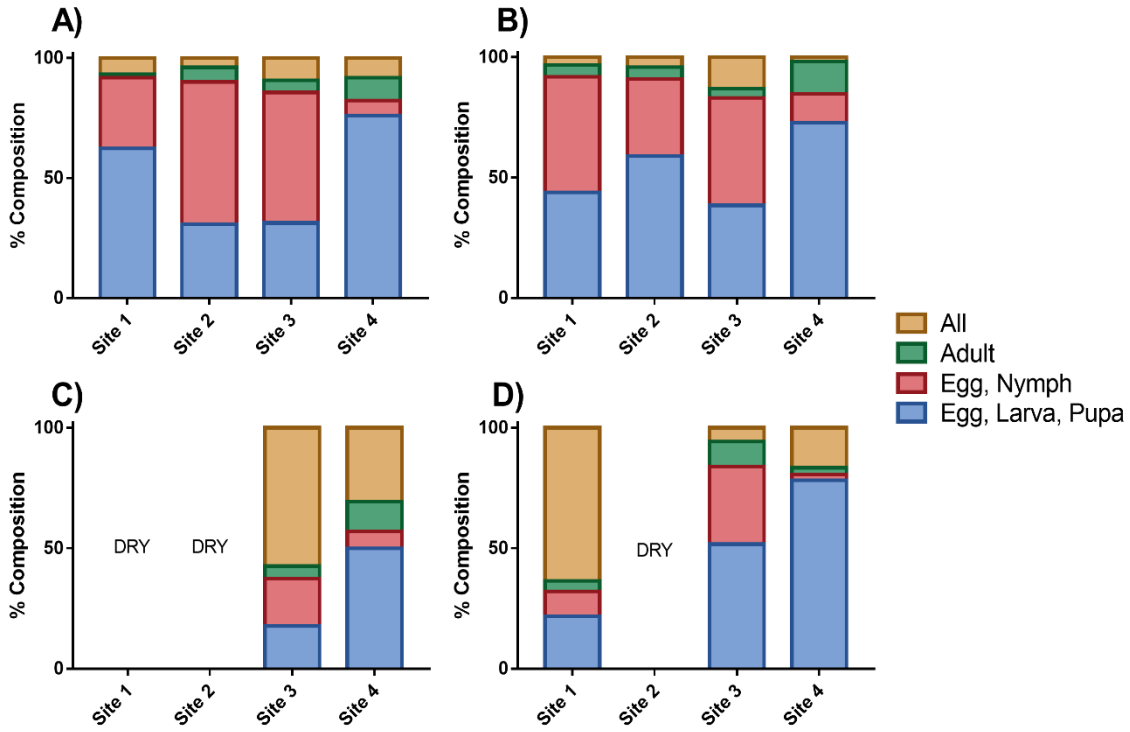


Figure 4.5: Percentage composition of the aquatic life stage present at four sites in the Hex River (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018).

4.3.2.4 Mode of respiration

The taxa present at all the river sites, were predominantly taxa that breathe by means of gills (73 – 89%), while at Sites 1 and 3 the second most dominant taxa were those that breathe through their tegument or cutaneous with 11% and 15%, respectively (Fig. 4.6). Sites 2 and 4 had taxa with spiracles, being the second dominant taxa that occurred with 7% and 15%, respectively. The percentage taxa that are air breathers increased from 5.5% at Site 1 to 15.2% at Site 4, and this corresponds with the decrease in percentage oxygen saturation from Site 1 (100.8%) to Site 4 (54.9%) (as seen in Chapter 3).

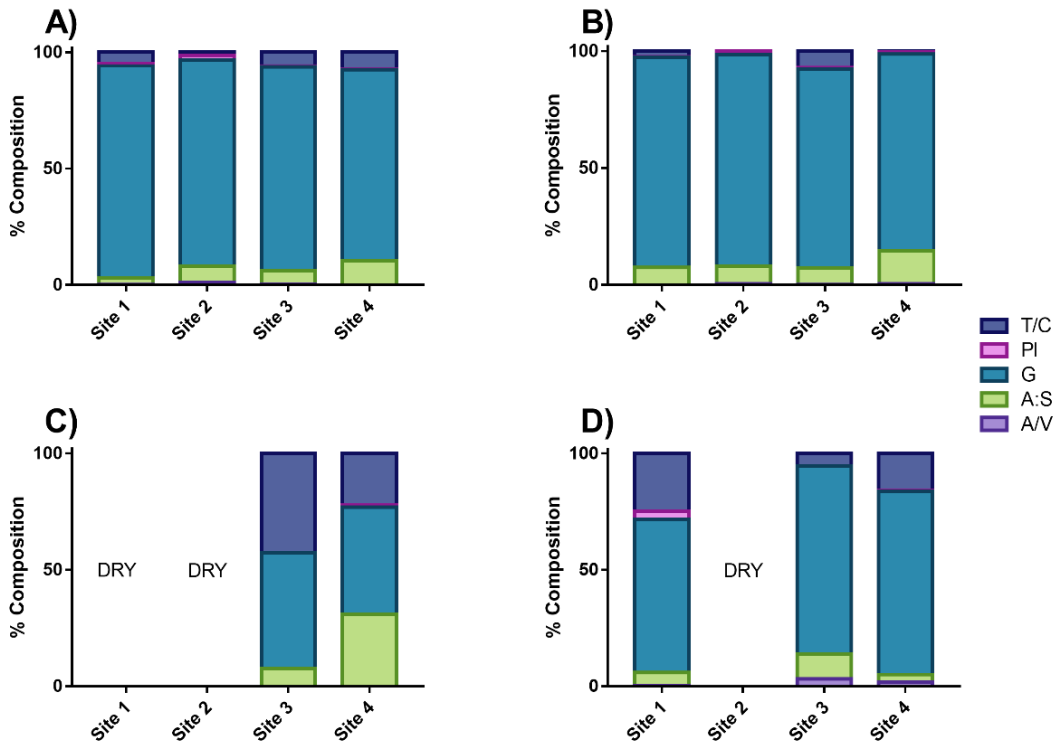


Figure 4.6: Percentage composition of the mode of respiration [tegument/cutaneous (T/C), plastron (Pl), gills (G), aerial: spiracle (A:S) and aerial/vegetation: breathing tube, straps/ other apparatus (A/V)] present at four sites in the Hex River (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018).

4.3.2.5 Orders

The order Ephemeroptera decreased in abundance from the reference sites within the river (Site 1, 23.9%; Site 2, 32.7%) towards the impacted sites (Site 3, 29.5%; Site 4, 2.9%), while the Odonata varied between the sites (Site 1, 5.4%; Site 2, 12.8%; Site 3, 8.1% and Site 4, 4.0%). The Trichoptera increased from 3.1% at Site 1 to 16.4% at Site 4 (Fig. 4.7), and the Diptera increased from 39.4% at Site 1 to 52.8% at Site 4. These four orders made up 80 – 92% of the total community structures at all four river sites during the April, June and March surveys. The exception was the March survey at Site 1 when these four orders only made up 32% of the total community structure. During the November survey, these four orders only made up 37 – 57% of the total community structure of Sites 3 and 4.

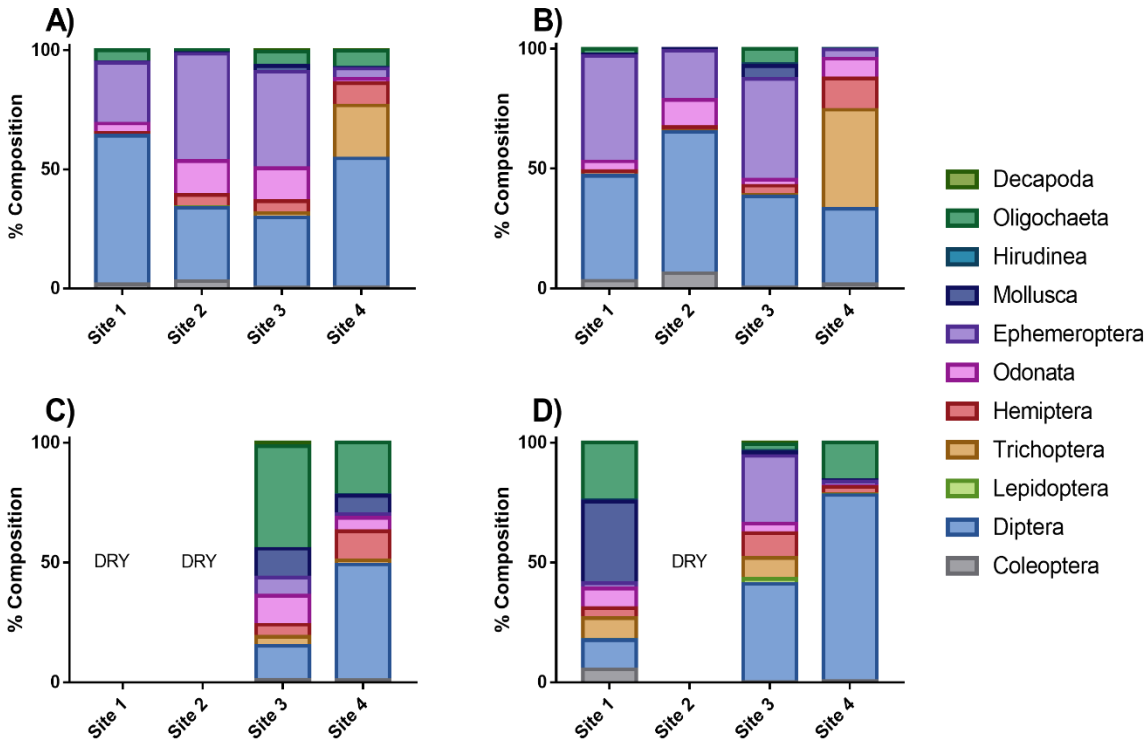


Figure 4.7: Percentage composition of Decapoda, Oligochaeta, Hirudinea, Mollusca, Ephemeroptera, Odonata, Hemiptera, Trichoptera, Lepidoptera, Diptera and Coleoptera present at four Hex River sites (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018).

4.3.2.6 Habitat preference

The macroinvertebrates that prefer the stones biotope at Sites 1 and 2 were dominant with 50.4% and 49.4%, while at Sites 3 and 4 the GSM biotope dominated with 40.4% and 55.5%, respectively (Fig. 4.8). Except for Site 2, the free living macroinvertebrates were consistently the lowest between the sites with 5.7%, while at Site 2 it was 7.6%. During the April 2017 survey, the macroinvertebrates that prefer the stones biotope were dominant at Sites 1 and 2 but declined in the June 2017 survey. At Site 3 the variance between surveys was consistent, except for the November survey, when the macroinvertebrates that prefer the stones biotope, declined and the GSM macroinvertebrates increased, however, recovered in the March 2018 survey. At Site 4 the same trend as Site 3 was evident, but macroinvertebrates that prefer the stones biotope almost disappeared in the November 2017 survey (0.9%), while the macroinvertebrates in the GSM biotope dominated and did not recover to the structure of the previous surveys, but rather further increased during the March 2018 survey.

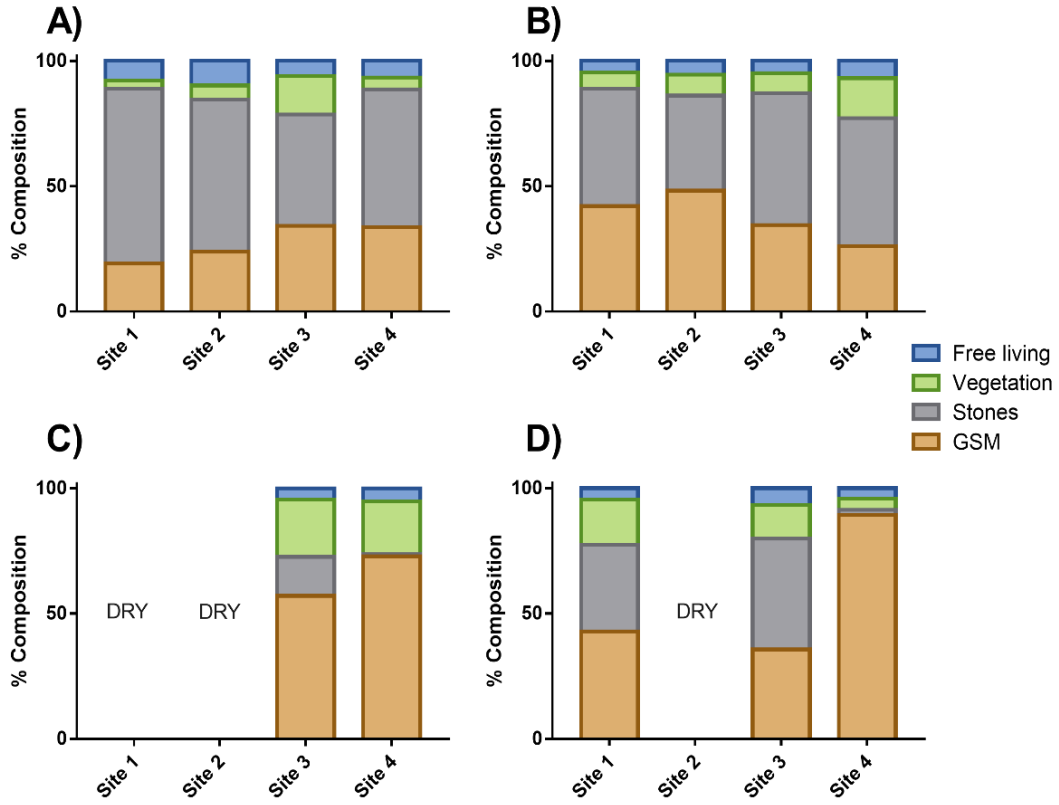


Figure 4.8: Percentage composition of macroinvertebrate habitat preference (free living; vegetation; stones; GSM – gravel, sand, and mud) present at four sites in the Hex River (Site 1; Site 2; Site 3; Site 4) during four surveys (A – 04/2017; B – 06/2017; C – 11/2017; D – 03/2018).

In the species based RDA, all of the factors were included, but only PO_4, NH_4, TDS, as well as concentrations of Ni and Cd in the water, were significant (p = 0.002, 0.018, 0.016, 0.004 and 0.01, respectively) (Fig. 4.9). The species composition between Sites 1 – 3 were similar between the sites and surveys, and all grouped together, while Site 4 not only differed from the other sites, but also between surveys.

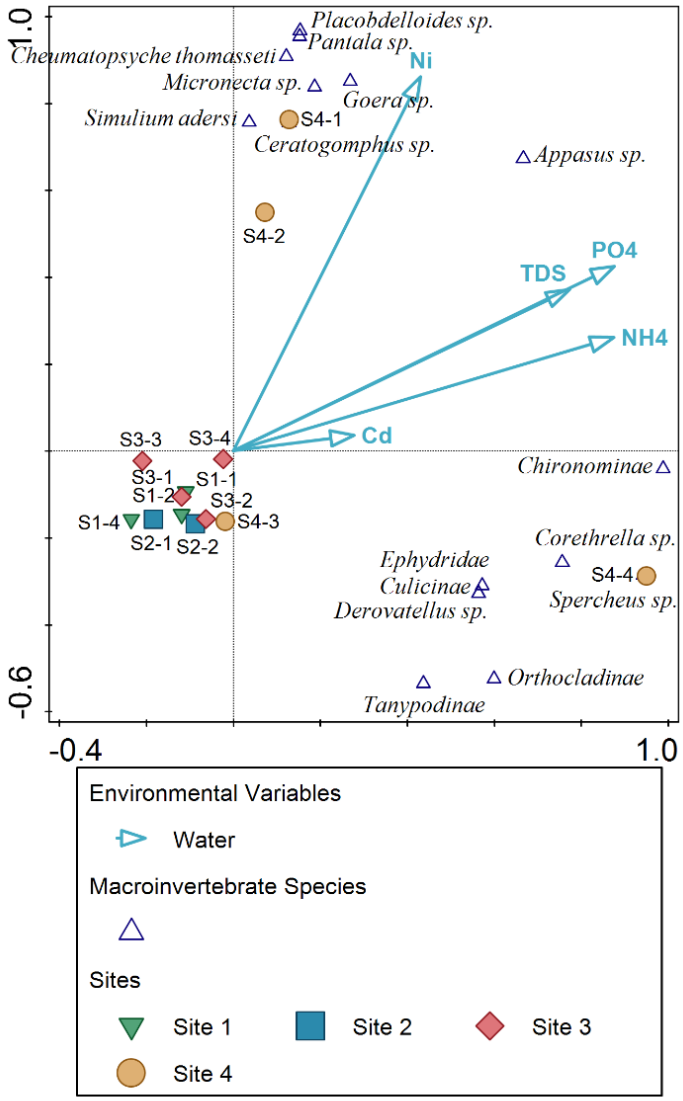


Figure 4.9: A RDA triplot illustrating associations between macroinvertebrate species, selected abiotic factors (pH, EC, temperature, turbidity, TDS, DO), nutrients (NH_4, NO_3, NO_2, SO_4, PO_4, TH) and metals in water and sediment (Cr, Ni, Cu, Zn, Cd, Pt, Pb) from four sites in the Hex River during four surveys (2017 – 2018). The triplot describes 78.9% of the variation with 58.9% on the first axis and 20.0% on the second axis. Only species with a 60% fit of the response variable are shown. Sites and surveys are indicated as Site – Survey (*e.g.* S1-1).

In the trait-based RDA all of the factors were included, however, only PO_4, as well as concentrations of Ni, Cd, and Pt in the water and concentrations of Cr in the sediment, were significant ($p = 0.002$, 0.01, 0.006, 0.012 and 0.048, respectively) (Fig 4.10). Sites 1, 2 and 3 grouped together during the first two surveys and indicated slight or no differences between the sites or the surveys, while during the third and fourth surveys, Site 1 and 3 differed from the first two surveys. Site 4 differed completely from the other sites, while large variation also occurred within the site between surveys. These differences at Site 4 can be explained by the higher PO_4, concentrations of Ni and Cd in the water, while a higher percentage of highly tolerant taxa and taxa that prefer the GSM biotope, were also present at this site.

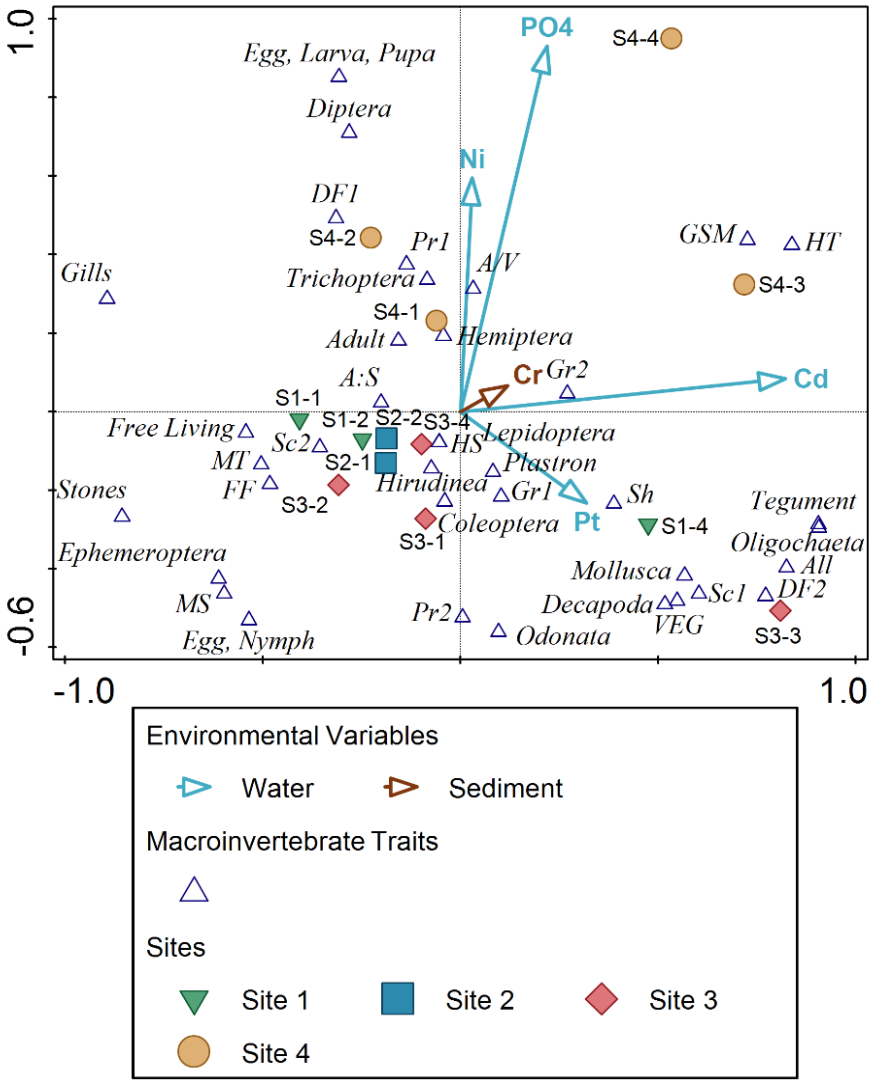


Figure 4.10: A RDA triplot illustrating associations between macroinvertebrate traits {sensitivity [highly tolerant (HT), moderately tolerant (MT), moderately sensitive (MS), highly sensitive (HS)], FFGs [scarpers (Sc 1 & 2), grazers (Gr 1 & 2), shredders (Sh), deposit feeders (DF 1 & 2), filter feeders (FF), predators (Pr 1 & 2)], aquatic life stage (egg, larva, pupa; egg, nymph; adult; all) mode of respiration [aerial/vegetation: breathing tube (A/V), aerial: spiracle (A:S), gills, plastron, tegument], Orders (Decapoda, Oligochaeta, Hirudinea, Trombidiformes, Mollusca, Ephemeroptera, Odonata, Hemiptera, Trichoptera, Lepidoptera, Diptera, Coleoptera), habitat preference (GSM, vegetation, stones, free living)}, selected abiotic factors (pH, EC, temperature, turbidity, TDS, DO), nutrients (NH_4, NO_3, NO_2, SO_4, PO_4, TH) and metals in water and sediment (Cr, Ni, Cu, Zn, Cd, Pt, Pb) from four sites in the Hex River during four surveys (2017 – 2018). The triplot describes 62.6% of the variation with 39.5% on the first axis and 23.1% on the second axis. Sites and surveys are indicated as Site – Survey (*e.g.* S1-1).

4.3.3 Species Response Curves the lotic systems

The species responses curves represent positive or negative correlations between the abundance of taxa and the different environmental variables. In the river sites, total hardness and Cl had a significant negative response on *Acanthiops* sp. (p = 0.036; p = 0.024), while *Physella acuta* (p = 0.024; p = 0.042), *Appasus* sp. (p = 0.001; p = 0.0006) and Muscidae (p = 0.007; p = 0.0004) had a significant positive response, whereas Cl also had a significant positive response on *Laccobius* sp. (p = 0.0034) (Fig. 4.11). Sulphates also had a significant negative response on *Acanthiops* sp. (p = 0.013) and *Caenis* sp. (p = 0.032), while having a significant positive response on *Tubifex* sp. (p = 0.027), *Appasus* sp. (p = 0.0004), Chironominae (p = 0.043) and Muscidae (p = 0.023). Concentrations of Pt in the water had a significant negative response on *Simulium nigritarse* (p = 0.028), while *Physella acuta* (p = 0.046), *Burnupia mooiensis* (p = 0.0002), *Bezzia* sp. (p = 0.013) and *Potamonautes warreni* (p = 0.012) all had a significant positive response. Concentrations of Pt in the sediment had a significant positive response on *Potamonautes warreni* (p = 0.0023), *Helobdella stagnalis* (p = 0.0024), *Burnupia mooiensis* (p = 0.009), *Cloeon* sp. (p = 0.030), *Agriocnemis pinheyi* (p = 0.049), *Anax* sp. (p = 0.049), *Sympetrum fonscolombii* (p = 0.049), *Paraphyrynovelia* sp. (p = 0.0017), *Enithares* sp. (p = 0.027), *Laccocoris* sp. (p = 0.015), *Bezzia* sp. (p = 0.00034) and Tabanidae (p = 0.020). *Caenis* sp. (p = 0.046) had a significant negative response towards concentrations of Ni in the water, while *Placobdelloides* sp. (p = 0.00009), *Pantala* sp. (p = 0.00011), *Rhagodotarsus* sp. (p = 0.041), *Micronecta* sp. (p = 0.0054), *Laccothrephes* sp. (p = 0.041), *Appasus* sp. (p = 0.00001), *Cheumatopsyche thomasseti* (p = 0.0026) and *Goera* sp. (p = 0.0053) all had a significant positive response. The concentrations of Ni in the sediment had a significant positive response on *Helobdella stagnalis* (p = 0.0056), *Agriocnemis pinheyi* (p = 0.0037), *Anax* sp. (p = 0.0037), *Sympetrum fonscolombii* (p = 0.0037), *Paraphrynovelia* sp. (p = 0.0049), *Ranatra* sp. (p = 0.0081), *Laccocoris* sp. (p = 0.0072), *Ecnomus thomasseti* (p = 0.014), Crambidae (p = 0.0043) and *Corethrella* sp. (p = 0.0074). Concentrations of Cr in the water had a significant positive response on *Burnupia mooiensis* (p = 0.020), *Pseudagrion* sp. (p = 0.0012) and *Bezzia* sp. (p = 0.044), while *Helobdella stagnalis* (p = 0.0011), *Agriocnemis pinheyi* (p = 0.0011), *Anax* sp. (p = 0.0011), *Sympetrum fonscolombii* (p = 0.0011), *Paraphrynovelia* sp. (p = 0.0018), *Enithares* sp. (p = 0.0008), *Laccocoris* sp. (p = 0.0017), *Ecnomus thomasseti* (p = 0.013), Crambidae (p = 0.0014) and *Corethrella* sp. (p = 0.0056) all responded significantly positive to concentrations of Cr in the sediment.

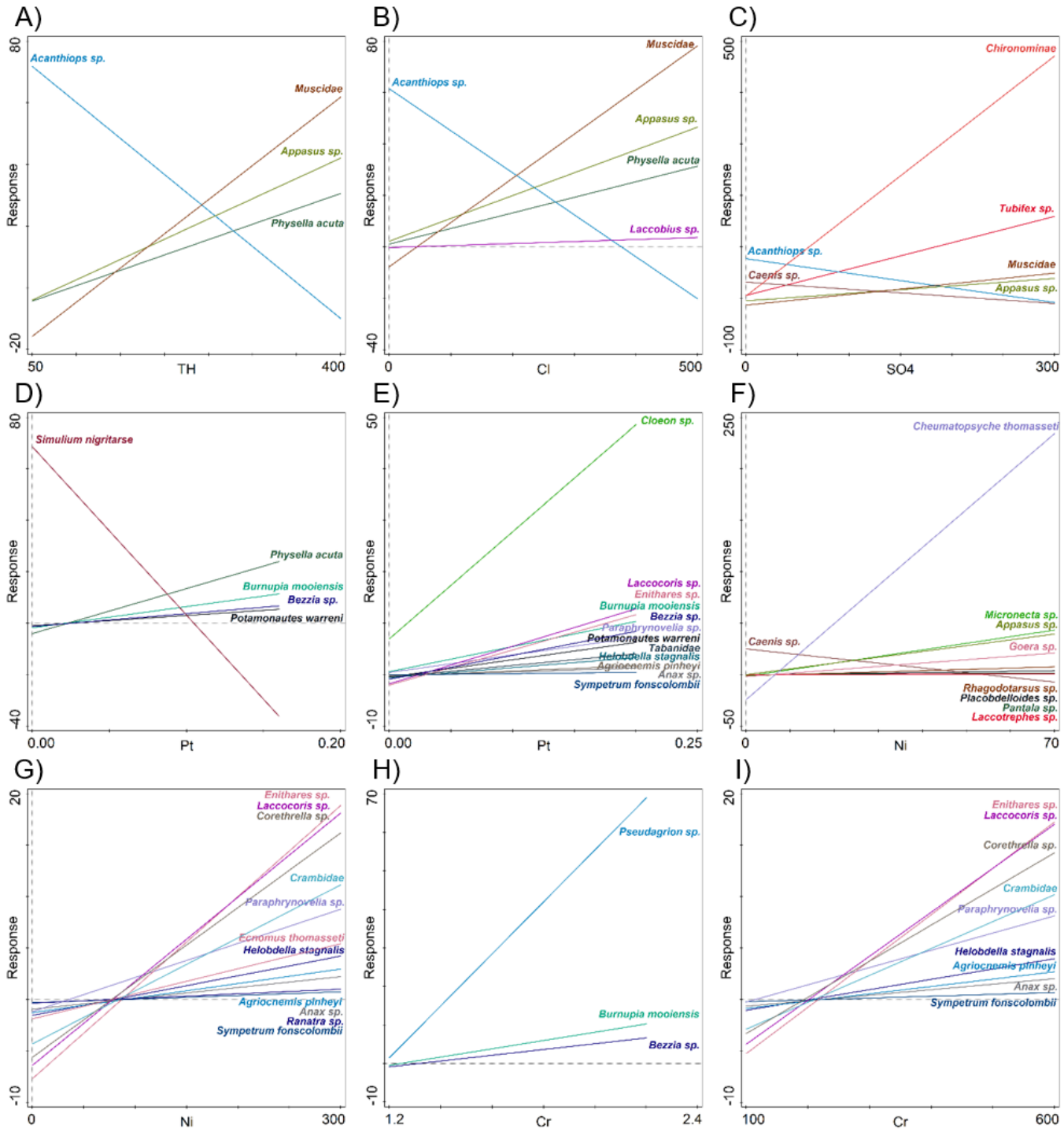


Figure 4.11: Species response curves of macroinvertebrate species (abundance) to environmental factors from the Hex River. These environmental factors include: A) total hardness (TH), B) chloride (Cl), C) sulphates (SO_4), D) Pt in water, E) Pt in sediment, F) Ni in water, G) Ni in sediment, H) Cr in water, I) Cr in sediment. Only species with a significant response ($p < 0.05$) were included.

The species response curves of the traits are representative of a decrease of increase of the trait and corresponding environmental variable. Regarding the traits in the river sites, total hardness had a significant negative response on filter feeders (p = 0.014), gill breathers (p = 0.039), Coleoptera (p = 0.044) and taxa that prefer the stones biotope (p = 0.008), while highly tolerant taxa (p = 0.007), air breathers using a spiracle (p = 0.013) and taxa that prefer the GSM biotope (p = 0.016), all had a significant positive response (Fig. 4.12). Chloride and SO_4 had a significant negative response on moderately sensitive taxa (p = 0.036; p = 0.010), egg, nymph aquatic life cycle stage (p = 0.036; p = 0.0051), gill breathers (p = 0.017; p = 0.035), Ephemeroptera (p = 0.040; p = 0.011) and taxa that prefer the stones biotope (p = 0.009; p = 0.009), while highly tolerant taxa (p = 0.006; p = 0.003), air breathers using a spiracle (p = 0.0006; p = 0.021), Hemiptera (p = 0.024; p = 0.042) and taxa that prefer the GSM biotope (p = 0.023; p = 0.011) all had a significantly positive response. Chloride also had a significant negative response on filter feeders (p = 0.037) and a positive response on the adult aquatic life cycle stage (p = 0.044). Concentrations of Pt in the water and sediment had a significant negative response on Coleoptera (p = 0.010; p = 0.042) and a significant positive response on Decapoda (p = 0.010; p = 0.005), while concentrations of Pt in the water also had a negative response on scrapers 2 (p = 0.033). Concentrations of Ni in the water had a significant negative response on moderately sensitive taxa (p = 0.018), egg, nymph aquatic life cycle stage (p = 0.017) and Ephemeroptera (p = 0.021), while grazers 2 (p = 0.012), predators 1 (p = 0.002), egg, larva, pupa (p = 0.037), as well as adult aquatic life cycle stage (p = 0.004), air breathers using a spiracle (p = 0.028), Hemiptera (p = 0.002) and Trichoptera (p = 0.006) all had a significantly positive response. Concentrations of Cr in the water had a significant positive response on Decapoda (p = 0.049) and taxa that prefer the vegetation biotope (p = 0.018), while concentrations of Cr and Ni in the sediment had a significant positive response on highly sensitive taxa (p = 0.0023; p = 0.0061), grazers 1 (p = 0.0020; p = 0.0012), air breathers using breathing tubes, straps or other apparatus (p = 0.0017; p = 0.0058) and Lepidoptera (p = 0.0023; p = 0.0061).

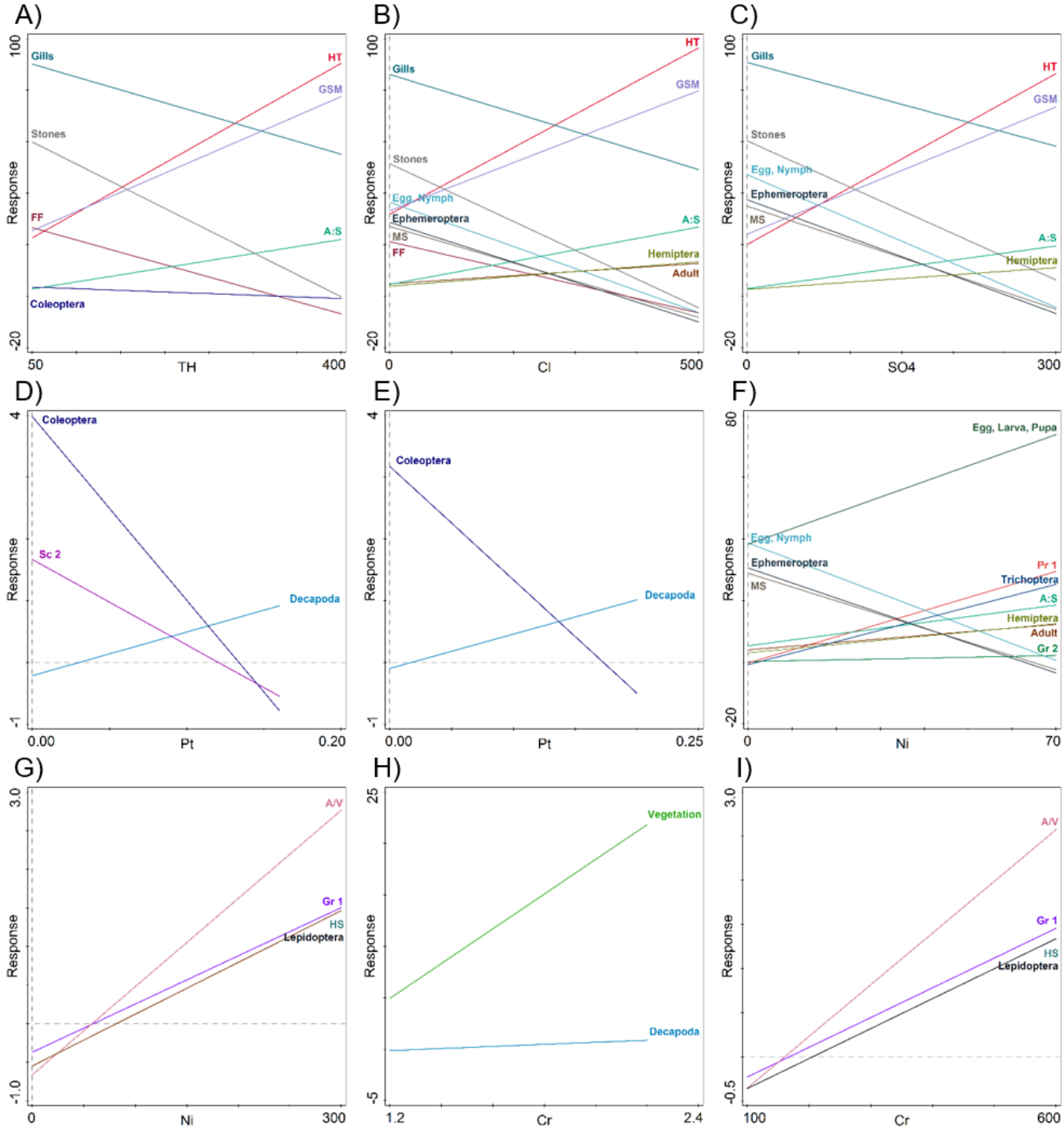


Figure 4.12: Species response curves of (increase or decrease) macroinvertebrate traits to environmental factors from the Hex River. These environmental factors include: A) total hardness (TH), B) chloride (Cl), C) sulphates (SO_4), D) Pt in water, E) Pt in sediment, F) Ni in water, G) Ni in sediment, H) Cr in water, I) Cr in sediment. Only traits with a significant response ($p < 0.05$) were included.

4.3.4 Macroinvertebrate community structures in the lentic sites

For statistical purposes, the mean diversity index scores of the three surveys were calculated per site and the temporal variation within sites is indicated by the standard error. Comparing the two impoundment sites, opposite trends were visible compared to the river sites, where the species richness at the reference impoundment (Olifantsnek Dam; mean of 13) was lower than the impacted impoundment (Bospoort Dam; mean of 21) (Fig. 4.13A). The number of individuals, as well as the diversity index, were higher at Bospoort Dam compared to Olifantsnek Dam (Fig. 4.13B and 4.13C), while the evenness index was higher at Olifantsnek Dam compared to Bospoort Dam (Fig. 4.13D). However, there were no significant differences between the two impoundments for species richness, number of organisms, diversity index or the evenness index. During the November 2017 survey, a dominant taxon (*Micronecta* sp.) occurred at Bospoort Dam with 75% dominance, whereas the macroinvertebrates from the other surveys and Olifantsnek Dam were more evenly distributed (Fig 4.14).

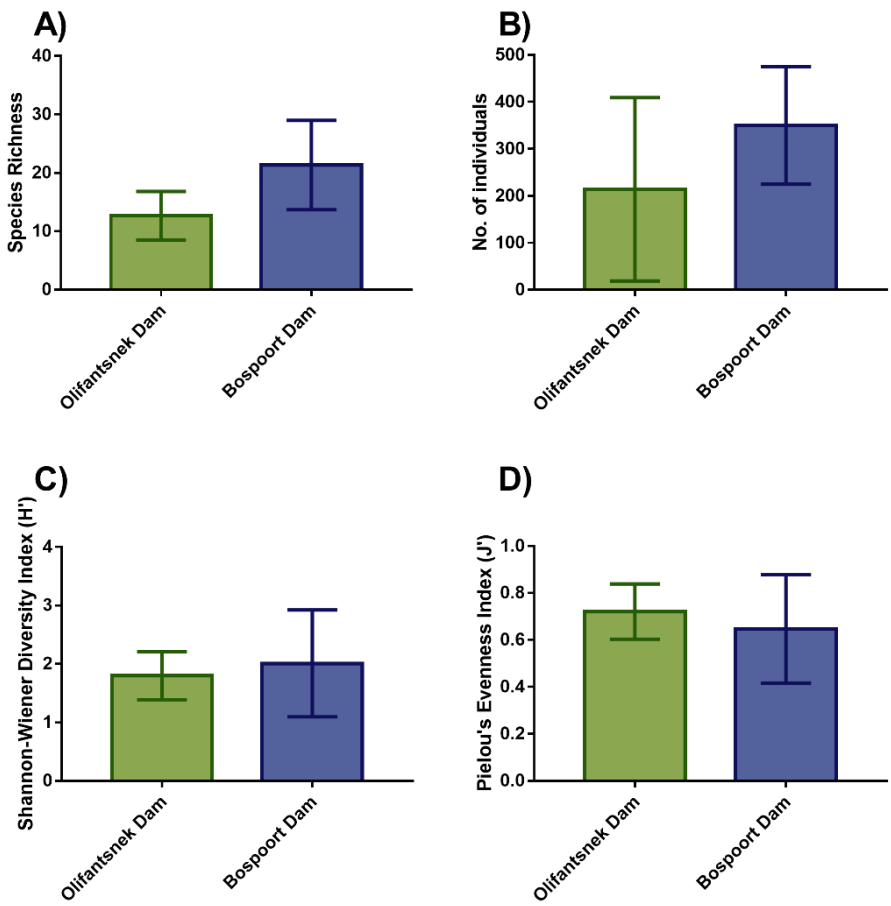


Figure 4.13: The mean ± standard deviation of the calculated biodiversity indices between two impoundment sites during three surveys (04/2017; 11/2017; 03/2018). The indices include A) Species Richness, B) Number of individuals, C) Shannon-Wiener Diversity Index and D) Pielou's Evenness Index. The biodiversity indices had no significant differences between sites.

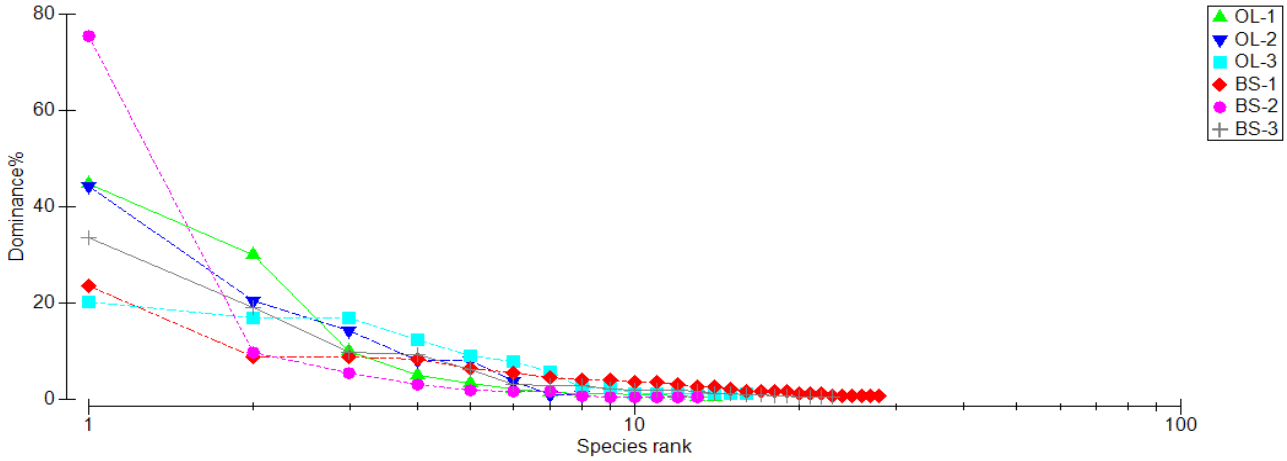


Figure 4.14: K-dominance plot of the macroinvertebrate species collected at two impoundment sites during three surveys (04/2017; 11/2017; 03/2018). Sites and surveys are indicated as Site – Survey (*e.g.* S1-1).

4.3.5 Traits in the lentic sites

4.3.5.1 Sensitivity values

The sensitivity also varied between the two impoundments, as well as between the surveys (Fig. 4.15), whereas highly tolerant taxa were the predominant taxa. The highly tolerant and moderately tolerant taxa increased from an average of 68% and 5.1% at Olifantsnek Dam to an average of 75% and 15% at Bospoort Dam, respectively. Moderately sensitive taxa decreased from Olifantsnek Dam (26%) to Bospoort Dam (7.4%), while no highly sensitive taxa were collected at either of the two impoundments. At both impoundments, during the November 2017 survey, highly tolerant taxa increased and, to some extent recovered during the March 2018 survey at Olifantsnek Dam, but not at Bospoort Dam.

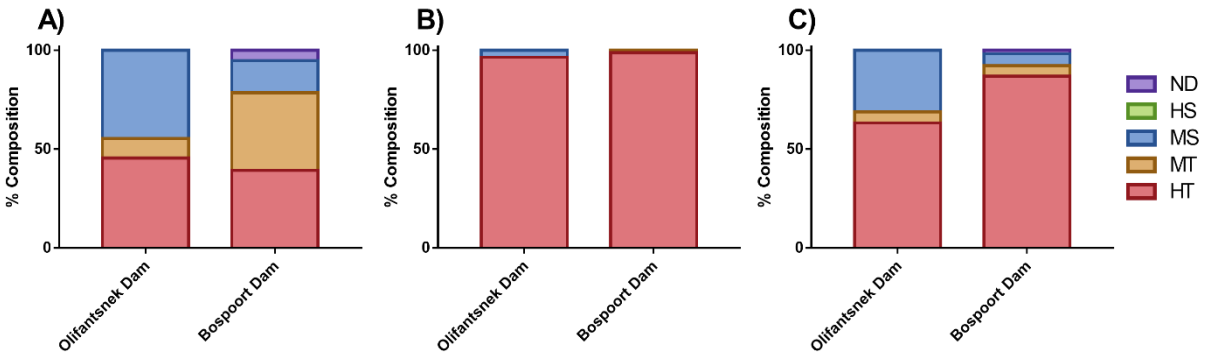


Figure 4.15: Percentage composition of highly sensitive (HS), moderately sensitive (MS), moderately tolerant (MT) and highly tolerant (HT) taxa collected at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018). Taxa with no sensitivity values allocated were also included as not determined (ND).

4.3.5.2 Functional feeding groups

Between the two impoundment sites, the functional feeding groups also varied between the surveys within a site, and between the sites (Fig. 4.16). The dominant feeding group at Olifantsnek Dam was deposit feeders 2 (DF 2) (52%), whereas at Bospoort Dam it was predators 1 (Pr 1) (39%). The second dominant feeding group at Olifantsnek Dam was deposit feeders 1 (DF 1) (21%), while at Bospoort Dam it was predators 2 (Pr 2) (33%). During the November 2017 survey, there was a small change in the functional feeding group composition at Olifantsnek Dam, while a major change occurred at Bospoort Dam. At Olifantsnek Dam, during the March 2018 survey, the community fully recovered to a similar composition as April 2017, while at Bospoort Dam the community only partially recovered.

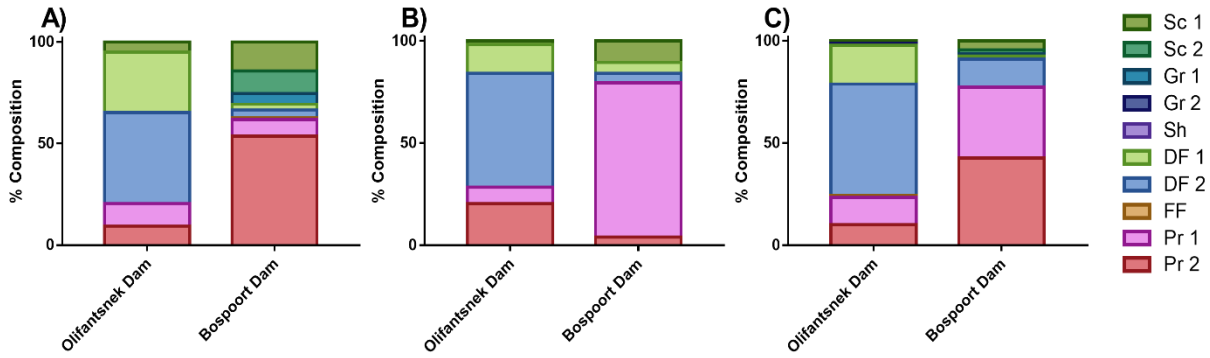


Figure 4.16: Percentage composition of different functional feeding groups [scrapers 1 (Sc 1), scrapers 2 (Sc 2), grazers 1 (Gr 1), grazers 2 (Gr 2), shredders (Sh), deposit feeders 1 (DF 1), deposit feeders 2 (DF 2), filter feeders (FF), predators 1 (Pr 1) and predators 2 (Pr 2)] collected at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018).

4.3.5.3 Aquatic life cycle stage

At the impoundment sites, the percentage composition completely changed (Fig. 4.17). The dominant aquatic life cycle stage at Olifantsnek Dam was all life stages (mean of 39.1%), while at Bospoort Dam it was the adult only life stage (mean of 61.3%). The second most abundant life stage at Olifantsnek Dam was the egg and nymph life stage (mean of 25.4%), while at Bospoort Dam it was all life stages (mean of 20.8%). Changes in the community structure were evident during the November 2017 survey at both impoundments, while the community structure recovered to some extent during the March 2018 survey.

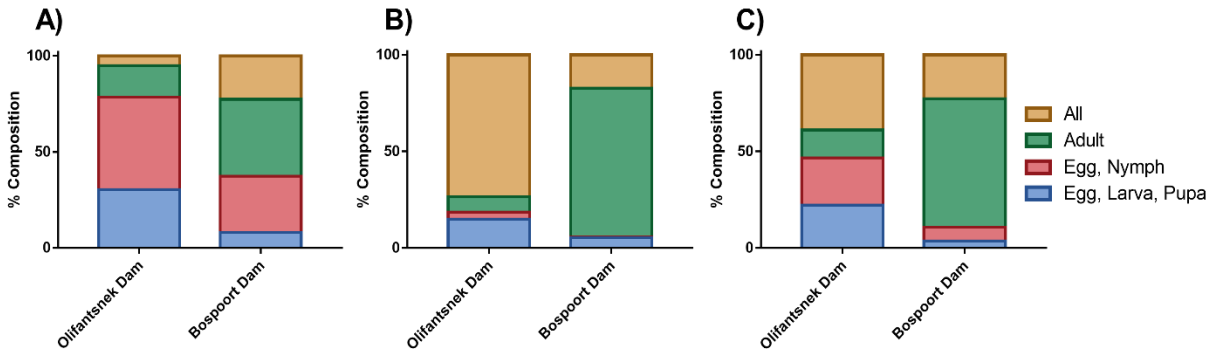


Figure 4.17: Percentage composition of the aquatic life stage present at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018).

4.3.5.4 Mode of respiration

The percentage gill breathers in the two impoundments decreased compared to the river sites, but at Olifantsnek Dam the gill breathers were still the dominant taxa present with 52%, while at Bospoort Dam the gill breathers were the second dominant with 24% (Fig. 4.18). The taxa with spiracles were dominant at Bospoort Dam and the taxa that breathe through their tegument or cutaneous was the second most dominant at Olifantsnek Dam. The number of taxa that are air breathers were higher in the impoundment sites, compared to the river sites, and comprised of 13% at Olifantsnek Dam and 64% at Bospoort Dam. This increase in air breathers from Olifantsnek Dam to Bospoort Dam also, like those in the river sites, correlates with the decrease in dissolved oxygen from 67.4% to 42.4%, at the impoundments, respectively. The same trend occurred at the two impoundment sites, where the community structure changed during the November 2017 survey and, to some degree recovered during the March 2018 survey.

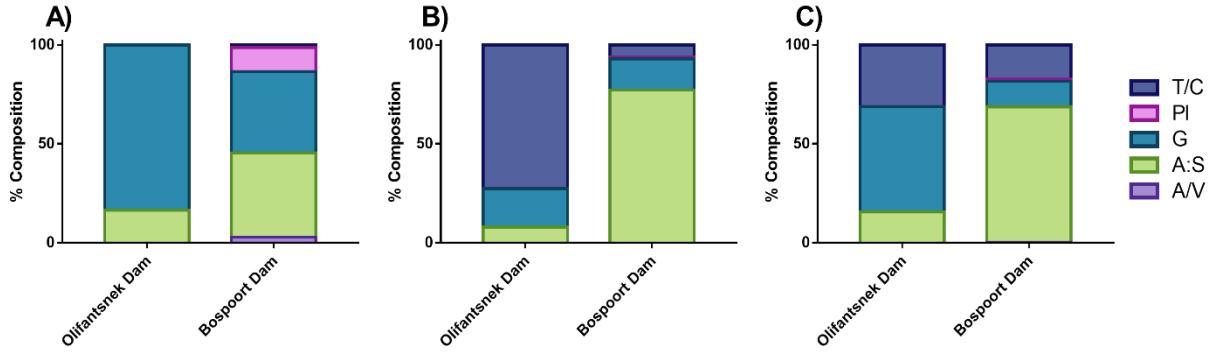


Figure 4.18: Percentage composition of the mode of respiration [tegument/cutaneous (T/C), plastron (Pl), gills (G), aerial: spiracle (A:S) and aerial/vegetation: breathing tube, straps/ other apparatus (A/V)] present at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018).

4.3.5.5 Orders

The Ephemeroptera decreased from 23.1% at Olifantsnek Dam to 2.2% at Bospoort Dam (Fig. 4.19). The Odonata and Trichoptera increased from 2.3% and 0.7% at Olifantsnek Dam to 10.0% and 1.0% at Bospoort Dam, respectively. The Diptera decreased from Olifantsnek Dam (21.8%) to Bospoort Dam (5.7%). The community composition in the impoundments is completely different from the composition in the river sites and these four orders only comprised 13 – 79% during the April 2017 and March 2018 surveys. The same trend occurred during the November 2017 survey where these four orders declined to 7% and 19% at Bospoort Dam and Olifantsnek Dam, respectively.

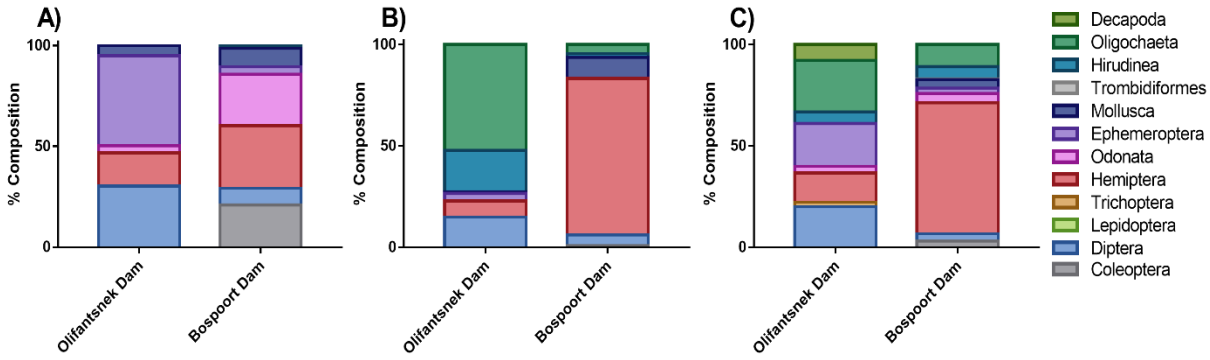


Figure 4.19: Percentage composition of Decapoda, Oligochaeta, Hirudinea, Trombidiformes, Mollusca, Ephemeroptera, Odonata, Hemiptera, Trichoptera, Lepidoptera, Diptera and Coleoptera present at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018).

4.3.5.6 Habitat preference

The macroinvertebrates that prefer the GSM biotope were dominant at Olifantsnek Dam (71.8%), while at Bospoort Dam the macroinvertebrates that are free living, dominated (56.0%). The macroinvertebrates that prefer vegetation were the lowest at Olifantsnek Dam (5.2%), while at Bospoort Dam it was second dominant (17.9%). At Olifantsnek Dam the macroinvertebrates that prefer the GSM biotope were consistent between surveys, while the macroinvertebrates that prefer the stones and vegetation biotopes, as well as free living organisms, varied (Fig. 4.20). At Bospoort Dam, on the other hand, the free living organisms increased during the November 2017 survey and, to some extent recovered during the March 2018 survey.

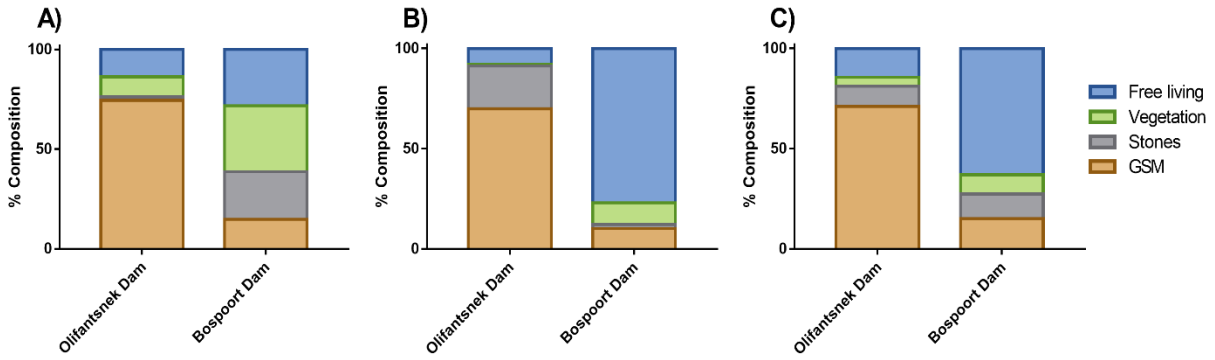


Figure 4.20: Percentage composition of macroinvertebrate habitat preference (free living; vegetation; stones; GSM – gravel, sand, and mud) present at two impoundment sites (Olifantsnek Dam; Bospoort Dam) during three surveys (A – 04/2017; B – 11/2017; C – 03/2018).

In the species based RDA all of the factors were included, however, only COD was significant ($p = 0.008$) (Fig 4.21). A temporal variation was evident between the surveys at both impoundments with Olifantsnek Dam during the April 2017 survey, and Bospoort Dam during the November 2017 survey, varied considerably from the other surveys.

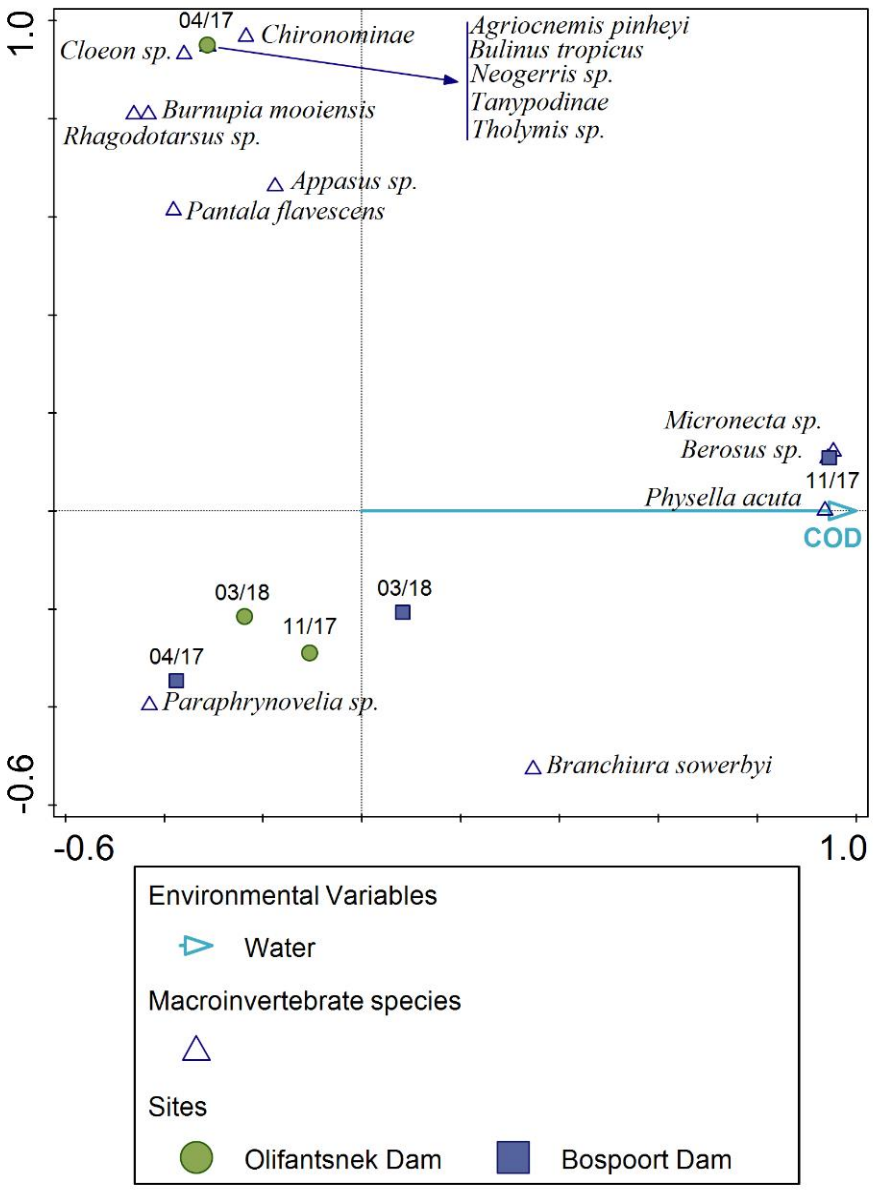


Figure 4.21: A RDA triplot illustrating associations between macroinvertebrate species, selected abiotic factors (pH, EC, temperature, turbidity, TDS, DO), nutrients (NH_4, NO_3, NO_2, SO_4, PO_4, TH) and metals in water and sediment (Cr, Ni, Cu, Zn, Cd, Pt, Pb) from two impoundment sites during three surveys (2017 – 2018). The triplot describes 87.3% of the variation with 55.0% on the first axis and 32.3% on the second axis. Only species with a 70% fit of the response variable are shown.

Considering all the factors in the trait-based RDA, only EC and concentrations of Cd in the sediment were significant ($p = 0.004$ and $p = 0.048$, respectively) (Fig 4.22). A clear spatial difference was evident between the sites, while temporal differences were also present. The Bospoort Dam site associated with higher EC values, a higher abundance of highly tolerant taxa, a higher percentage of taxa that are air breathers, taxa that are predators, as well as taxa that prefer the vegetation biotope and free living taxa. Olifantsnek Dam, on the other hand, associated with a higher abundance of moderately sensitive taxa, a higher percentage of taxa that are gill breathers, taxa that are deposit feeders and taxa that prefer the GSM biotope.

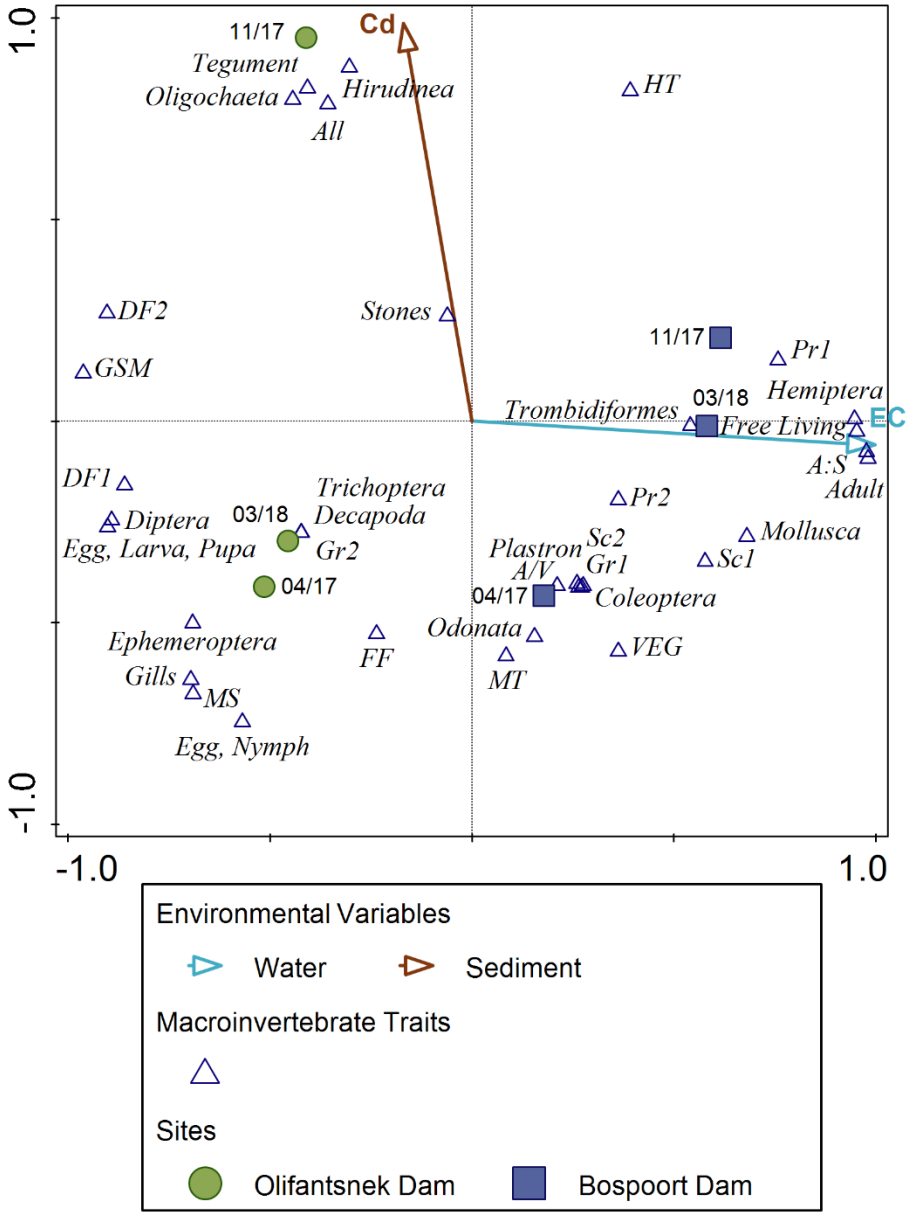


Figure 4.22: A RDA triplot illustrating associations between macroinvertebrate traits {sensitivity [highly tolerant (HT), moderately tolerant (MT), moderately sensitive (MS), highly sensitive (HS)], FFGs [scarpers (Sc 1 & 2), grazers (Gr 1 & 2), shredders (Sh), deposit feeders (DF 1 & 2), filter feeders (FF), predators (Pr 1 & 2)], aquatic life stage (egg, larva, pupa; egg, nymph; adult; all) mode of respiration [aerial/vegetation: breathing tube (A/V), aerial: spiracle (A:S), gills, plastron, tegument], Orders (Decapoda, Oligochaeta, Hirudinea, Trombidiformes, Mollusca, Ephemeroptera, Odonata, Hemiptera, Trichoptera, Lepidoptera, Diptera, Coleoptera), habitat preference (GSM, vegetation, stones, free living)}, selected abiotic factors (pH, EC, temperature, turbidity, TDS, DO), nutrients (NH_4, NO_3, NO_2, SO_4, PO_4, TH) and metals in water and sediment (Cr, Ni, Cu, Zn, Cd, Pt, Pb) from two impoundment sites during three surveys (2017 – 2018). The triplot describes 80.8% of the variation with 57.6% on the first axis and 23.2% on the second axis.

4.3.6 Species Response Curves in the lentic sites

In the impoundment sites, total hardness, Cl and SO_4, as well as concentrations of Cr and Ni in the sediment had no significant responses on any species. Concentrations of Pt in the water had a significant negative response on Orthocladinae ($p = 0.012$), while concentrations of Pt in the sediment, as well as concentrations of Ni and Cr in the water, had a significant positive response on Chrionominae ($p = 0.04$), *Laccophilus* sp. ($p = 0.014$) and *Lymnaea collumella* ($p = 0.0013$), respectively (Fig 4.23).

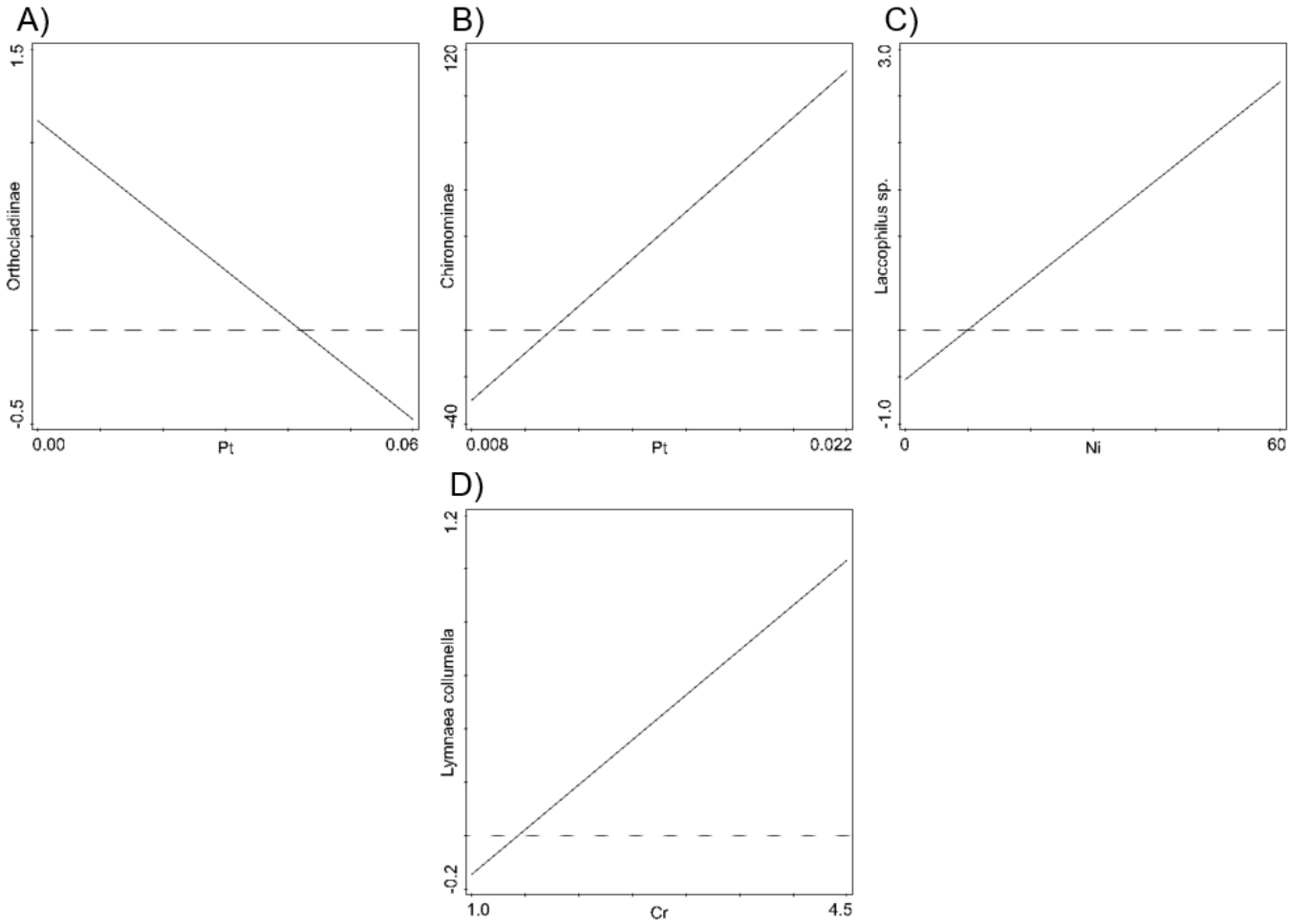


Figure 4.23: Species response curves of macroinvertebrate species (abundance) to environmental factors from Olifantsnek Dam and Bospoort Dam. These environmental factors include: A) Pt in water, B) Pt in sediment, C) Ni in water, D) Cr in water. Only species with a significant response were included.

Total hardness, Cl and SO_4 had a significant negative response on deposit feeders 1 (p = 0.028; p = 0.049; p = 0.013) and deposit feeders 2 (p = 0.0066; p = 0.032; p = 0.003), egg, larva, pupa aquatic life cycle stage (p = 0.027; p = 0.029; p = 0.015), Diptera (p = 0.031; p = 0.033; p = 0.016) and taxa that prefer the GSM biotope (p = 0.0014; p = 0.010; p = 0.00004), while the adult aquatic life cycle stage (p = 0.0095; p = 0.002; p = 0.011), air breathers using a spiracle (p = 0.0069; p = 0.0015; p = 0.0082), Hemiptera (p = 0.024; p = 0.0042; p = 0.028) and free living taxa (p = 0.030; p = 0.0054; p = 0.033) all had a significantly positive response (Fig 4.24). Concentrations of Pt in the water had a significant negative response on deposit feeders 2 (p = 0.0031), Oligochaeta (p = 0.045) and taxa that prefer the GSM biotope (p = 0.028), whereas scrapers 1 (p = 0.011) and Mollusca (p = 0.0048) had a significantly positive response, while concentrations of Pt in the sediment had no significant response. Concentrations of Ni in the water had a significant negative response on deposit feeders 2 (p = 0.014) and taxa that prefer the GSM biotope (p = 0.032), while scrapers 1 (p = 0.0014), Mollusca (p = 0.015) and taxa that prefer the vegetation biotope (p = 0.040) had a significantly positive response, whereas concentrations of Ni in the sediment only had a significant positive response on highly tolerant taxa (p = 0.015). Concentrations of Cr in the water had a significant positive response on all aquatic life cycle stages (p = 0.044), taxa that breath through their tegument (p = 0.029), Oligochaeta (p = 0.039) and Hirudinea (p = 0.016), while concentrations of Cr in the sediment had a significant negative response on scrapers 1 (p = 0.049), adult aquatic life cycle stage (p = 0.026) and air breathers that use a spiracle (p = 0.021), and a significant positive response on deposit feeders 1 (p = 0.025) and deposit feeders 2 (p = 0.00034), egg, larva aquatic life cycle stage (p = 0.033), Diptera (p = 0.038) and taxa that prefer the GSM biotope (p = 0.00016).

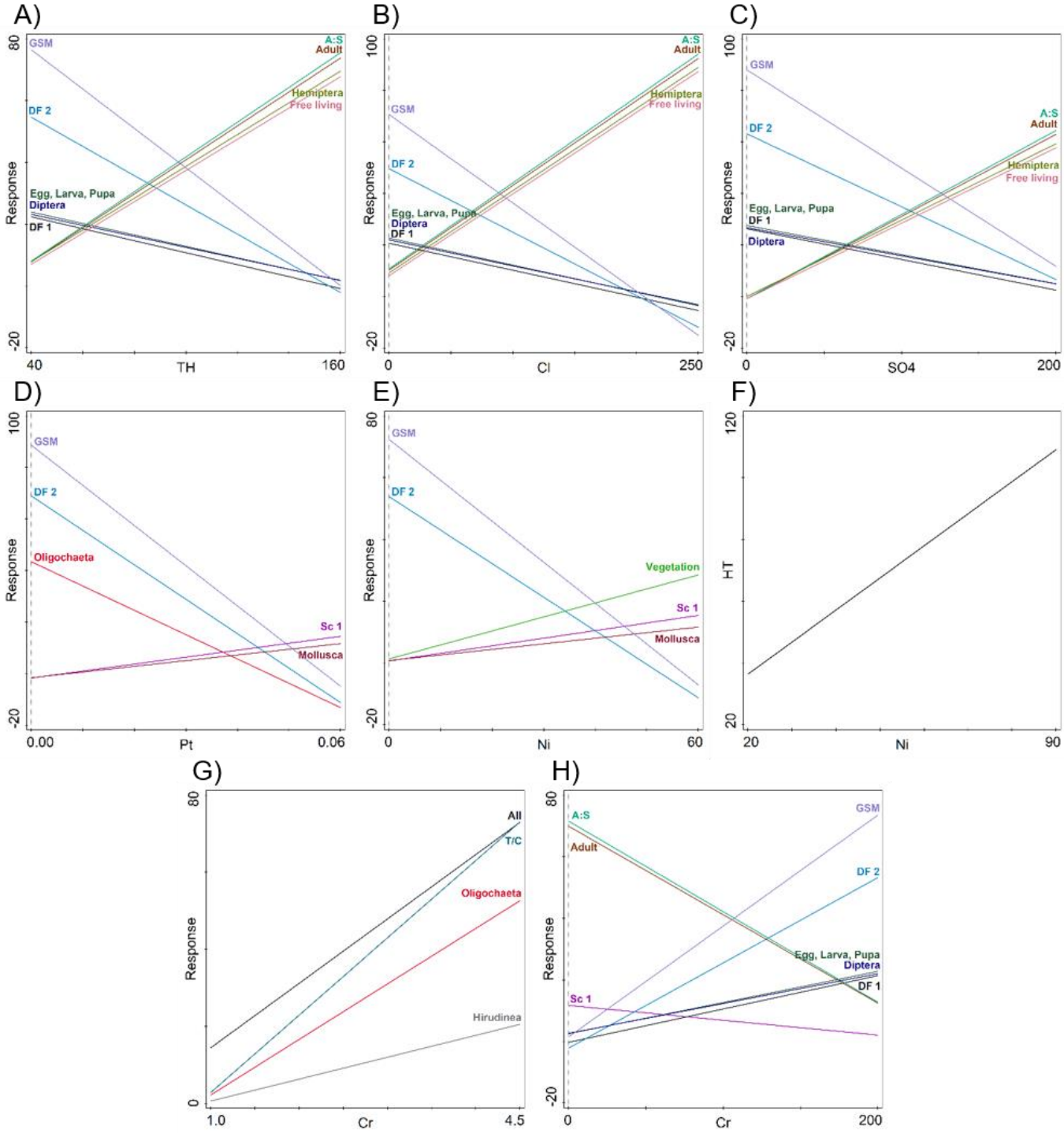


Figure 4.24: Species response curves of (increase or decrease) macroinvertebrate traits to environmental factors from Olifantsnek Dam and Bospoort Dam. These environmental factors include: A) total hardness (TH), B) chloride (Cl), C) sulphates (SO_4), D) Pt in water, E) Ni in water, F) Ni in sediment, G) Cr in water, H) Cr in sediment. Only traits with a significant response were included.

4.4 Discussion

The effects of environmental factors on the diversity of freshwater macroinvertebrates are well documented in North America and Europe, yet information on these effects from the rest of the world is still understudied, especially in Africa (Leszczyńska *et al*., 2017; Schmera *et al*., 2017). Recent studies focusing on environmental factors that affect macroinvertebrates in African rivers are available (Dalu *et al*., 2012; Bere *et al*., 2016; Mwedzi *et al*., 2016; Dalu *et al*., 2017a; 2017b; Mangadze *et al*., 2019). These effects of environmental factors on the macroinvertebrate community structures can be easily assessed by using trait-based community descriptors, rather than traditional taxonomy-based descriptors (Menezes *et al*., 2010). These traits include biological traits (*e.g.* behavioural and physiological characteristics, such as feeding, mode of respiration and, reproduction strategies) and ecological traits (tolerance to organic pollution and other abiotic factors, habitat preference and, biogeographic distribution) (Menezes *et al*., 2010).

4.4.1 Platinum mining effects on macroinvertebrate communities in a lotic system

Metals deriving from mining activities can affect macroinvertebrates in a direct (uptake via ingestion, adsorption and absorption, as well as precipitation on the gills or tegument) and indirect (metals altering food and habitat availability, competition and predation) manner (Luoma and Rainbow, 2008; Jones *et al*., 2016). Metals can affect changes in macroinvertebrate community structures, by eliminating or reducing the abundance of sensitive taxa and subsequently increase the abundance of tolerant taxa (Jones *et al*., 2016). While this chapter focuses on the indirect effects of metals on macroinvertebrates, Chapter 5 will focus on the direct effects on macroinvertebrates.

Sites 1 and 2 located in the upper reaches of the Hex River had the highest biodiversity index scores. These high biodiversity indices can be ascribed to limited anthropogenic activities (game farms and small-scale agricultural activities) occurring in the upper reaches at Sites 1 and 2 and were thus considered as reference conditions relative to mining activities. As the anthropogenic activities (mining, urban and industrial activities) increase towards the lower reaches of the Hex River, the different biodiversity index values significantly declined, especially at Site 4. The increase in numbers of individuals was from highly tolerant taxa that dominated the community structure at Site 4 and thrive in polluted water. These taxa include *Tubifex* sp., *Simulium adersi,* and Chironominae. Chironominae increased from a mean of 9.1% of the community structure at Site 1 to a mean of 36.5% of the community structure at Site 4. The Chironominae dominated (70.6%) the community structure at Site 4 during the last survey. This substantially higher percentage can be due to raw sewage from Rustenburg that was observed entering the Hex River approximately 2 km upstream of Site 4, between the last two surveys. River systems that are modified by anthropogenic activities are mostly dominated by Oligochaeta, Chironomidae, and Hirudinea, as was found by Azrina *et al*. (2006) in the Langat River, Malaysia, by Głowacki *et al*. (2011) and Grzybkowska *et al*. (2015) in the

Bzura River, Poland, by Orendt *et al*. (2012) in the Bílina River, Czech Republic, as well as by Bere *et al*. (2016) in the Manyame catchment, Zimbabwe.

From the sensitivity values that indicate the macroinvertebrates' response towards organic enrichment, and that are not a factor in metal pollution, it can be seen that highly tolerant taxa were the predominant taxa that occurred at all the sites, as well as during all the surveys. This can be explained by the fact that the dominant families at all the river sites ranged from Baetidae (*Acanthiops* sp., *Baetis* sp.), Chironomidae (Chironominae), Hydropsychidae (*Cheumatopsyche thomasseti*), Simuliidae (*Simulium adersi*, *Simulium nigritarse*) and Tubificidae (*Tubifex* sp.). Except for Baetidae and Hydropsychidae, all of these families are considered to be highly tolerant towards organic pollution (Day *et al*., 2002; Day and de Moor, 2002b; de Moor, 2003; de Moor *et al*., 2003a; 2003b). Within the order Ephemeroptera, the family Baetidae is considered to be the most tolerant family towards pollution (Awrahman *et al*., 2016; Bervoets *et al*., 2016). The abundance of highly tolerant taxa increased from Site 1 towards Site 4, due to the nutrient and metal concentrations that increased in the direction of Site 4.

Specialised feeders (*i.e.* scrapers and shredders) are more sensitive to changes in the environment, while the generalists (*i.e.* gatherers and filterers) are more tolerant towards pollution that might change the availability of certain food sources (Rawer-Jost *et al*., 2000). The Hex River can thus be regarded as modified, even in the upper reaches (Sites 1 and 2), due to filterers and gatherers that are the dominant taxa present at all the river sites. Site 1 was, however, the only site where shredder taxa were present. According to Rawer-Jost *et al*. (2000), the percentage of scrapers and shredders will decline with anthropogenic impacts, while the percentage of predators will increase. Results from the present study corroborate the findings of Rawer-Jost *et al*. (2000), as the scrapers declined from 9.6% at Site 1 to 3.1% at Site 4, the shredders only occurred at Site 1, while the predators increased from 14.6% at Site 1 to 34.9% at Site 4. At Site 4 during the last survey, the percentage predators declined and the deposit feeders increased, primarily due to Chironomidae that dominated the community structure with 70.6%. Rawer-Jost *et al*. (2000) reported the same trend where the benthic community structure was so depleted that *Chironomus thummi* was essentially the only species that remained during their surveys in 11 streams in southern Germany.

The importance of aquatic life stages as a trait is correlated as follows: the greater the number of aquatic life stages, the larger the effect of pollution on the macroinvertebrates. At all the river sites, the taxa varied between egg, larva, and pupa or the egg and nymph life stages, except for Site 4, where taxa with all life stages were present. This can be ascribed to the increased water volume, where water flow increased due to wastewater treatment plant effluent return, as well as mining and industrial effluent that enters the Hex River, resulting in a more stable water volume at Site 4. The taxa present in the river sites were predominantly gill breathing taxa. This reflects

the high percentage oxygen saturation recorded in the Hex River. However, as the dissolved oxygen decreased from Site 1 to Site 4, the respiration mode at Site 4 changed to air breathers, rather than gill breathers.

During the present study, the percentage of Ephemeroptera decreased from the reference sites towards the impacted sites, while Trichoptera increased. This result can be explained by the fact that Trichoptera taxa prefer fast flowing streams (de Moor *et al*., 2003b) and the increase in water quantity towards Site 4 facilitated the increase in abundance. The Diptera increased from Site 1 towards Site 4 and reflects the increase in anthropogenic activities downstream of Site 1 (Clements, 2004; Bere *et al*., 2016; Dalu *et al*., 2017b; Mabidi *et al*., 2017). The decrease in the abovementioned four orders during the November 2017 survey, was due to below average rainfall during the rainy season and resulted in below average water levels in the river (Baudoin *et al*., 2017). The recovery potential of macroinvertebrates following a drought event (Wallace, 1990; Zhao *et al*., 2018) was evident by the increase recorded in abundance after the prolonged dry spell. At Site 1, the recovery potential increased from 0%, due to the river being completely dry in the November 2017 survey, to 31.6% within the four months prior to the March 2018 survey. Sites 3 and 4 had a recovery increase from 37.4% and 57.1% in the November 2017 survey, to 81.8% and 80.2% in the March 2018 survey, respectively.

From the taxonomic and trait approaches it is clear that Pt mining, in combination with urban and sewage effluent had a negative effect on the macroinvertebrate community structure of the Hex River. The pollutants derived from Pt mining activities (Cr, Ni, Cd, Pt), as well as sewage effluent (NH_4, PO_4, TDS, EC), significantly affected the occurrence of moderately sensitive taxa, while significantly promoting the occurrence of highly tolerant taxa. Although these effects were evident, there is sufficient resilience in the river to sustain sensitive macroinvertebrates and the taxa present has the potential to recover from disturbances in this lotic system.

4.4.2 Platinum mining effects on macroinvertebrate communities in a lentic system

The species richness, number of individuals, as well as the Shannon Diversity Index, were higher at the impacted impoundment (Bospoort Dam) compared to the reference impoundment (Olifantsnek Dam). This effect can be due to a lack of habitat at Olifantsnek Dam where there was no marginal or aquatic vegetation available, compared to Bospoort Dam with various marginal and aquatic vegetation present, as well as the invasive water hyacinth (*Eichhornia crassipes*). Although the macroinvertebrates present at Olifantsnek Dam had no vegetation habitat, the community structure is balanced between the taxa, whereas Bospoort Dam had a lower Pielou's Evenness index due to the dominant taxa *Micronecta* sp. that comprised up to 75.4% of the community structure. This taxon is also highly tolerant and can occur in a variety of waterbody types as found by Mabidi *et al*. (2017) who sampled 33 waterbodies in the Eastern Cape, South Africa.

At the impoundment sites, the same trends occurred as for the river sites where highly tolerant taxa were the most predominant taxa that occurred at these two sites, over the three surveys. The dominant families were Baetidae (*Cloeon* sp.) and Tubificidae (*Tubifex* sp.) at Olifantsnek Dam, while in Bospoort Dam the dominant families were Corixidae (*Micronecta* sp.) and Notonectidae (*Enithares* sp.). All of these families are also considered to be highly tolerant towards organic pollution (Day and de Moor, 2002b; de Moor *et al*., 2003a; 2003b). Although the nutrient concentrations were all higher in Bospoort Dam, it is possible that the absence of the vegetation biotope at Olifantsnek Dam could be the reason why no highly sensitive taxa were present.

The FFG trends at the impoundment sites are slightly different to the river sites, where the percentage of scrapers increased from 2.6% at Olifantsnek Dam to 14.2% at Bospoort Dam, while at both of these sites, no shredders were present. However, the percentage of predators increased from 24.1% at Olifantsnek Dam to 72.9% at Bospoort Dam. Rawer-Jost *et al*. (2000) found that the percentage of predators were the most abundant functional feeding group metric, indicating the presence of anthropogenic impacts on a system. The impoundment sites also varied between all life stages and adult life stages. This can be due to sustained water volume, where taxa are able to complete their life cycles within the impoundments. Bospoort Dam had the highest presence of only adult life stages and can be ascribed to the dominant families Corixidae (*Micronecta* sp.) and Notonectidae (*Enithares* sp.), that only spend their adult lives in the water.

Even though Bospoort Dam had plenty of vegetation biotopes available, the dissolved oxygen was lower than Olifantsnek Dam. The taxa present at Bospoort Dam were all adapted to air breathers rather than through gill respiration. Ephemeroptera decreased from Olifantsnek Dam towards Bospoort Dam, while the Odonata and Trichoptera increased from Olifantsnek Dam towards Bospoort Dam. Diptera, however, decreased from the reference impoundment to the impacted impoundment. This can be attributed to Simuliidae being an important food source for various predators, especially Coleoptera and Odonata (de Moor, 2003; Werner and Pont, 2003), where Coleopterans and Odonata increased from 0% and 2.3% at Olifantsnek Dam to 8.3% and 10.0% at Bospoort Dam, respectively. During the November 2017 survey, these orders also decreased due to the drought. Nonetheless, they recovered from 18.6% and 6.6% during the November 2017 survey, to 46.7% and 12.7% during the March 2018 survey at Olifantsnek Dam and Bospoort Dam, respectively.

If only taxonomic approaches were followed, the effects of Pt mining activities would most likely not be detected, as the pollutants derived from Pt mining had little to no effect on the species' response. However, when trait-based approaches were applied, effects could be observed, as well as the extent to which the occurrence of highly tolerant taxa was stimulated by these stressors. These effects seemed to be less within the impoundments than those in the Hex River and can be explained by a more stable water volume. The water volume probably dilutes the

pollutants and due to the non-flowing conditions, pollutants can precipitate into the sediments, where they will remain until flushed out.

4.5 Conclusions

The macroinvertebrate community structures were altered from the reference conditions (Sites 1 and 2, Olifantsnek Dam) towards the impacted conditions (Sites 3 and 4, Bospoort Dam). These impacts include mining activities (especially Pt and Cr), urban, industrial, as well as informal settlements. The results indicate that the Hex River is largely organically enriched from the sources mentioned above and that enables the tolerant taxa to thrive, albeit not to the extent that they prohibit the occurrence of moderately sensitive taxa. Although the sensitivity towards organic enrichment should be considered, several other factors can also influence the macroinvertebrate community structure. Such factors include, amongst others, habitat availability, abiotic factors, biological traits (behavioural and physiological characteristics, life cycle stages) and ecological traits (habitat preference, tolerance towards organic enrichment, abiotic factors, and biogeographic distribution) of the macroinvertebrates. The trait-based approach proved valuable in assessing the influences of Pt mining on macroinvertebrate communities within the Hex River, South Africa. The impacted river sites (Sites 3 and 4) had a lower species richness and diversity due to the anthropogenic activities within this system, and even though, the impacted impoundment had a higher species richness and Shannon's diversity index value, these results were ascribed to factors such as habitat preference. The hypotheses stated for this chapter, namely, that macroinvertebrate community structures will be altered from the reference conditions due to Pt mining related responses, and those macroinvertebrate traits will be transformed as a result of changes in water and sediment quality, were supported by the data. The associated aim of this chapter, namely, to determine whether spatial and temporal changes have occurred within the aquatic macroinvertebrate community structures due to Pt mining, was subsequently achieved.

CHAPTER 5: METAL ACCUMULATION IN RIVERINE MACROINVERTEBRATES FROM A PLATINUM MINING REGION

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5.1 Introduction

Aquatic macroinvertebrates are effective biomonitoring organisms, especially to assess metal pollution in water and sediments of aquatic systems (Ding *et al*., 2017; Wang *et al*., 2019). This is due to their relatively long lifecycles, lower mobility, and their range in sensitivity to pollution (Mangadze *et al*., 2019; Pastorino *et al*., 2020). Macroinvertebrates comprise of a variety of different taxa, such as insects, crustaceans, bivalves, oligochaetes, as well as gastropods (Merritt *et al*., 2008); each of these taxa exhibit different feeding strategies and occupy different trophic levels (Moore, 2006; Ojija *et al*., 2017). According to Kenney *et al*. (2009) and Deborde *et al*. (2016), these organisms are responsible for several important ecological functions (*e.g.* nutrient cycling, decomposition), but also represents an important link in aquatic food webs, being both consumers and prey. Macroinvertebrates, therefore, provide valuable information on the bioavailability and transfer of metals within food webs.

Macroinvertebrates associated with the sediments in aquatic systems are considered good sentinels for pollution (Dalu *et al*., 2017a). Due to their low mobility, they can provide valuable information about the conditions in the localities where they are found (Kenney *et al*., 2009; Song *et al*., 2015). These organisms can accumulate metals from the environment via dietary (*i.e.* ingestion of contaminated sediment and food particles), as well as non-dietary routes (*i.e.* absorption through and adsorption onto their exoskeleton, respiration, and binding to their digestive system) (Solà and Prat, 2006). Metal pollution can affect macroinvertebrates by impeding their growth and reproduction, alter the biological diversity and density, can cause population declines in sensitive organisms, and can even alter the food web structures (Gray and Delaney, 2008; Wang *et al*., 2019).

The aims of the present study were to 1) determine the behaviour of metals associated with Pt mining in the Hex River system, 2) determine the metal concentrations in water, sediment and aquatic macroinvertebrates at different impacted sampling sites along the Hex River and 3) evaluate the bioaccumulation of metals in various macroinvertebrate families. The hypotheses tested in this chapter are that macroinvertebrate families from the impacted sites will accumulate higher metal concentrations compared to the reference sites and that predator families will accumulate higher metal concentrations than lower trophic level families.

5.2 Materials and Methods

5.2.1 Field sampling

Water, sediment and aquatic macroinvertebrate samples were collected at five sites during March 2018 in the Rustenburg area, North West Province, South Africa (Fig. 5.1). These included three sites within the Hex River, one site in a settling pond (Mine) located on a Pt mine and one site in a tributary downstream of the city of Rustenburg (Urban, Chapter 2, DS 2). The sites in the Hex River included a reference site (Site 1; Chapter 3, Site 1), a site located directly downstream of the Pt mining activities (Site 2; Chapter 3, Site 4), and a site located further (approximately 6 km) downstream of the mining area, that is additionally influenced by urban effluent and a wastewater treatment works (Site 3; Chapter 3, Site 5).

At each sampling site, *in situ* water quality variables were measured according to the methods in Chapter 2. Water samples for metal analysis were collected in triplicate in pre-cleaned polyethylene containers. Sediment samples from the upper 10 cm were also collected in triplicate in pre-cleaned polyethylene containers. Macroinvertebrates were sampled (using sample method as described in Chapter 4) with a sweep net (1 mm mesh size) and placed into a sorting tray. The macroinvertebrates were rinsed with MilliQ® water and sorted according to families in pre-cleaned polyethylene containers, and frozen at -20 °C until further analyses. Families collected at all three Hex River sites were the Lymnaeidae (pond snails), Baetidae (mayflies), Potamonautidae (river crabs), Coenagrionidae (sprites, damselflies) and Libellulidae (skimmers, dragonflies). Hydropsychidae (caddisflies) and Tubificidae (aquatic earthworms) were only found at Site 2 and Chironomidae (common midges) only at Site 3. A single recently emerged dragonfly (Libellulidae) was collected at the mine settling pond, while no macroinvertebrates were collected in the urban effluent. These taxa represented different functional feeding groups namely scraper-grazers (Lymnaeidae), collector-gatherers (Potamonautidae, Hydropsychidae, Tubificidae, and Chironomidae), shredders (Baetidae) and predators (Coenagrionidae and Libellulidae).

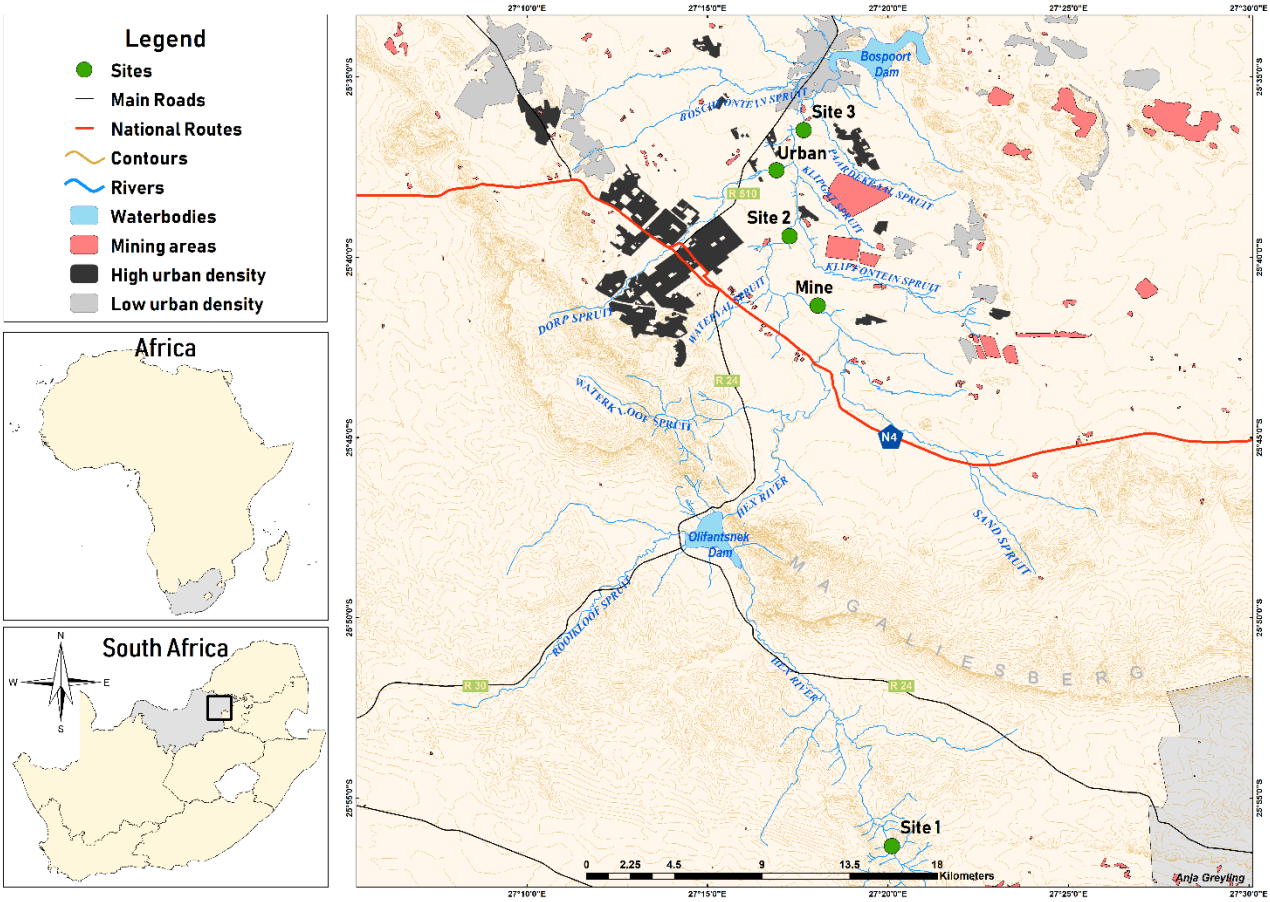

Figure 5.1: Map of the study area, indicating the sampling sites in the Hex River (Site 1 – 3), in the mine settling pond (Mine) and in the urban effluent (Urban), as well as mining activities, urban and informal settlements in the surrounding area.

5.2.2 Sample preparation

Water and sediment samples were prepared for element analysis using the same methods that are described in Chapter 2. After thawing, each family of macroinvertebrates from each site was homogenised with a dispersing tool (Ultra-Turrax T25, Janke and Kunkel) and freeze-dried. For molluscs (Lymnaeidae) and crabs (Potamonautidae), shell and carapace, respectively, were removed before homogenisation, so that only the soft tissue was used for metal analysis. Samples of 0.1 g (dry weight) were weighed into 20 mL TFM® vessels (MarsXpress, CEM) and digested in a mixture of 3 mL HNO_3 (65%, supra pure quality, Merck) and 1 mL HCl (37%, supra pure quality, Merck) adapted from Djingova *et al*. (2003) using a Mars 6 microwave digestion system (CEM Corporation, Kamp-Lintfort). Taxa with enough sample mass were analysed in replicates (see Appendix D, Table D1). Following digestion, the solution was transferred to a 5 mL volumetric flask and brought to volume with MilliQ® water. All samples were stored at room temperature until element analysis.

5.2.3 Element analysis

In all of the samples, the concentrations of Cr, Ni, Cu, Zn, Cd, Pt, and Pb were determined using the same methods as described in Chapter 2. Detection limits for the macroinvertebrate samples (related to a mean sample weight of 136 mg) were determined as three times the standard deviation of the blank measurements and were found to be 0.21 µg/g for Cr, 0.02 µg/g for Ni and Cu, 0.38 µg/g for Zn, 0.00063 µg/g for Cd, 0.0008 µg/g for Pt and 0.021 µg/g for Pb. Quality control of the element analysis was performed using applicable standard reference materials for all of the metals (see Table 5.1). Reference materials, IAEA-407 (fish homogenate reference material; International Atomic Energy Agency, Monaco), DORM-3 (fish protein certified reference material, National Research Council, Canada) and DOLT-3 (dogfish liver certified reference material, National Research Council, Canada) were used to validate the analytical procedure of biota samples. The quality control method for sediment samples can be found in Chapter 2. As Pt was not certified in the biological reference materials, a spiking experiment was performed by adding Pt to the sample matrix before and after the digestion in a concentration range of 0.1 to 100 µg/L. The recovery rates were evaluated from the ratios of the slopes calculated for the Pt addition series before and after digestion. All recovery rates were within a 10% deviation range. In order to assess the potential mass interferences during the detection of Pt, after the measurement series, a matrix-adapted addition with Hf was performed as described by Ek *et al*. (2004). However, obtained interferences rates were always less than 2% and therefore no Hf-correction was applied.

Table 5.1: Recovery rates (%) of the elements of interest obtained for the different certified reference materials used in the analysis.

Elements	Recovery rates (%)		
	IAEA-407	DORM-3	DOLT-3
Cd	101	110	96
Cr	84	96	119
Cu	97	100	110
Ni	90	118	93
Pb	108	91	85
Pt	n.c.	n.c.	n.c.
Zn	86	86	96

n.c. – not certified.

5.2.4 Data analysis

Data were tested for normality and homogeneity of variance using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively. One-way analysis of variance with Tukey's multiple comparison test was used to test for significant differences in metal concentrations between sites. The level of significance was set at $p < 0.05$. A Principle Component Analysis (PCA) (Canoco v5.12) was constructed to assess the spatial patterns associated with the water and sediment quality, as well as a PCA with supplementary variables to assess the associations between bioaccumulation in macroinvertebrates and abiotic metal concentrations. Data for the PCA was log transformed $y = \log(x + 1)$. Bioconcentration factor ($BCF = c_{invertebrate} / c_{water}$) and biota-sediment accumulation factor ($BSAF = c_{invertebrate} / c_{sediment}$) were calculated as the ratio of the average metal concentration in the invertebrates per quantified metal concentration of the filtered water and sediment, respectively.

5.3 Results

5.3.1 Water and sediment quality

The water and sediment quality results collected from the three sites in the Hex River, as well as the mine settling pond and urban effluent, are presented in Table 5.2 and Appendix D, Table D2. The data from the three river sites, mine settling pond and urban effluent were subjected to a PCA biplot and Fig. 5.2 presents an integrated spatial ordination of the abiotic variables. Distinct spatial differences were evident, while Site 3, the mine settling pond and urban effluent displayed more similar water quality characteristics than Sites 1 and 2. Sites 1 and 2 were characterized by higher concentrations of Cu, Zn, and Pb in the water, with higher dissolved oxygen and organic content, as well as finer substrate composition. Site 3, the mine settling pond and urban effluent were characterized by higher concentrations of Cr, Ni, and Pt in the water, as well as higher turbidity and electrical conductivity. From the metal concentrations in the sediment, Site 2 associated with higher concentrations of Cr, Ni, Cu, Cd, and Pb, while the mine settling pond and urban effluent associated with high concentrations of Zn and Pt.

Table 5.2: Water and sediment quality variables analysed at the three Hex River sites (Site 1 – 3), as well as the mine settling pond (Mine) and urban effluent (Urban). The codes for each variable are used in the PCA biplot.

	Code	Site 1	Site 2	Site 3	Mine	Urban
Water quality variables						
Dissolved oxygen (mg/L)	DO	7.3	5.5	1.5	n.m.	3.8
Electrical conductivity (µS/cm)	EC	375	548	1 646	1 775	3 950
pH	pH	8.2	8.3	7.8	8.0	7.6
Suspended solids (mg/100mL)	SS	1.8	1.5	3.6	9.9	1.7
Temperature (°C)	TEMP	23.8	24.9	23.8	25.6	23.0
Turbidity (FAU)	TURB	12	13	14	12	17
Sediment quality variables						
Organic content (%)	OC	7.7	5.7	3.2	n.m.	1.4
Gravel (%)	GR	16.0	13.4	3.4	n.m.	6.7
Very course sand (%)	VCS	6.7	11.4	2.5	n.m.	27.4
Course sand (%)	CS	22.6	35.1	20.3	n.m.	52.8
Medium sand (%)	MS	19.0	18.5	43.8	n.m.	9.7
Fine sand (%)	FS	16.9	14.7	25.1	n.m.	3.0
Mud (%)	M	18.8	7.0	5.0	n.m.	0.4

n.m. – not measured.

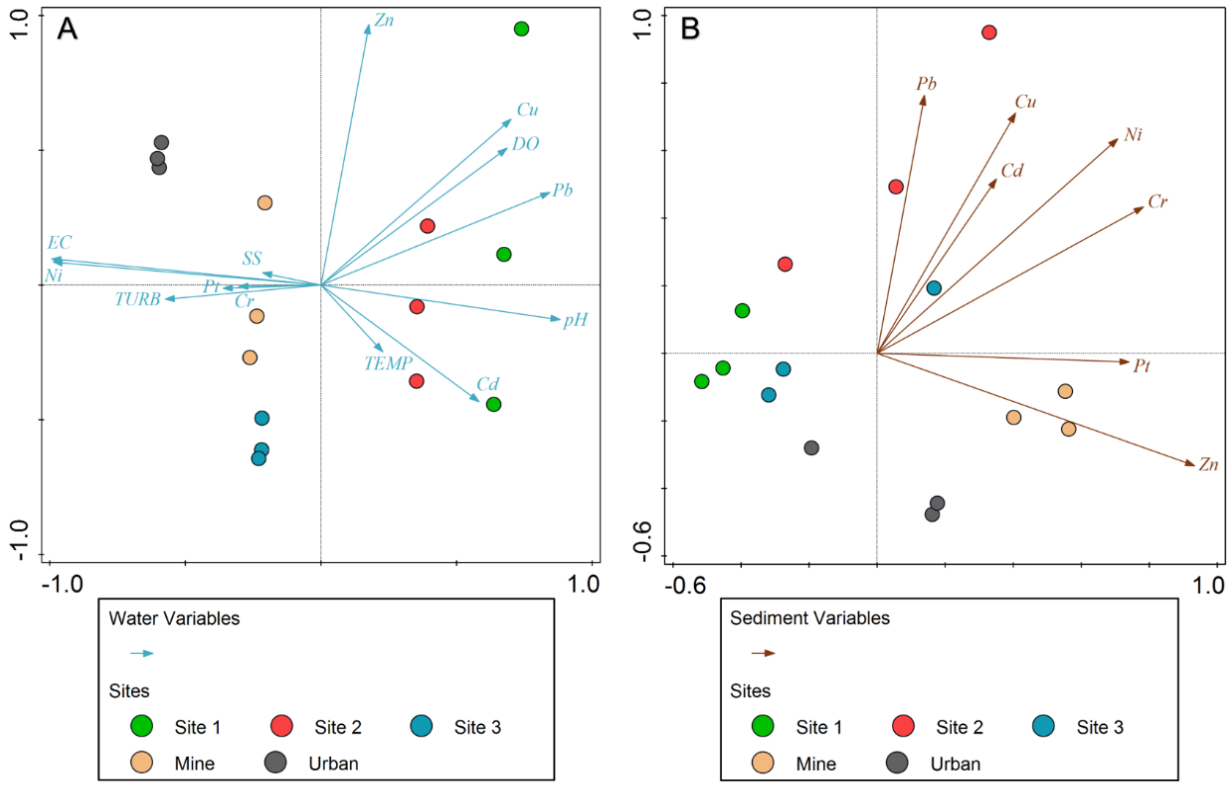

Figure 5.2: PCA biplot of the water (A) and sediment (B) quality variables measured at sites in the Hex River (Sites 1 – 3), a mine settling pond (Mine) and an urban effluent (Urban) during March 2018. The water (A) biplot describes 93.2% of the variation with 81.2% on the first axis and 12.0% on the second axis. The sediment (B) biplot describes 84.1% of the variation with 58.0% on the first axis and 26.1% on the second axis.

5.3.2 Metal concentrations in the water

Among the four sampling sites, the water of the reference site (Site 1) had the highest concentrations of Cu (21.2 µg/L), Zn (20.6 µg/L) and Pb (2.12 µg/L) (Fig. 5.3). The concentrations of these metals tend to decrease further downstream of the Hex River. However, statistical analysis revealed no significant differences between the different sampling sites for these elements. The water of the mine settling pond (Mine) contained the highest concentrations of Cd (0.16 µg/L) and Pt (0.47 µg/L) (Fig. 5.3). The urban effluent (Urban) had the highest concentrations of Ni (115.3 µg/L) and significantly increased in the Hex River from Site 2 to Site 3. As expected, the water of the mine settling pond showed significantly higher concentrations of Pt than all of the other sampling sites. The highly impacted site (Site 2) had significantly higher concentrations of Pt than Sites 1 and 3, while Site 3 differed significantly from Site 1. The concentrations of Cr in the water of all sampling sites were below the detection limit.

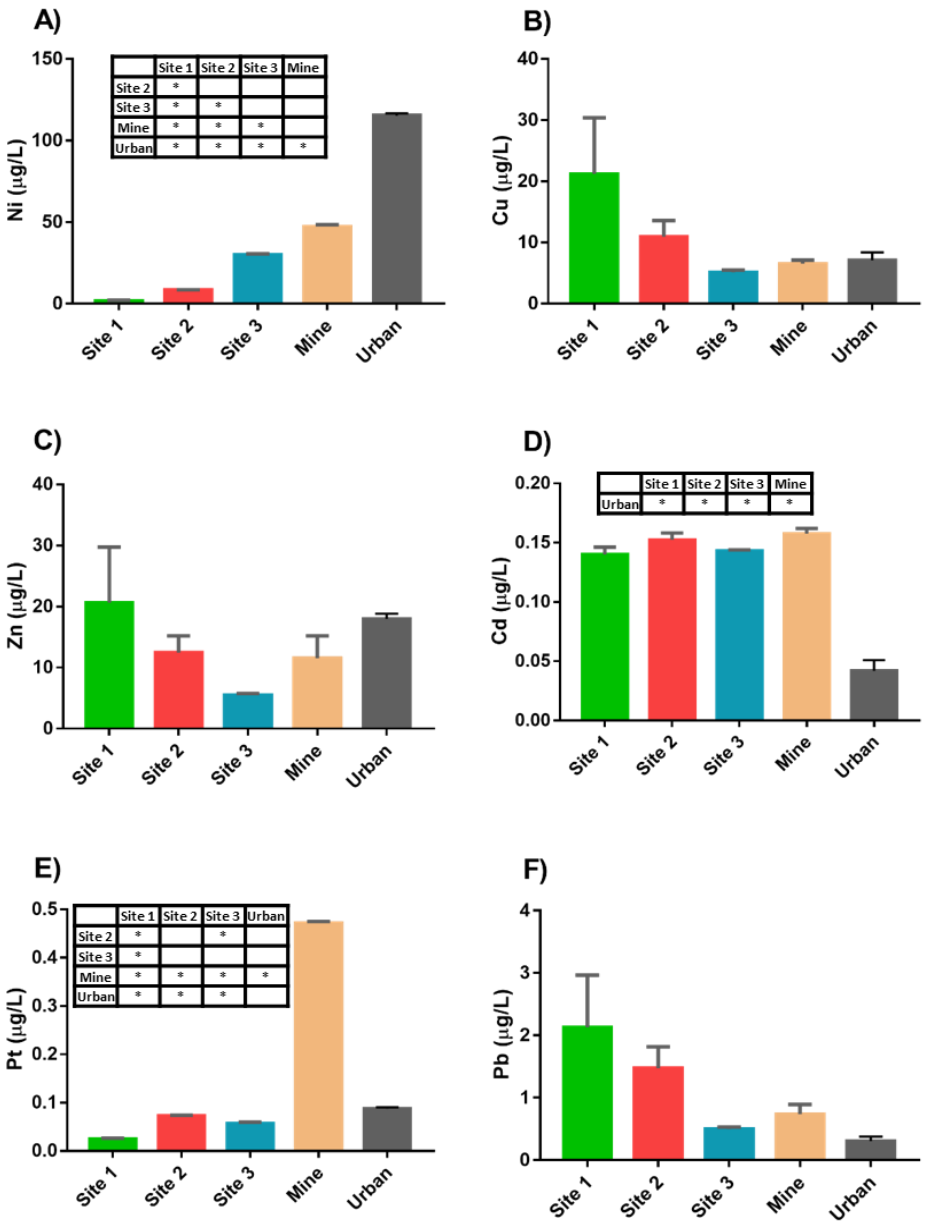

Figure 5.3: Mean concentrations (µg/L) of Ni (A), Cu (B), Zn (C), Cd (D), Pt (E) and Pb (F) with standard error of the mean in water collected from selected sites in the Hex River (Site 1 – 3), a mine settling pond (Mine) and an urban effluent (Urban). Concentrations of Cr were below the detection limit of 0.19 µg/L. Significant differences ($p < 0.05$) between sites are indicated in table format by means of an asterisk.

5.3.3 Metal concentrations in sediment

The sediments of the highly impacted site (Site 2) contained the highest concentrations of Ni (256 µg/g), Cu (119 µg/g), Cd (0.39 µg/g) and Pb (41 µg/g). However, statistical analysis revealed no significant differences between the sites for these metals. In contrast, the mine settling pond (Mine) demonstrated the highest concentrations of Cr (1 609 µg/g), Zn (391 µg/g) and Pt (0.61 µg/g) (Fig. 5.4). In the sediments of the Hex River, the concentrations of both, Cr and Pt, were significantly higher at Site 2 than at Sites 1 and 3, respectively. The urban effluent site (Urban) contributes to high concentrations of Ni, Zn, and Pb.

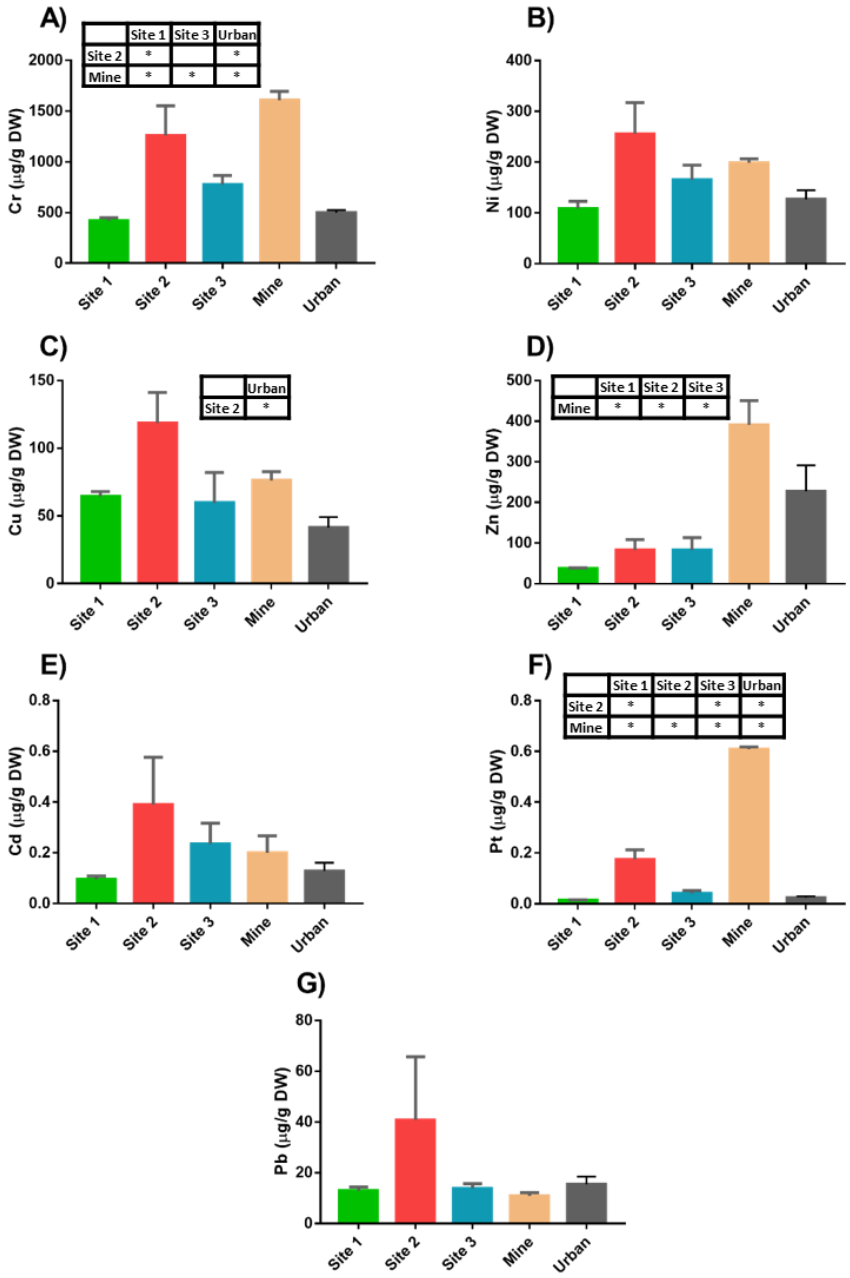

Figure 5.4: Mean concentrations (µg/g DW) of Cr (A), Ni (B), Cu (C), Zn (D), Cd (E), Pt (F) and Pb (G) with standard error of the mean in sediments (DW: dry weight) collected from selected sites in the Hex River (Site 1 – 3), a mine settling pond (Mine) and an urban effluent (Urban). Significant differences ($p < 0.05$) between sites are indicated in table format by means of an asterisk.

5.3.4 Metal concentrations in macroinvertebrate families

A PCA with supplementary variables to assess the associations between bioaccumulation in macroinvertebrates and abiotic metal concentrations was constructed where the metal concentrations in the macroinvertebrates varied, depending on the family and the sampling site (Fig. 5.5). Most of the macroinvertebrate families accumulated the highest metal concentrations at Site 2, where the concentrations of Cr, Cd, and Pt in the water and all of the metals concentrations in the sediment were the highest. The family Coenagrionidae accumulated higher concentrations of Ni, Cr, and Pb at Site 1 than the other sites, while Libellulidae accumulated higher concentrations of Pb at Site 1 than the other sites. At Site 3, Lymnaeidae and Potamonautidae accumulated higher concentrations of Zn, while Coenagrionidae and Libellulidae accumulated higher concentrations of Pt and Ni, respectively compared to the other sites.

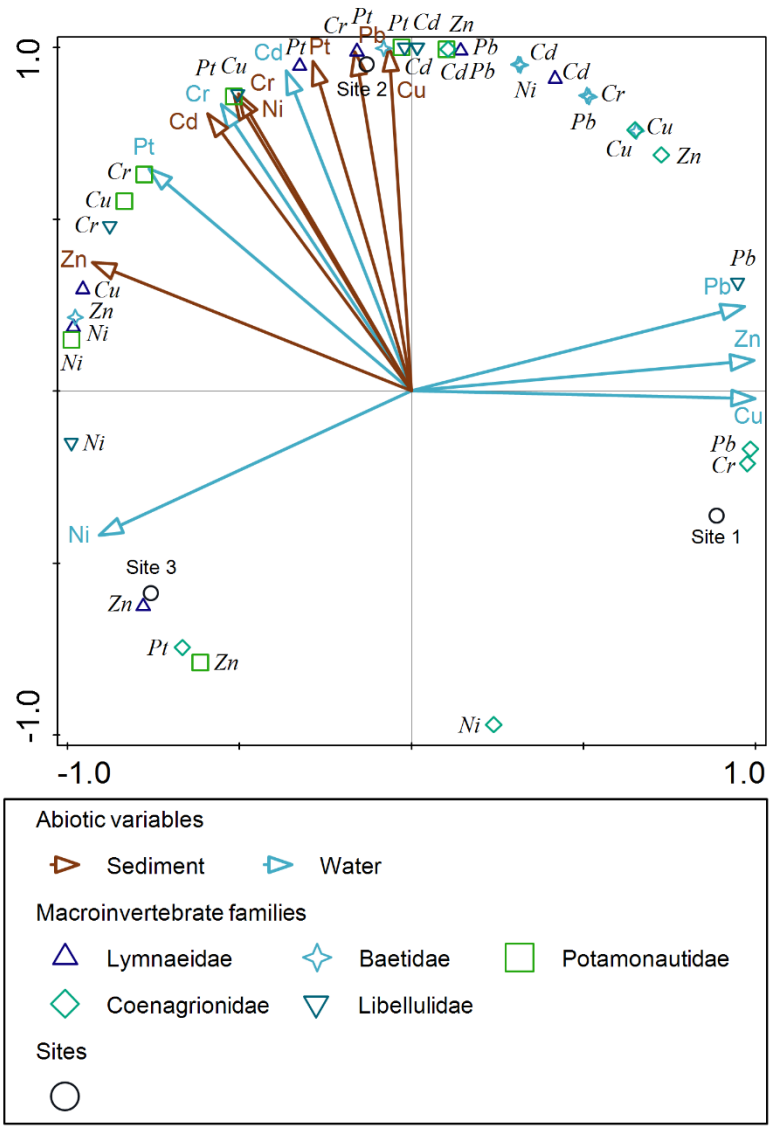

Figure 5.5: A PCA biplot with supplementary variables of the metal concentrations in water, sediment and macroinvertebrate families, respectively, collected from the three sites in the Hex River (Site 1 – 3) during March 2018. The biplot describes 100% of the variation with 59.2% on the first axis and 40.8% on the second axis.

The highest concentrations of Cr were found in the families Tubificidae (92 µg/g DW) from Site 2 and the Libellulidae sample (69 µg/g DW) collected from the mine settling pond (Fig. 5.6A). Concentrations of Cr were higher for most taxa at Site 2 and only the Coenagrionidae showed higher concentrations at Site 1. The highest concentrations of Ni were determined in the Lymnaeidae (59 µg/g DW) of Site 3 and in the Libellulidae sample of the mine settling pond (59 µg/g DW). Concentrations of Ni were higher in most of the families at Site 3, except for Baetidae at Site 2 and Coenagrionidae at Site 1. Both Lymnaeidae and Potamonautidae showed the highest concentrations of Cu regardless of the location (Fig. 5.6C). Concentrations of Cu ranged from 16 µg/g DW in Libellulidae of Site 1 and 88 µg/g DW in Potamonautidae of Site 2. In contrast, the Lymnaeidae contained the lowest concentrations of Zn within the macroinvertebrate families with the lowest mean value of 22 µg/g DW of Site 2. The highest concentrations of Zn were found in the Libellulidae sample collected at the mine settling pond (180 µg/g DW) (Fig. 5.6D). However, at all sampling sites of the Hex River, the highest concentrations of Zn and Cd were obtained for the family Baetidae. In all families, the mean concentrations of Cd were higher at Site 2 (the site directly downstream of Pt mining activities) in comparison to the other sites. The same trend was evident for Pt with the exception of the Libellulidae sample collected at the mine settling pond. For both Pt and Pb (Fig. 5.6 F – G), the concentrations were the highest in Tubificidae, whereas Site 2 showed the highest metal concentrations compared to the other sites, except for Coenagrionidae and Libellulidae.

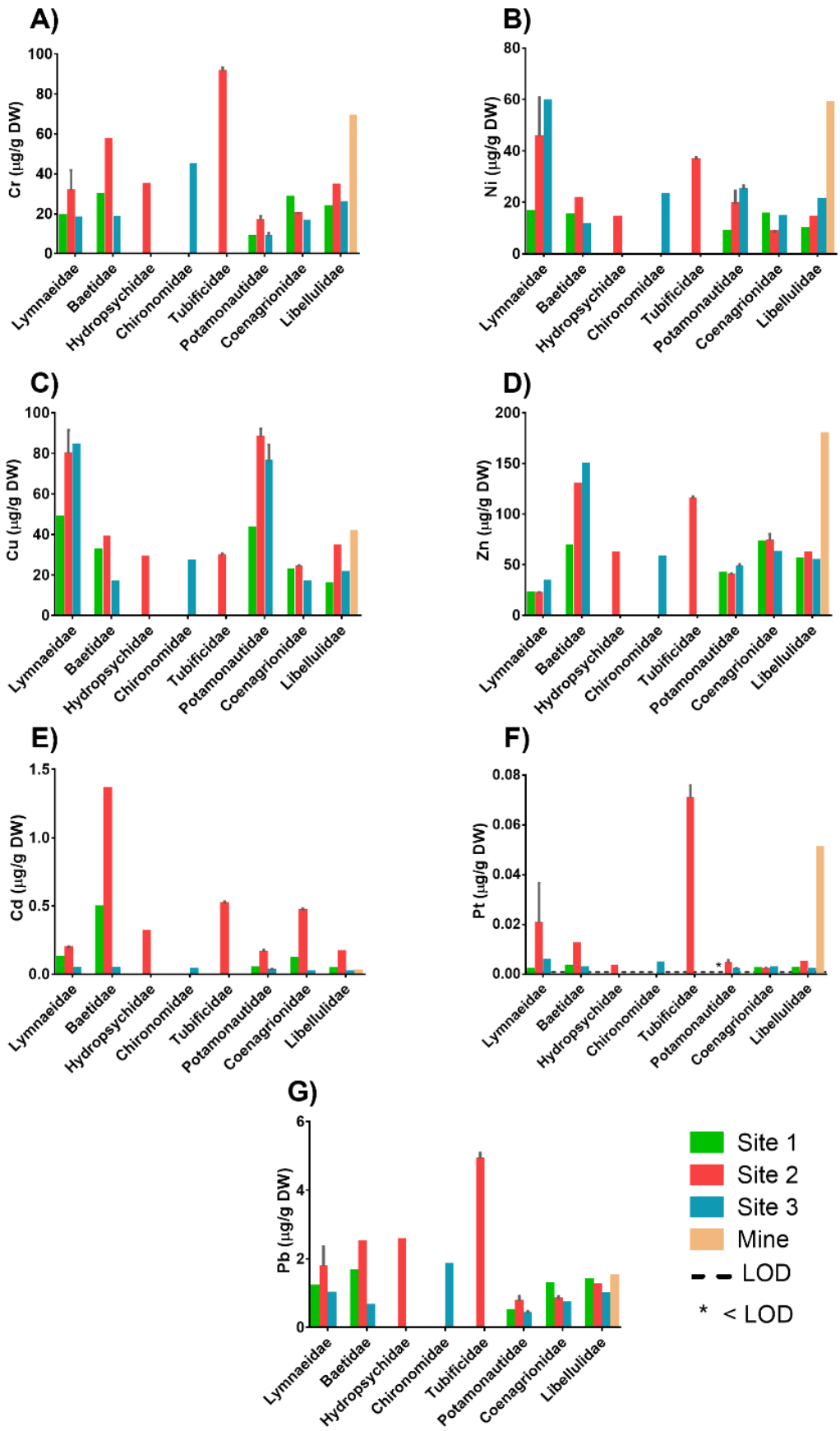

Figure 5.6: Mean concentrations (µg/g DW) of Cr (A), Ni (B), Cu (C), Zn (D), Cd (E), Pt (F) and Pb (G) with standard error of the mean in macroinvertebrate families (DW: dry weight) collected from selected sites in the Hex River (Site 1 – 3) and a mine settling pond (LOD: limit of detection).

5.3.5 Bioaccumulation in macroinvertebrate families

The BCF ranged from 23 (Pt in Potamonautidae) to 27 143 (Zn in Baetidae), whereas the BSAF ranged from 0.012 (Cr in Potamonautidae) to 11.17 (Zn in Baetidae) (Tables 5.3 and 5.4). The mean BCF between metals and invertebrate families decreased in the order Zn > Cu > Ni > Cd > Pb > Pt, while the order of mean BSAF was Zn > Cu > Cd > Ni > Pt > Pb > Cr. Of all the invertebrate families collected within the Hex River, the families with the highest BCF and BSAF were Lymnaeidae for Ni and Cu, and Baetidae for Zn and Cd, while Chironomidae had the highest for Cr and Pb, and Tubificidae for Cr, Pt, and Pb. In contrast, when considering only the families collected at all the Hex River sites, Lymnaeidae had the highest BCF and BSAF for Ni, Cu, and Pt, while Baetidae had the highest for Cr, Zn, Cd and Pb. Comparing the sites, the highest BCF continuously occurred in the invertebrates at Site 1 for Ni, Site 2 for Cd and Pt, and Site 3 for Cu, Zn, and Pb, while the highest BSAF continuously occurred at Site 1 for Cr, Zn, Cd, Pt and Pb, and Site 3 for Ni and Cu. The BCF differed between sites. The BCF decreased in the order Ni > Zn > Cu > Cd > Pb > Pt (Site 1), Zn > Cu > Cd > Ni > Pb > Pt (Site 2) and Zn > Cu > Pb > Ni > Cd > Pt (Site 3). On the other hand, the BSAF for Sites 1 and 2 showed the same order (Zn > Cu > Cd > Ni > Pt > Pb > Cr), while at Site 3 the BSAF decreased in the order Zn > Cu > Ni > Cd > Pt > Pb > Cr.

Table 5.3: Bioconcentration factors (BCF) calculated as the ratio of the metal concentrations in macroinvertebrate families and metal concentrations in water collected from selected sites in the Hex River (Site 1 – 3) and a mine settling pond (Mine).

Metal	Site	Macroinvertebrate families							
		Lymnaeidae	Baetidae	Hydropsychidae	Chironomidae	Tubificidae	Potamonautidae	Coenagrionidae	Libellulidae
Ni	Site 1	9 031	8 239	-	-	-	4 761	8 446	5 323
	Site 2	5 387	2 541	1 695	-	4 315	2 288	1 008	1 681
	Site 3	1 979	377	-	765	-	830	482	698
	Mine	-	-	-	-	-	-	-	1 241
Cu	Site 1	2 285	1 533	-	-	-	2 038	1 060	748
	Site 2	7 304	3 535	2 639	-	2 705	8 052	2 221	3 149
	Site 3	16 435	3 234	-	5 271	-	14 845	3 283	4 189
	Mine	-	-	-	-	-	-	-	6 330
Zn	Site 1	1 079	3 326	-	-	-	2 035	3 523	2 696
	Site 2	1 774	10 421	4 980	-	9 268	3 208	5 948	4 969
	Site 3	6 176	27 143	-	10 529	-	8 816	11 380	9 905
	Mine	-	-	-	-	-	-	-	15 629
Cd	Site 1	872	3 522	-	-	-	333	820	294
	Site 2	1 275	8 927	2 073	-	3 389	1 065	3 062	1 081
	Site 3	302	303	-	251	-	224	119	129
	Mine	-	-	-	-	-	-	-	155
Pt	Site 1	89	155	-	-	-	23	108	117
	Site 2	283	170	45	-	972	61	31	66
	Site 3	99	47	-	82	-	36	47	36
	Mine	-	-	-	-	-	-	-	108
Pb	Site 1	565	777	-	-	-	229	597	653
	Site 2	1 209	1 702	1 742	-	3 338	519	563	844
	Site 3	2 011	1 301	-	3 692	-	814	1 435	1 963
	Mine	-	-	-	-	-	-	-	2 052

Table 5.4: Biota-sediment accumulation factors (BSAF) calculated as the ratio of the metal concentrations in macroinvertebrate families and metal concentrations in sediment collected from selected sites in the Hex River (Site 1 – 3) and a mine settling pond (Mine).

Metal	Site	Macroinvertebrate families							
		Lymnaeidae	Baetidae	Hydropsychidae	Chironomidae	Tubificidae	Potamonautidae	Coenagrionidae	Libellulidae
Cr	Site 1	0.05	0.07	-	-	-	0.02	0.07	0.06
	Site 2	0.03	0.05	0.03	-	0.07	0.01	0.02	0.03
	Site 3	0.02	0.02	-	0.06	-	0.01	0.02	0.03
	Mine	-	-	-	-	-	-	-	0.04
Ni	Site 1	0.88	0.81	-	-	-	0.47	0.83	0.52
	Site 2	0.97	0.46	0.31	-	0.78	0.41	0.18	0.30
	Site 3	2.27	0.43	-	0.88	-	0.95	0.55	0.80
	Mine	-	-	-	-	-	-	-	0.82
Cu	Site 1	4.34	2.91	-	-	-	3.87	2.02	1.42
	Site 2	3.82	1.85	1.38	-	1.42	4.21	1.16	1.65
	Site 3	8.81	1.73	-	2.83	-	7.96	1.76	2.25
	Mine	-	-	-	-	-	-	-	1.50
Zn	Site 1	3.38	10.42	-	-	-	6.37	11.04	8.44
	Site 2	1.48	8.71	4.16	-	7.75	2.68	4.97	4.15
	Site 3	2.54	11.17	-	4.33	-	3.63	4.68	4.07
	Mine	-	-	-	-	-	-	-	1.25
Cd	Site 1	1.73	6.99	-	-	-	0.66	1.63	0.58
	Site 2	0.62	4.32	1.00	-	1.64	0.52	1.48	0.52
	Site 3	0.23	0.23	-	0.19	-	0.17	0.09	0.10
	Mine	-	-	-	-	-	-	-	0.15
Pt	Site 1	0.18	0.30	-	-	-	0.05	0.21	0.23
	Site 2	0.14	0.09	0.02	-	0.50	0.03	0.02	0.03
	Site 3	0.17	0.08	-	0.14	-	0.06	0.08	0.06
	Mine	-	-	-	-	-	-	-	0.10
Pb	Site 1	0.12	0.17	-	-	-	0.05	0.13	0.14
	Site 2	0.05	0.08	0.08	-	0.15	0.02	0.03	0.04
	Site 3	0.09	0.06	-	0.16	-	0.04	0.06	0.09
	Mine	-	-	-	-	-	-	-	0.17

5.4 Discussion

5.4.1 Key metals for mining, by-products, and waste

Platinum mining in South Africa provide efficient supplies to the world's demand for Pt (Johnson-Matthey, 2018), but are a major source of metal contamination within aquatic ecosystems associated with these mining regions (Rauch and Fatoki, 2013). The key metals for mining in the Rustenburg area consist primarily of PGE (especially Pt) and Cr, while Ni and Cu are recovered as by-products from the mined ores. In the ores of the Bushveld Igneous Complex, the PGE occur naturally in high concentrations, while the concentrations of Cu, Cr, and Ni were found to be 12-, 22- and 22-fold higher, respectively, than in the normal upper earth crust (Barnes and Maier, 2002). Although these metals are naturally present in the environment, the intensive mining activities (Site 2 and mine settling pond) within this region can increase the concentrations within the aquatic ecosystem. Accordingly, in the sediment samples of the present study, the concentrations of both, Cr and Pt, were significantly higher for the settling pond, as well as for the highly impacted site (Site 2) than for the reference site (Site 1). The concentrations of Ni, Cu, Cd, and Pb showed no significant differences between the sites. This clearly confirms the entry of mining associated Pt and Cr into the river ecosystem.

When comparing the data of the present study with results of previous studies in the Hex River near Rustenburg, the concentrations of Pt in the water of the highly impacted site (Site 2) and the mine settling pond were four-fold and 28-fold higher than the concentrations reported by Somerset *et al*. (2015) (see Appendix D, Table D3). The concentrations of Pt in the sediment at Site 2 were four-fold higher than the concentrations recorded by Almécija *et al*. (2017). Rauch and Fatoki (2013) reported concentrations of Pt of up to 0.653 µg/g in topsoil collected near a Pt smelter within the Hex River catchment, indicating that Pt can also be transported via air.

Downstream from the reference in the Hex River, concentrations of Cd and Pt in the water increased at Site 2 and decreased at Site 3. These metal concentrations can be attributed to the intensive mining activities, but also to industrial and urban activities from the city of Rustenburg. The decrease in these metal concentrations can be due to a dilution effect as the wastewater treatment plants are located between Sites 2 and 3, and add a large volume of water (31.5 ML/day; DWA, 2011) to the river system. Furthermore, the increase of the concentrations of Ni from Site 2 to Site 3 can be attributed to wastewater treatment effluents, as domestic wastewater effluents are considered a major source of Ni together with industrial effluent (Cempel and Nikel, 2006). On the other hand, concentrations of Cu, Zn, and Pb in the water were the highest at Site 1 and decreased further downstream, which indicates that these metals are geological in origin.

The concentrations of Cr, Ni, Zn, Cd, and Pt in the sediments were higher at Site 2 than Sites 1 and 3. The concentrations of Cu and Pb followed the same trend but decreased at Site 3 to almost the same concentrations as at Site 1. This increase in all metal concentrations at Site 2 indicates a point source of pollution, being mainly mining activities, whereas downstream at Site 3, the pollution source is remediated. This decrease in metal concentrations downstream from Site 2 can possibly be ascribed to the metals that precipitate or adsorb to organic matter (Blais *et al*., 2008) and decrease steadily downstream of the pollution source.

Sources of these metals into the environment include natural weathering of the geology, mining activities, industrial and domestic wastewater effluent, as well as urban runoff. According to Kelly *et al*. (2012), Ni sulphide deposits usually contain high concentrations of PGE and Cu. The high concentrations of Cu can be attributed to natural weathering or dissolution of Cu minerals, mining activities, sewage treatment plant effluent, as well as runoff from the surrounding settlements. Zinc is present in aquatic ecosystems due to natural weathering and erosion of rocks and ores, but Zn can also enter the system by means of industrial effluent, mining activities, as well as sewage treatment plant effluent (DWAF, 1996). According to DWAF (1996), Cd can usually be found in association with Cu, Pb, and Zn sulphide ores, that are present in the Bushveld Igneous Complex. Although Pb occurs naturally within the geology of this region, the concentrations of Pb within the sediment at Site 2 can be attributed to the intensive mining activities. Wittmann and Förstner (1976) reported that Pb carbonate was used in the PGE refining process. The high concentrations of Pb at Site 2 may, therefore, be historical Pb waste that is still present in the environment, while road runoff from heavy traffic (Rauch and Fatoki, 2009; Hwang *et al*., 2016) in the adjacent area, may also contribute to the Pb contamination.

Dissolved metal concentrations were compared to the Australian and New Zealand's guidelines for fresh water quality (ANZECC) (ANZECC, 2000), European Union environmental quality standards (EU-EQS) (EU-EQS, 2008), South Africa's target water quality range (TWQR) (DWAF, 1996), as well as the United States Environmental Protection Agency national recommended water quality criteria for aquatic life (USEPA) (USEPA, 2009). Concentrations of Ni in the water at Site 3 was the highest in the river sites and even higher at the mine settling pond and exceeded ANZECC of 11 µg/L, as well as EU-EQS of 20 µg/L. Concentrations of Cu in the water at all the sites exceeded ANZECC and TWQR of 1.4 µg/L for very hard water, as well as the chronic effect value (CEV) of 2.8 µg/L, while the concentrations of Cu at Site 1 exceeded the acute effect value (AEV) of 12 µg/L for aquatic ecosystems in South Africa. Although these concentrations exceeded these values, it indicates that the toxicity of Cu is potentially mitigated due to increased water hardness and dissolved oxygen (Table 1), as well as the presence of high concentrations of Zn (DWAF, 1996). Concentrations of Zn in the river water at all of the sampling sites exceeded the TWQR (2 µg/L) and the CEV (3.6 µg/L), while the concentrations of Zn at most of the sites, except Site 3, exceeded ANZECC (8 µg/L). However, as discussed before with regard to Cu, the toxicity of Zn in aquatic systems is similarly reduced with an increase in water hardness

(DWAF, 1996). Therefore, although the concentrations of Zn exceeded even the acute effect value, it may not be toxic to the biota within this river system due to the high water hardness. High concentrations of Pb at Site 1 in the water, exceeded the TWQR (1.2 µg/L) and can be attributed to the natural weathering of sulphide ores (DWAF, 1996).

5.4.2 Metal bioaccumulation in macroinvertebrates

The trends in metal concentrations of the macroinvertebrate families were similar to those seen for the sediment. Organisms collected at Site 2, generally had the highest metal concentrations that again decreased at Site 3, with the exception of Ni and Zn, that were higher in organisms collected at Site 3. Although the Libellulidae individual was the only macroinvertebrate found in the mine settling pond, its exceptionally high concentrations of Cr, Ni and Pt clearly demonstrate the biological availability of these metals emitted by the mine. Thus, to our knowledge, the present study demonstrated for the first time that Pt from mine activities is bioavailable to aquatic macroinvertebrates.

However, in contrast to the present work, a few field studies focused on the uptake of Pt from automobile traffic by macroinvertebrates in urban aquatic systems. Previous field studies completed on the accumulation of Pt by freshwater crustaceans and clams reported concentrations ranging from below detection limit to 0.0013 µg/g (Ruchter *et al.*, 2015). In addition, concentrations of Pt of up to 4.5 µg/g have been reported in the asselid *Asellus aquaticus* from urban river systems in Germany and Sweden (Rauch and Morrison, 1999; Moldovan *et al*., 2001; Haus *et al*., 2007). In particular, families associated with the sediment (Lymnaeidae, Baetidae, and Tubificidae) accumulated Pt at Site 2, with intensive Pt mining activities. At this site, an average concentration of Pt of 0.071 µg/g was recorded in Tubificidae, that was 55 times higher than reported values in freshwater clams in urban environments (Ruchter *et al.*, 2015). Furthermore, Haus *et al*. (2007) determined a BSAF of 0.11 for *A. aquaticus* from an urban pond in Germany, that is in the same range as for the different macroinvertebrate families of the present study (0.02 – 0.50). Even if different macroinvertebrate families were compared in terms of their metal accumulation patterns, it emerges that traffic-related Pt and Pt from mine activities are biologically available to a similar degree.

The high concentrations of Cu in Lymnaeidae and Potamonautidae at all the sites could be explained by the fact that these families have haemocyanin (Cu based metalloproteins) for oxygen transportation rather than haemoglobin (Fe based metalloproteins) (van Aardt, 1993; Brown, 1994). Even though these families have naturally increased concentrations of Cu, their higher concentrations at Sites 2 and 3 were most probably due to anthropogenic activities (mining and settlement) that are present in the catchments upstream of these sites and are reflected in the BCF and BSAF of both families. Somerset *et al*. (2015) found concentrations of Cu of 139.1 µg/g DW in Potamonautidae collected from the Hex River, while the highest concentration of Cu recorded in the present study is 88.2 µg/g DW.

The bioaccumulation factors clearly varied with the sampling site. According to John and Leventhal (1995), the bioavailability of metals in aquatic ecosystems can be influenced by several factors, such as total concentration and speciation of the metal, pH, total organic content, redox potential, volume and velocity of the water. For example, although Site 1 had the lowest concentrations of Ni, the families collected at this site had the highest BCF, while the same trend occurred with Zn, Cd, Pt and Pb in the BSAF. Thus, the metals at this site, even though occurring at lower concentrations in the matrices, might be more bioavailable to the macroinvertebrates, compared to the other sampling sites.

5.4.3 Macroinvertebrates as a biomonitoring tool

Macroinvertebrates can be used as an effective biomonitoring tool. In the present study, the families Lymnaeidae, Baetidae, Tubificidae, and Chironomidae accumulated the highest metal concentrations showing the highest BCF and BSAF. The same was found by Kemp *et al*. (2017) in the Marico River, South Africa. These families are associated with the sediment (Harrison, 2002; van Hoven and Day, 2002; Barber-James and Lugo-Ortiz, 2003), except for Lymnaeidae, that are generally associated with aquatic vegetation (Appleton, 2002). All of these families feed on detritus, by means of either ingesting sediment or scraping it from the substratum (Appleton, 2002; Harrison, 2002; van Hoven and Day, 2002; Barber-James and Lugo-Ortiz, 2003).

According to Bervoets *et al*. (2016), more tolerant families (*e.g.* Chironomidae and Tubificidae) should rather be regarded as suitable indicator organisms for ecological effects in aquatic ecosystems, than sensitive families (*e.g.* Ephemeroptera, Plecoptera and Trichoptera). Results of their field surveys between 1990 and 2010 in the Scheldt and Meuse River basin, showed that metal concentrations within the family Tubificidae were generally higher than in the family Chironomidae (Bervoets *et al*., 2016). This was also true of the present study. Bervoets *et al*. (2016) calculated the critical body burden of metals for Tubificidae and Chironomidae (see Appendix D, Table D4) based on accumulated concentrations that were related to macroinvertebrate community changes. In the present study, the concentrations of Cr and Ni of the Tubificidae and Chironomidae exceeded the calculated critical body burdens. Nevertheless, both families were found in high numbers at the sampling sites.

In the present study, the family Baetidae had the highest concentrations of Zn and Cd, as well as relatively high concentrations of Cr. Various authors regard this family as the most tolerant family towards metal pollution within the order Ephemeroptera (Awrahman *et al*., 2016; Bervoets *et al*., 2016). Metal concentrations within lower trophic families (*e.g.* scraper-grazers, collector-gatherers, and shredders) were generally higher as compared with predator families (Coenagrionidae and Libellulidae) (Liess *et al*., 2017). This is supported by the work of Goodyear and McNeill (1999), Gray (2002), Cardwell *et al*. (2013), Kemp *et al*. (2017), Liess *et al*. (2017) and Rodriquez *et al*. (2018) where these metals were reported to not biomagnify within the food

chain. According to Liess *et al*. (2017), possible reasons for lower metal concentrations in predator families can be that (i) predator families have the ability to regulate their internal metal concentrations better than lower trophic families, and (ii) predator families also have higher body mass to surface ratios that can account for lower metal uptake rates. Although these metals (Zn, Cd, Cr) are considered as potentially toxic to aquatic biota at the high concentrations found in the present study, we did not observe any obvious lethal effects. This is possibly due to protective mechanisms that aquatic invertebrates utilize to reduce metal exposure, such as elimination (excretion), detoxification (*e.g.* metallothioneins) and sequestration (Hoffman *et al*., 2002; Rainbow, 2002; Ahearn *et al*., 2004; Cardwell *et al*., 2013; Mugwar and Harbottle, 2016; Kemp *et al*., 2017). Kemp *et al*. (2017) found that metallothioneins were even present in macroinvertebrates from a region where metal contamination was not present, but naturally high metal concentrations from geological background, that indicates the inherent natural protective role of metallothioneins in macroinvertebrates.

5.5 Conclusion

The aims of the present study were to determine the concentrations of metals in water, sediment and aquatic macroinvertebrates associated with Pt mining in the Hex River, and based on the bioaccumulation in the biota, the biological availability of metals was evaluated at river sites affected by mining activities. From the present results, it can be concluded that Pt mining activities are a major source of Cr and Pt contamination within the Hex River system. However, urban and informal settlements, as well as the geological background also contribute to the metal concentrations found in this system. These results provide valuable information and bridge the gap regarding the concentrations associated with Pt mining and processing activities, as well as the concentrations within biota occurring within these systems. The results also demonstrate the bioaccumulation of these metals in macroinvertebrates in relation to the metal concentrations in water and sediment, respectively, at specific sites. Although Pt mining in South Africa provides efficient supplies to the world's demand for Pt, it is a major source of metal contamination within aquatic ecosystems located in these mining regions. These aquatic ecosystems can be monitored by using macroinvertebrates as a biomonitoring tool, especially the families Lymnaeidae, Baetidae, Tubificidae, as well as Chironomidae. Although high metal concentrations were recorded within selected families, there were no obvious detrimental effects on them, leading to the assumption that they possess strategies to cope with these concentrations.

CHAPTER 6: THE EFFECTS OF METAL POLLUTION ON FISH HEALTH AND HUMAN HEALTH RISKS ASSOCIATED WITH THE CONSUMPTION OF FISH

6.1 Introduction

The use of fish as biomonitoring indicator to assess impacts on and ecological state of aquatic ecosystems have been proven effective in the past and is still the preferred method to date (Barbour *et al*., 1999; Moiseenko *et al*., 2008; Wepener *et al*., 2011; Lima *et al*., 2017). Fish present information on long term exposure to pollutants when compared to invertebrates. Fish may also biomagnify pollutants such as metals, since they are generally considered to be at the top of aquatic food webs (Zeitoun and Mehana, 2014). Metals are accumulated predominantly in the liver as the metabolic organ which stores the metals and detoxifies it by means of metallothioneins (Bervoets *et al*., 2005; Zeitoun and Mehana, 2014). High metal concentrations in fish can have negative health effects and can cause deferred embryonic development, adversely affect several metabolic pathways, stimulate the production of reactive oxygen species (ROS). This can cause damage to the fish, reduce the growth and condition of the fish and malformation in adult fish, or death in sensitive individuals (Bervoets *et al*., 2005; Singh and Kalamdhad, 2011; Jooste *et al*., 2014; Authman *et al*., 2015).

According to Adams *et al*. (2000), pollutants such as metals are consequentially incorporated into aquatic food webs, which can potentially biomagnify in the food web posing risks to organisms that consume them (*e.g.* predatory fish species, piscivore birds, mammals, and humans). Fish are an important food source for people across the world (Sayer and Cassman, 2013), with low-income groups particularly consuming fish due to its availability from local rivers and impoundments, and is an inexpensive option compared to other protein sources (Addo-Bediako *et al*., 2014). In South Africa, numerous rural communities and low-income groups depend on fish that are harvested by subsistence fishers from local rivers and its impoundments as a nutritional protein source (Ellender *et al*., 2009; McCafferty *et al*., 2012; Coetzee *et al*., 2015). However, communities that consume contaminated fish on a regular basis are susceptible to genotoxic, non-carcinogenic and carcinogenic health risks from chronic exposure to toxic pollutants, especially metals (du Preez *et al*., 2003; Castro-González and Méndez-Armenta, 2008; Jooste *et al*., 2014)

Local subsistence fishers from rural areas across South Africa (Rouhani and Britz, 2011), and more specifically the subsistence fishers from the study area selected for the present study, generally target three fish species. These species are the non-native common carp, *Cyprinus carpio* Linnaeus, 1758, indigenous sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), and indigenous Mozambique tilapia, *Oreochromis mossambicus* (Peters, 1852). These species also represent various functional feeding groups, *i.e. C. carpio* omnivore – grub in

sediments, *C. gariepinus* omnivore – preys, scavenge and grubs and *O. mossambicus* algaevore and detritivore (Skelton, 2001). These three species were selected as indicator species for the present study due to their high abundance in South Africa and in the study area, resilience, general association with the sediments, and value as food source to people (Skelton, 2001; Addo-Bediako *et al*., 2014; Musa *et al*., 2017; Pheiffer *et al*., 2018).

In the present study, the metal bioaccumulation and fish health assessment of the three fish species mentioned above from two impoundments in the Hex River system were determined. It was hypothesised that fishes collected from the impacted impoundment (Bospoort Dam) downstream of the Pt mining and urban activities will accumulate higher metal concentrations and have impaired health when compared to fishes from the reference impoundment (Olifantsnek Dam) upstream of all the mining activities. It was further hypothesised that fish from the impacted system will consequently pose a higher human health risk to people that consume these fish. The aims of this chapter of the study were, therefore, to determine the metal accumulation in three important fish species from impoundments in the Hex River system and to assess the potential human health risk posed by consuming these fish species.

6.2 Materials and Methods

6.2.1 Field sampling

Surveys were completed in May and November 2017, as well as November 2018 in Olifantsnek Dam and Bospoort Dam. The necessary permits (HO 09/03/17-125 NW; HO 20/02/18-057 NW, North West Province, Department of Rural, Environmental and Agricultural Development) and ethical clearance (NWU-00282-17-A5) were obtained prior to sampling. Fish were collected using multifilament gill nets (45 m x 3 m, consisting of five 9 m x 3 m sections with stretch mesh sizes of 45, 60, 75, 100 and 145 mm), that were deployed shortly after dawn for nine hours and checked every three hours. Other sampling equipment used, included fyke nets (5 m total length, a height of 50 cm (D-ring) with a mesh size of 15 mm) and seine nets (25 m x 1 m x 25 mm mesh) (as described in Erasmus *et al*., 2019). The specimens were identified using the key by Skelton (2001) and were subsequently humanely killed by percussive stunning followed by pithing (SOP NWU-00267-17-S5). The fish health assessment index (FHAI), condition factor (CF), as well as the organosomatic indices (OSI) following Adams *et al*. (1993) and Heath *et al*. (2004) were calculated. Muscle and liver samples were collected from each individual for metal analysis and stored in pre-cleaned polyethylene containers and frozen at -20 °C until further analysis.

6.2.2 Water and sediment quality

Water and sediment samples were collected at the two impoundments during the three surveys in accordance with the methods described in detail in Chapter 2. *In situ* abiotic parameters were measured, and nutrient and dissolved metal concentrations were determined in the water samples as described in Chapter 2 and the results obtained see Chapter 3, Tables 3.7 and 3.8. Sediment quality variables included particle size distribution and organic content, as well as total metal concentrations in the sediment and were determined using the methods described in Chapter 2 (see Chapter 3, Tables 3.7 and 3.8 for the results).

6.2.3 Fish health assessment index, condition factor, and organosomatic indices

The fish health assessment index (FHAI) developed by Adams *et al*. (1993) is a qualitative index, which evaluates how stressors influence fish health. A numerical value (0; 10; 20; 30) is given to fish tissues and organs that are examined and are dependent on the degree of stressor-induced anomalies (Watson *et al*. 2012), while the total of these values will represent the index value. An increase in this index value correlates with a decrease in water quality, with concomitant increased stress in the fish (Watson *et al*. 2012). Externally, the eyes, skin, fins, gills, and opercula were inspected for abnormalities. Internally, the bile, mesenteric fat, liver, spleen, hindgut, kidney and visceral parasites were assessed. A blood sample was collected to assess the haematocrit of the fish. The condition factor (CF), as well as organosomatic indices (OSI), can be used to assess the general health of the fish, both on an individual level and population level (Hoque *et al*., 1998; Dekić *et al*., 2016). The CF represents a degree of the individual fish's response to environmental influences (*i.e.* quantity and quality of nutrients, pollutants or toxins and the presence of pathogens), and can change the mass of the individual and its organs when compared to individuals from reference conditions (Dekić *et al*., 2016). The OSI is the ratio of organ weight over the body weight and can be directly linked to some environmental changes (Ronald and Bruce, 1990). Environmental factors have the potential to increase or decrease the organ size with the size and weight of the liver, spleen, and gonads indicating the general health status of the fish (Dekić *et al*., 2016).

The condition factor (CF) was calculated by using the formula:

$$CF = W \times 100/L^3$$

where W is the weight of the fish (g) and L is the standard length of the fish (cm). The liver, spleen, and gonads were carefully removed and weighed. The hepatosomatic index (HSI), spleen somatic index (SSI) and gonadosomatic index (GSI) were calculated as follows:

$$OSI = [\text{weight of organ (g)} / \text{weight of fish (g)}] \times 100$$

6.2.4 Sample preparation for metal analysis

Muscle and liver samples for element analysis were freeze-dried (FreeZone® 6, Labconco). Subsequently, approximately 0.2 g (dry weight) of the muscle and liver samples were digested in a mixture of 3 mL HNO_3 (65%, supra pure quality, Merck) and 1 mL HCl (37%, supra pure quality, Merck) adapted from Djingova *et al*. (2003). All of the samples were digested in a Mars 6 microwave digestion system (CEM Corporation, Kamp-Lintfort), with 20 mL TFM® vessels (MarsXpress, CEM). Following digestion, the solution was transferred to a 5 mL volumetric flask and brought to volume with MilliQ® water and was stored at room temperature until element analysis.

6.2.5 Metal measurement

In all samples, the concentrations of Cr, Ni, Cu, Zn, As, Cd, Pt, and Pb were determined using quadrupole inductively coupled plasma-mass spectroscopy (ICP-MS) (PerkinElmer, Elan 6000) equipped with an auto-sampler system (PerkinElmer, AS-90). For the quantification, the following mass lines were evaluated: ^{52}Cr, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{111}Cd, ^{194}Pt, ^{206}Pb. Due to several interferences for the mass line of Cr on the ICP-MS, Cr was analysed on an atomic absorption spectrometer (AAS) (PerkinElmer, AAnalyst 600) equipped with Zeeman-effect background correction, following the method of Nawrocka and Szkoda (2012).

Prior to measurements, the digested solutions of the fish samples were diluted 1:5 with 1% HNO_3 (supra pure quality, Merck). Yttrium and Tm (both Certipur®, Merck) were added as an internal standard at a concentration of 10 µg/L. The ICP-MS operated at 1000 W plasma power, 14 L/min plasma gas flow, 0.95 L/min nebuliser gas flow and a sample flow-rate of 1 mL/min regulated by a peristaltic pump. Calibration of the ICP-MS was performed using a series of 11 dilutions of a multi-element standard solution. With this calibration, the concentrations of the analytes were calculated in the samples using corresponding regression lines with a correlation factor of ≥ 0.999.

Detection limits for the fish samples were determined as three times the standard deviation of the blank measurements and were found to be 0.19 µg/g for Cr, 0.08 µg/g for Ni, 0.24 µg/g for Cu, 1.59 µg/g for Zn, 0.36 µg/g for As, 0.013 µg/g for Cd, 0.000094 µg/g for Pt and 0.29 µg/g for Pb. Quality control of the ICP-MS analysis was performed using standard reference materials for most of the elements (see Table 6.1). Reference materials such as IAEA-407 (fish homogenate reference material; International Atomic Energy Agency, Monaco), DORM-4 (fish protein certified reference material, National Research Council, Canada) and DOLT-3 (dogfish liver certified reference material, National Research Council, Canada) were used to validate the analytical procedure of biota samples. All elements showed recovery rates within the 20% deviation range (Table 6.1). As Pt was not certified in the biological reference materials, a spiking experiment was performed by adding Pt to the sample matrix before and after the digestion in a concentration range of 0.1 to 100 µg/L. The recovery rates were evaluated from the ratios of the slopes calculated for the Pt addition series before and after digestion. All recovery rates were within a

10% deviation range. In order to assess the potential mass interferences during the detection of Pt, after the measurement series a matrix-adapted addition with Hf was performed as described by Ek *et al*. (2004). However, obtained interferences rates were always less than 2% and therefore no Hf-correction was applied.

Table 6.1: Recovery rates (%) of the elements of interest obtained for different certified reference materials.

Elements	Recovery rates (%)		
	IAEA-407	DORM-4	DOLT-3
As	93	97	87
Cd	101	104	96
Cr	84	98	119
Cu	97	102	110
Ni	90	98	93
Pb	108	91	85
Pt	n.c.	n.c.	n.c.
Zn	86	91	96

n.c. – not certified

6.2.6 Human health risk assessment

Several international organisations have established a number of standards and instructions on the estimation of potential risks to human health from fish that are contaminated with environmental pollutants (USEPA, 2016). The risk of chronic non-cancer health effects from oral exposure was calculated using the average daily dose (ADD) and expressed in mg/kg body mass per day:

$$ADD = \frac{(average\ metal\ concentration\ in\ fish\ muscle\ (WW) \times (mass\ of\ portion)}{(adult\ body\ mass) \times (no.of\ days\ between\ fish\ meals)}$$

where the average metal concentration is in mg/kg wet weight, the mass of portion is 150 g, adult body mass is 60 kg and no. of days between fish meals as 3 days (Addo-Bediako *et al*., 2014).

Risk assessments evaluating non-carcinogenic toxic effects of pollutants use reference doses (RfDs) as thresholds above which adverse health impacts could be expected. A hazard quotient (HQ) was calculated to estimate the risk to human health:

$$HQ = \frac{ADD}{RfD}$$

where HQ < 1 suggests unlikely adverse health effects and HQ > 1 suggests a high probability of adverse health effects. RfD levels used in the present study were published by the USEPA (USEPA, 2000).

6.2.7 Data analysis

The statistical significance of the spatial and temporal variation of metal concentrations in fishes was determined at $p < 0.05$. Normality and homogeneity of variance were tested using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test in GraphPad Prism v7. When the data were parametric, 2-way ANOVA and Tukey's multiple comparison test and Sidak multiple comparison tests were performed. In the case of non-parametric data, Kruskal-Wallis tests with Dunn's multiple comparison tests were performed, to test for significant differences between species, sites, and surveys. A Principle Component Analysis (PCA) (Canoco v5.12) was created to assess the temporal and spatial patterns associated with the water and sediment quality, while a Redundancy Analysis (RDA) was created to assess the bioaccumulation of metals in fish tissues and environmental metal concentrations. All of the data used for PCA and RDA analyses were log transformed $x = \log(y + 1)$, as well as centred and standardised. Bioconcentration factors ($BCF = c_{fish}/ c_{water}$) and biota-sediment accumulation factors ($BSAF = c_{fish}/ c_{sediment}$) were calculated as the ratio of the average metal concentration in a fish species per quantified metal concentration of the filtered water and sediment, respectively.

6.3 Results

6.3.1 Water and sediment quality

The water and sediment quality variables collected from the two impoundments are presented in Table 6.2. The water and sediment quality data were reported and discussed in depth previously in Chapter 3. An integrated multivariate assessment of the data was done for the purpose of this chapter.

Table 6.2: Mean water and sediment quality variables analysed at Olifantsnek Dam (OL) and Bospoort Dam (BS) in the Hex River catchment during three surveys (05/2017; 11/2017; 11/2018).

	Code	OL1	OL2	OL3	BS1	BS2	BS3
Water quality variables							
Ammonium (mg/L)	NH4	0.07	0.11	0.02	0.14	0.09	0.13
Chemical oxygen demand (mg/L)	COD	12	16	13	10	38	21
Chloride (mg/L)	Cl	10	9	12	95	164	240
Dissolved oxygen (mg/L)	O2	5.6	7.1	10.5	3.8	4.0	8.1
Electrical conductivity (µS/cm)	EC	162	183	189	808	1180	1552
Nitrate (mg/L)	NO3	4.2	1.4	0.3	5.0	1.3	0.4
Nitrite (mg/L)	NO2	0.012	0.010	0.007	0.062	0.063	0.004
pH	pH	7.4	8.8	7.9	8.7	7.8	7.2
Phosphate (mg/L)	PO4	0.09	0.18	0.04	0.09	0.19	0.20
Sulphate (mg/L)	SO4	30	42	86	189	196	233
Temperature (°C)	TEMP	21.4	25.6	23.3	21.6	23.9	23.1
Total hardness (mg/L)	TH	51	44	61	127	130	201
Turbidity (FAU)	TURB	22	48	38	12	21	9
Arsenic (µg/L)	As	2.3	2.2	1.1	1.9	2.5	2.6
Cadmium (µg/L)	Cd	0.052	0.711	0.026	0.048	0.529	0.022
Chromium (µg/L)	Cr	0.25	0.71	0.51	0.19	0.33	0.29
Copper (µg/L)	Cu	5.6	20.5	7.0	13.0	8.9	4.8
Lead (µg/L)	Pb	0.62	20.97	0.35	1.01	4.11	0.23
Nickel (µg/L)	Ni	8.0	8.7	6.1	53.4	41.9	33.5
Platinum (µg/L)	Pt	0.0285	0.0085	0.0059	0.0503	0.0443	0.0254
Zinc (µg/L)	Zn	7.7	41.7	23.3	9.5	17.6	8.0
Sediment quality variables							
Organic content	OC	7.3	6.2	2.0	5.4	4.6	2.3
Gravel (%)	GR	1.0	1.7	3.3	6.7	9.9	1.1
Very coarse sand (%)	VCS	1.2	3.6	2.5	8.2	7.3	1.7
Coarse sand (%)	CS	23.7	20.2	13.1	25.9	20.9	11.0
Medium sand (%)	MS	20.5	25.1	16.7	33.2	33.8	27.5
Fine sand (%)	FS	40.0	43.7	56.4	23.2	25.1	55.0
Mud (%)	M	13.6	5.8	8.0	2.9	3.0	3.7
Arsenic (µg/g DW)	As	10.5	2.2	15.5	7.5	7.1	17.7
Cadmium (µg/g DW)	Cd	0.034	0.238	0.040	0.012	0.098	0.030
Chromium (µg/g DW)	Cr	380.3	389.6	432.1	34.2	176.0	68.1
Copper (µg/g DW)	Cu	12.1	20.2	22.5	2.2	13.0	10.1
Lead (µg/g DW)	Pb	6.4	9.0	6.5	2.0	4.9	2.6
Nickel (µg/g DW)	Ni	39.5	66.4	56.6	20.9	81.2	67.5
Platinum (µg/g DW)	Pt	0.0205	0.0152	0.0091	0.0086	0.0158	0.0072
Zinc (µg/g DW)	Zn	17.6	24.7	42.1	5.5	16.2	22.9

A PCA biplot (Fig. 6.1) was created with all of the water and sediment quality variables and presents an integrated temporal and spatial ordination of the environmental variables. The biplot explains 59.8% of the variance within the data on the two axes, which indicates that other factors that were not tested explain the remaining 40.2% of the variation between these two sites. Distinct temporal differences in the water and sediment quality were evident, where the May and November 2017 surveys at both impoundments displayed more similar quality characteristics than the November 2018 survey. Clear spatial differences were also evident, where Olifantsnek Dam was characterised by finer substrate composition and higher metal concentrations in the sediment, with increased dissolved oxygen, turbidity, temperature and metals in the water (Cd, Cr, Cu, Zn, Pb). Bospoort Dam, on the other hand, was characterised by

increased nutrient and salt concentrations, gravel and coarse sand, as well as increased metal concentrations in the water (As, Ni, Pt).

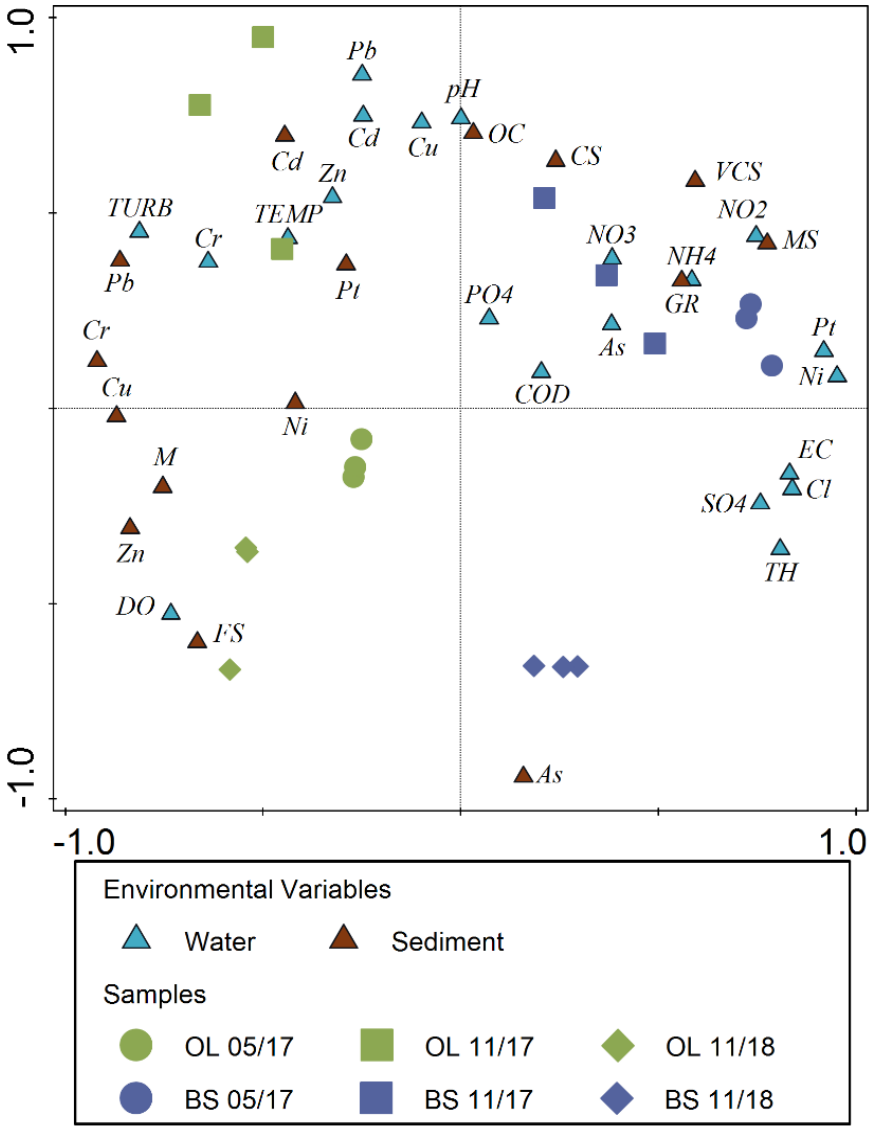

Figure 6.1: A PCA biplot of the water and sediment quality variables measured at Olifantsnek Dam (OL) and Bospoort Dam (BS) during three surveys (05/2017; 11/2017; 11/2018). The biplot describes 59.8% of the variation with 37.5% on the first axis and 22.3% on the second axis.

6.3.2 Fish health assessment index, condition factor, and organosomatic indices

Fishes collected in Bospoort Dam were significantly larger than the specimens collected from Olifantsnek Dam (*C. carpio* p = 0.023; *C. gariepinus* p < 0.0001), with the exception of specimens of *O. mossambicus*, which were larger in Olifantsnek Dam compared to Bospoort Dam (Table 6.3).

Table 6.3: The number of individuals (n), as well as the mean and standard deviation of the weight and the total length (TL) of the three fish species (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*), collected during three surveys from Olifantsnek Dam and Bospoort Dam.

Date	Species	Olifantsnek Dam			Bospoort Dam		
		n	Weight (g)	TL (mm)	n	Weight (g)	TL (mm)
05/2017	*C. carpio*	-	-	-	11	1 714.0 ± 931.7	448.2 ± 119.0
	C. gariepinus	2	301.8 ± 336.8	330.0 ± 162.6	2	5 750.0 ± 975.8	960.0 ± 70.7
	O. mossambicus	23	34.7 ± 7.4	127.1 ± 9.9	11	27.2 ± 8.9	116.0 ± 11.1
11/2017	*C. carpio*	23	256.3 ± 247.2	227.2 ± 86.0	10	700.0 ± 555.2	333.5 ± 75.3
	C. gariepinus	10	1 406 ± 395.8	593.0 ± 62.0	1	1 900	630
	O. mossambicus	8	479.4 ± 234.5	298.8 ± 52.5	-	-	-
11/2018	*C. carpio*	10	990.8 ± 722.3	381.5 ± 123.1	14	1 018.9 ± 2 242.7	306.4 ± 166.1
	C. gariepinus	8	1 029 ± 626.3	511.9 ± 176.2	13	3 019.2 ± 1 488.1	697.7 ± 112.6
	O. mossambicus	1	168	215	9	80.1 ± 39.9	165.9 ± 29.8

- no fish of the species were collected during the survey.

From the FHAI scores, the fish collected in Olifantsnek Dam were healthier (*C. carpio* – 14.5, *C. gariepinus* – 34.1, *O. mossambicus* – 5.3) than the fish collected from Bospoort Dam (*C. carpio* – 17.7, *C. gariepinus* – 60.3, *O. mossambicus* – 8.8), with a few exceptions such as *C. gariepinus* during the surveys of November 2017 and 2018 (Table 6.4). However, the CF of the fish was higher in all of the species collected in Bospoort Dam, while *O. mossambicus* were higher in Olifantsnek Dam during the November 2018 survey. There were no significant temporal differences in the CF at both impoundments and the only significant spatial difference (p = 0.0394) in CF was in *O. mossambicus*. The CF differed significantly (p < 0.0001) between all the species at each impoundment. The HSI, SSI, and GSI were mostly higher in the fish collected from Bospoort Dam, except for *C. gariepinus* and *O. mossambicus* (May 2017), *O. mossambicus* (May 2017) and *C. carpio* (November 2017) which were higher in Olifantsnek Dam, respectively (Table 6.4).

Table 6.4: The mean and standard deviation of the fish health assessment index (FHAI), condition factor (CF), hepatosomatic index (HSI), spleen somatic index (SSI) and gonadosomatic index (GSI) of the three fish species (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*) collected during three surveys from Olifantsnek Dam and Bospoort Dam.

Date	Species	Olifantsnek Dam					Bospoort Dam				
		FHAI	CF	HSI (%)	SSI (%)	GSI (%)	FHAI	CF	HSI (%)	SSI (%)	GSI (%)
05/2017	*C. carpio*	-	-	-	-	-	14.0 ± 19.0	2.46 ± 0.13	1.70 ± 0.42	0.14 ± 0.04	6.77 ± 3.77
	C. gariepinus	20.0 ± 28.3	0.92 ± 0.11	0.90 ± 0.14	0.10 ± 0.04	0.04 ± 0.02	120.0 ± 28.3	0.94 ± 0.07	0.79 ± 0.17	0.25 ± 0.16	0.66 ± 0.20
	O. mossambicus	6.0 ± 9.4	2.91 ± 0.19	1.50 ± 0.27	0.21 ± 0.04	0.09 ± 0.03	10.9 ± 15.8	3.43 ± 0.21	1.34 ± 0.28	0.18 ± 0.05	0.22 ± 0.02
11/2017	*C. carpio*	10.0 ± 3.0	2.50 ± 0.16	1.66 ± 0.26	0.14 ± 0.04	0.81 ± 0.71	14.0 ± 7.0	2.69 ± 0.23	2.58 ± 0.38	0.18 ± 0.04	0.69 ± 1.40
	C. gariepinus	46.0 ± 20.7	0.97 ± 0.11	0.60 ± 0.26	0.13 ± 0.04	0.92 ± 0.80	30.0	1.00	0.78	0.12	7.08
	O. mossambicus	10.0	2.86 ± 0.22	0.92 ± 0.23	0.08 ± 0.02	0.37 ± 0.34	-	-	-	-	-
11/2018	*C. carpio*	19.0 ± 14.5	2.33 ± 0.29	1.62 ± 0.24	0.10 ± 0.07	1.73 ± 1.80	25.0 ± 7.1	2.84 ± 0.62	2.07 ± 0.47	0.21 ± 0.08	2.46 ± 2.94
	C. gariepinus	36.3 ± 20.0	0.90 ± 0.07	0.90 ± 0.31	0.14 ± 0.07	2.62 ± 3.58	30.8 ± 15.1	1.18 ± 0.25	1.38 ± 0.67	0.19 ± 0.12	5.93 ± 7.02
	O. mossambicus	0	3.13	0.57	0.07	*	6.7 ± 4.5	2.85 ± 0.37	1.46 ± 0.34	0.12 ± 0.03	0.90 ± 0.96

- no fish of the species were collected during the survey; * no GSI value, due to immature juvenile.

6.3.3 Metal bioaccumulation in Cyprinus carpio, Clarias gariepinus, and Oreochromis mossambicus

Metal bioaccumulation in tissues (muscle and liver) of *C. carpio*, *C. gariepinus,* and *O. mossambicus* were determined between the two impoundments (see Appendix E, Table E1; Fig. 6.2). Significant differences between fish tissues, fish species within an impoundment and the same species between impoundments were evident. Significantly higher ($p = 0.0034$ and $p = 0.048$) concentrations of Cr in *C. carpio* and *C. gariepinus* muscle tissue at Bospoort Dam were detected, while concentrations of Cr in *O. mossambicus* were significantly higher ($p < 0.0001$) than the other two fish species at both impoundments (Fig. 6.2 A). For Cr, there was a significant influence of site ($F = 20.13$; $p < 0.0001$) and species ($F = 21.11$; $p < 0.0001$), while the interaction between the two factors was not significant ($p = 0.36$).

Concentrations of Ni in muscle and liver tissue of *O. mossambicus* were significantly higher ($p = 0.0043$ and $p < 0.0001$) at Bospoort Dam, as well as significantly higher ($p = 0.0398$ and $p < 0.0001$) than *C. carpio* and *C. gariepinus*. There was a significant influence of site ($F = 98.56$; $p < 0.0001$) and species ($F = 121.1$; $p < 0.0001$), as well as the interaction between the two factors ($F = 54.94$; $p < 0.0001$) for Ni. Significantly higher ($p < 0.0001$) concentrations of Cu in the liver of *O. mossambicus* and between species at Olifantsnek Dam were recorded, while significantly higher ($p < 0.0001$) concentrations of Zn in the liver of *C. carpio* were detected at Bospoort Dam and between species at both impoundments. For Cu and Zn, there were a significant influence of site ($F = 19.63$; $p < 0.0001$ and $F = 5.019$; $p = 0.0259$) and species

($F = 24.62$; $p < 0.0001$ and $F = 171.9$; $p < 0.0001$), as well as for the interaction between the two factors ($F = 13.93$; $p < 0.0001$ and $F = 6.142$; $p < 0.0001$), respectively.

The concentrations of As were significantly higher in *C. carpio* muscle ($p = 0.0042$), *C. gariepinus* muscle ($p < 0.0001$) and liver ($p = 0.0001$), as well as *O. mossambicus* liver ($p = 0.0162$) at Bospoort Dam, compared to Olifantsnek Dam. Significantly higher concentrations of As were recorded in the liver tissue of *O. mossambicus* at both impoundments ($p < 0.0001$), while the muscle of *O. mossambicus* was significantly higher ($p = 0.0253$) than *C. carpio* at Olifantsnek Dam. There was a significant influence of site ($F = 57.98$; $p < 0.0001$) and species ($F = 22.1$; $p < 0.0001$), as well as the interaction between the two factors ($F = 3.601$; $p = 0.0036$) for As. Significantly higher ($p = 0.0191$) concentrations of Cd in the liver of *C. gariepinus* at Olifantsnek Dam were recorded, while only a significant influence of species ($F = 15.97$; $p < 0.0001$) was found.

Concentrations of Pt were significantly higher in muscle and liver tissue of *C. carpio* ($p < 0.0001$) and *C. gariepinus* ($p = 0.0228$ and $p = 0.0067$) at Bospoort Dam, while concentrations of Pt in the muscle of *C. carpio* were significantly higher ($p = 0.0063$) than *O. mossambicus* at Bospoort Dam. For Pt, there was a significant influence of site ($F = 38.26$; $p < 0.0001$) and species ($F = 3.057$; $p = 0.0105$), while the interaction between the two factors was also significant ($F = 5.067$; $p = 0.0002$). The concentrations of Pb had no significant differences between tissues, fish species or impoundments.

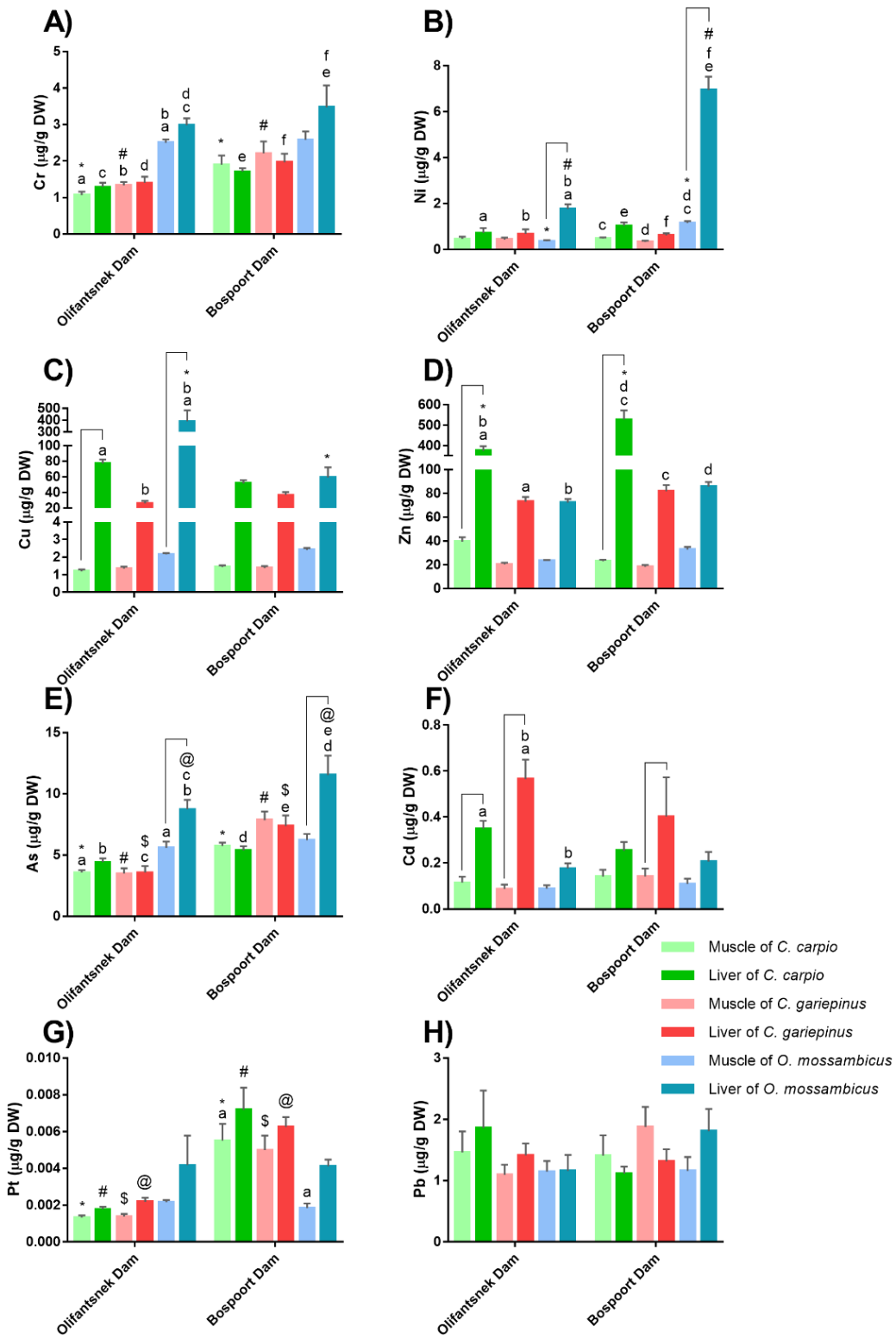

Figure 6.2: Mean concentrations (µg/g DW) of Cr (A), Ni (B), Cu (C), Zn (D), As (E), Cd (F), Pt (G) and Pb (H) with standard error of the mean in three fish species (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*) and two types of tissues (muscle and liver) (DW: dry weight) collected from two impoundments. Common symbol superscripts indicate significant differences between sites, while common alphabetical superscripts indicate significant differences between species within a site and brackets indicate significant differences between tissues within a species. See Table 6.3 for number (n) of specimens.

Strong positive correlations in bioaccumulation of metals in fish tissues, between the three fish species and impoundments, were evident (Fig. 6.3). The Mozambique tilapia, *O. mossambicus*, at both impoundments associated with higher concentrations of Cr, Ni, Cu, As and Pt in muscle and liver tissues, respectively, while *C. carpio* and *C. gariepinus* during the November 2017 and 2018 surveys at Bospoort Dam associated with higher concentrations of Cd, Pt, and Pb in muscle tissue. Metal accumulation in *C. carpio* and *C. gariepinus* from Olifantsnek Dam during all three surveys were more similar. The triplot indicated no, to very weak, correlations between the environmental concentrations and the subsequently accumulated tissue concentrations.

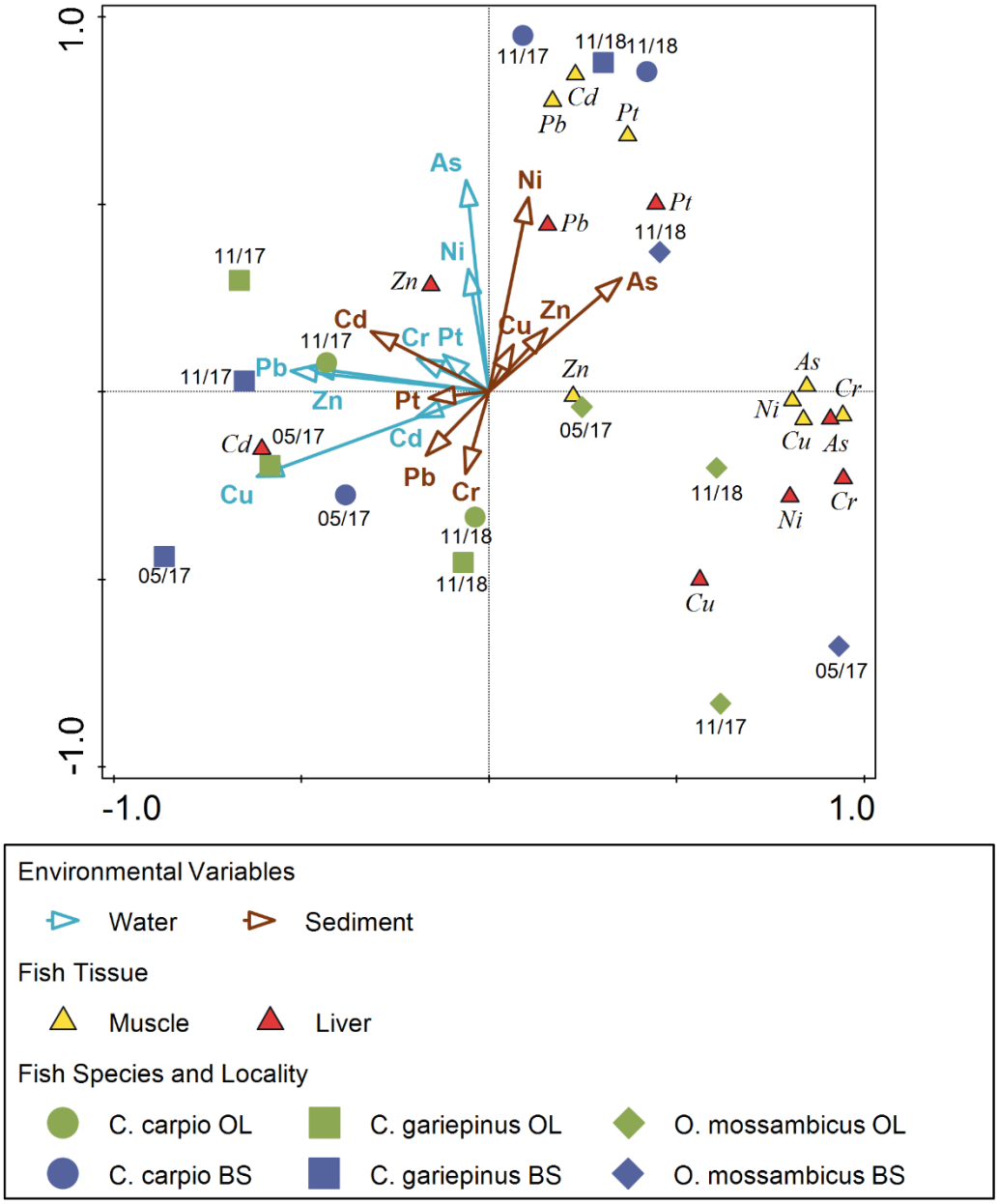

Figure 6.3: A RDA triplot of the metal concentrations in muscle and liver tissue of *Cyprinus carpio*, *Clarias gariepinus* and *Oreochromis mossambicus* collected from Olifantsnek Dam (OL) and Bospoort Dam (BS) during three surveys (05/2017; 11/2017; 11/2018). Water and sediment metal concentrations are overlaid. The triplot describes 57.9% of the variation with 40.8% on the first axis and 17.1% on the second axis.

6.3.4 Bioconcentration factor and biota-sediment accumulation factor in fish

The BCF_{muscle} ranged from 4.5 (Ni in *C. gariepinus*) to 11 437 (Cr in *O. mossambicus*) and the BCF_{liver} varied between 13 (Ni in *C. gariepinus*) and 52 428 (Zn in *C. carpio*), while the $BSAF_{muscle}$ ranged from 0.002 (Ni in *C. gariepinus*) to 5 (Cd in *O. mossambicus*) and the $BSAF_{liver}$ varied between 0.003 (Cr in *C. gariepinus*) and 61 (Zn in *C. carpio*) (Tables 6.5 and 6.6). The mean BCF for *C. carpio* decreased in the order of Zn > Cr > Cu > Cd > As > Pb > Pt > Ni, while for *C. gariepinus* it was Cd > Zn > Cr > Cu > As > Pb > Pt > Ni and for *O. mossambicus* Cu > Cr > As > Zn > Cd > Pb > Pt > Ni. The order of the mean BSAF for *C. carpio* was Zn > Cd > Cu > As > Pt > Pb > Ni > Cr, while for *C. gariepinus* it was Cd > Zn > Cu > As > Pt > Pb > Cr > Ni and for *O. mossambicus* Cu > Cd > Zn > As > Pb > Pt > Ni > Cr. The fish species with the highest BCF and BSAF were *O. mossambicus* for Cr, Ni, Cu, As, Pt and Pb, while *C. carpio* had the highest BCF and BSAF for Zn, and *C. gariepinus* for Cd, respectively.

Table 6.5: Bioconcentration factors (BCF) calculated as the ratio of the metal concentrations in fish muscle and liver tissue (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*) and metal concentrations in water collected from Olifantsnek Dam and Bospoort Dam.

		Cr	Ni	Cu	Zn	As	Cd	Pt	Pb
Muscle									
Olifantsnek Dam	*C. carpio*	2 212	87	131	994	2 965	1 508	202	1 387
	C. gariepinus	2 876	43	145	1 231	2 443	1 175	134	1 237
	O. mossambicus	5 988	76	222	1 397	4 653	1 774	348	1 841
Bospoort Dam	*C. carpio*	6 298	12	204	2 186	2 385	2 461	161	2 327
	C. gariepinus	4 938	5	165	1 723	1 897	2 642	97	3 174
	O. mossambicus	11 437	28	318	3 618	2 860	4 614	48	4 081
Liver									
Olifantsnek Dam	*C. carpio*	2 422	113	8 138	11 560	3 246	6 638	212	1 237
	C. gariepinus	2 854	83	3 493	5 220	2 607	7 854	217	1 487
	O. mossambicus	7 748	355	37 215	4 661	7 186	4 552	554	2 063
Bospoort Dam	*C. carpio*	6 373	24	7 097	52 428	2 173	4 658	184	1 987
	C. gariepinus	4 502	13	5 632	8 883	1 384	9 566	146	2 255
	O. mossambicus	18 371	171	7 427	10 104	5 964	7 088	122	5 551

Table 6.6: Biota-sediment accumulation factors (BSAF) calculated as the ratio of the metal concentrations in fish muscle and liver tissue (*Cyprinus carpio*, *Clarias gariepinus*, *Oreochromis mossambicus*) and metal concentrations in sediment collected from Olifantsnek Dam and Bospoort Dam.

		Cr	Ni	Cu	Zn	As	Cd	Pt	Pb
Muscle									
Olifantsnek Dam	*C. carpio*	0.003	0.010	0.059	1.207	0.823	1.194	0.124	0.121
	C. gariepinus	0.003	0.005	0.067	0.795	0.512	1.223	0.099	0.140
	O. mossambicus	0.006	0.010	0.117	0.886	1.812	1.785	0.238	0.146
Bospoort Dam	*C. carpio*	0.026	0.009	0.236	2.063	0.582	3.303	0.575	0.350
	C. gariepinus	0.020	0.002	0.205	1.707	0.377	2.829	0.364	0.425
	O. mossambicus	0.060	0.036	0.715	4.211	0.609	5.040	0.224	0.507
Liver									
Olifantsnek Dam	*C. carpio*	0.003	0.013	3.759	11.833	1.100	4.800	0.127	0.149
	C. gariepinus	0.003	0.009	1.537	3.151	0.519	6.460	0.158	0.134
	O. mossambicus	0.008	0.045	21.598	2.947	2.177	3.966	0.361	0.167
Bospoort Dam	*C. carpio*	0.027	0.028	10.546	61.235	0.518	10.638	0.662	0.317
	C. gariepinus	0.018	0.012	9.598	9.551	0.235	28.439	0.558	0.382
	O. mossambicus	0.098	0.258	27.119	10.514	1.316	8.632	0.533	0.648

6.3.5 Human health risk assessment

The total HQs of *C. carpio*, *C. gariepinus,* and *O. mossambicus* from Bospoort Dam were 1.2, 1.9 and 1.1 times greater than that for Olifantsnek Dam, respectively. The recommended HQ of 1 was exceeded for As for all three fish species at both impoundments (Table 6.7). At Olifantsnek Dam, *C. carpio* had the highest HQ for Zn, *C. gariepinus* had the highest HQs for Ni and Cd, while *O. mossambicus* had the highest HQs for Cr, Cu and As. However, at Bospoort Dam, *C. gariepinus* had the highest HQs for Cr, As, and Cd, while *O. mossambicus* had the highest HQs for Ni, Cu and Zn. No HQs were calculated for Pb and Pt as there are no RfD values for these metals.

Table 6.7: The hazard quotients (HQs) for *Cyprinus carpio*, *Clarias gariepinus* and *Oreochromis mossambicus* from Olifantsnek Dam and Bospoort Dam, calculated on the average metal concentration in the muscle tissue, supposing a person of 60 kg consumes one fish meal (150 g) twice a week. Highlighted values are HQ > 1, indicating a high probability of adverse health effects to humans that consume these fish.

		Cr	Ni	Cu	Zn	As	Cd
Olifantsnek Dam	***C. carpio***						
	Metal concentration (mg/kg WW)	0.25	0.11	0.28	9.23	0.84	0.027
	Average daily dose (ADD) (µg/kg)	0.21	0.090	0.23	7.74	0.70	0.022
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.070	0.005	0.006	0.026	2.33	0.007
	C. gariepinus						
	Metal concentration (mg/kg WW)	0.43	0.14	0.44	6.61	1.13	0.028
	Average daily dose (ADD) (µg/kg)	0.36	0.12	0.36	5.51	0.94	0.023
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.120	0.006	0.009	0.018	3.14	0.008
	O. mossambicus						
	Metal concentration (mg/kg WW)	0.67	0.098	0.58	6.27	1.50	0.024
	Average daily dose (ADD) (µg/kg)	0.56	0.082	0.48	5.23	1.25	0.020
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.187	0.004	0.012	0.017	4.17	0.007
Bospoort Dam	***C. carpio***						
	Metal concentration (mg/kg WW)	0.45	0.11	0.34	5.42	1.35	0.033
	Average daily dose (ADD) (µg/kg)	0.37	0.094	0.28	4.51	1.12	0.028
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.124	0.005	0.007	0.015	3.75	0.009
	C. gariepinus						
	Metal concentration (mg/kg WW)	0.72	0.11	0.45	5.94	2.54	0.046
	Average daily dose (ADD) (µg/kg)	0.60	0.092	0.37	4.95	2.12	0.038
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.199	0.005	0.009	0.017	7.06	0.013
	O. mossambicus						
	Metal concentration (mg/kg WW)	0.70	0.31	0.65	8.86	1.67	0.029
	Average daily dose (ADD) (µg/kg)	0.58	0.26	0.54	7.38	1.40	0.024
	Reference dose (RfD) (µg/kg)	3	20	40	300	0.3	3
	HQ	0.193	0.013	0.014	0.025	4.65	0.008

6.4 Discussion

The metal concentrations measured in the Hex River (water and sediment), which drains the Bushveld Igneous Complex, are elevated compared to normal upper continental crust values (Almécija *et al*., 2017). These naturally high levels in the parental rock (Barnes and Maier, 2002) are released into the river system and are available to aquatic biota. However, intensive Pt and Cr mining, urban and industrial effluent may contribute to an anthropogenic increase in metal concentrations over the already elevated background concentrations (Almécija *et al*., 2017). According to Rauch and Fatoki (2015), limited studies have been completed on Pt discharges from intensive Pt mining and production activities. Furthermore, data on Pt mining derived metals within aquatic biota occurring near Pt production sites are deficient (Rauch and Fatoki, 2015; Erasmus *et al*., 2020). The present study is the first to determine the Pt mining derived metal concentrations in fish occurring in this intensive Pt mining region. The anthropogenic increase in metal concentrations was evident in the fish muscle and liver tissue with metals significantly increasing from Olifantsnek Dam to Bospoort Dam, in particular, the concentrations of Cr, Ni, As and Pt. All of these metals are associated with the intensive Pt mining along the Hex River (as seen in Chapters 2 and 3). These metals are taken up by the fish from their environment (water and sediment), as well as potentially through their diet, which leads to accumulation in the tissues, and could potentially have detrimental effects on organisms that consume the fish (Marzouk, 1994; Adams *et al*., 2000; Addo-Bediako *et al*., 2014).

The fish health assessment index for all three the fish species was higher at Bospoort Dam compared to Olifantsnek Dam. This indicates that fish from Bospoort Dam are exposed to pollutants and organ damage due to environmental stressors (Marchand *et al*., 2009). The condition factor determines the condition of the fish on the basis of length: weight ratios, and it is assumed heavier fish, are in better condition. However, several factors potentially influence fish weight, *i.e.* seasonal changes can affect breeding behaviour, food availability and metabolic rates that are subjected to temperature (Marchand *et al*., 2009; Pieterse *et al*., 2010; van Dyk *et al*., 2012). From the results, fish collected from Bospoort Dam had higher condition factors than those collected from Olifantsnek Dam, indicating that fish in Bospoort Dam was in a better condition. This could be explained by the fact that Bospoort Dam had higher nutrient inputs, which resulted in higher food availability, leading to increased reproduction potential in these fish. Although the fish exhibited these elevated metal concentrations, the relative weight, fish health assessment index and condition factor indicated that the fish populations collected from both impoundments appeared to be in a healthy condition. These results can be indicative that fish occurring within the Hex River system, have adapted, and utilise various defensive mechanisms and strategies (*e.g.* sequestration, detoxification – metallothioneins and elimination) to protect themselves from the high metal concentrations (Hoffman *et al*., 2002; Cardwell *et al*., 2013).

6.4.1 Metal bioaccumulation in fish species

As expected, the metal concentrations in the liver tissue of all three species collected were higher than the concentrations in the muscle tissue. According to Karadede and Ünlü (1999), the liver for most metals accumulates the highest concentrations in the body due to its function as a detoxifying organ. The liver removes metals and other pollutants from the bloodstream, preventing it from reaching other vital organs (Musa *et al*., 2017). Even though the liver serves as a detoxifying organ, other organs and tissues still accumulate metals. Since muscle tissue is generally the part of the fish commonly consumed by humans, it is most important to assess these for metals, when looking to health risks for consumers.

The metals of concern in the Hex River system were those associated with the intensive Pt mining (Cr, Ni, As and Pt). However, geological borne Pb is also of concern due to the toxicity thereof. Chromium in the aquatic environment is most stable in the Cr^{3+} and Cr^{6+} oxidation states. Due to its ability to cross biological membranes and vigorous oxidative potential, Cr^{6+} is considered toxic (Lushchak *et al*., 2009). Nickel in surface waters freely binds and forms complexes with ligands, making it more mobile than most other metals (Palaniappan and Karthikeyan, 2009) and toxic in higher concentrations (0.1 mg/L) (Magyarosy *et al*., 2002). Arsenic compounds occurring in freshwater systems in the third oxidation state are rapidly absorbed into fish and are considered more toxic than the fifth oxidation state (Liao *et al*., 2004). Koch *et al*. (2001) reported that total As in various freshwater fish (whitefish, *Coregonus clupeaformis*; sucker, *Catostomus commersoni*; walleye, *Stizostedion vitreum*; pike, *Esox lucius*) from Great Slave Lake, Canada, which is impacted by gold mining effluent, ranged from 0.28 to 3.1 µg/g DW. Concentrations of As found during the present study ranged from 3.5 to 7.9 µg/g DW in *C. gariepinus*. According to Fortin *et al*. (2011), there are limited studies available on the bioaccumulation of platinum group elements (PGE) derived from auto catalyst emissions by fish in urban systems, while bioaccumulation of PGE derived from mining and production activities are even scarcer. In a review by Ruchter *et al*. (2015) on field studies of PGE in aquatic ecosystems, concentrations of Pt in muscle tissue of the common barbel, *Barbus barbus*, were 0.4 ng/g DW from urban systems in the Danube River, Hungary. In the present study, concentrations of Pt ranged from 1.3 to 5.5 ng/g DW in *C. carpio*. These results can possibly illustrate that Pt deriving from mining effluent is more bioavailable than Pt deriving from auto catalysts. Lead is a persistent metal in aquatic ecosystems and has been classified as a priority hazardous substance (Sfakianakis *et al*., 2015). According to Cretì *et al*. (2010), fish accumulate Pb more from contaminated water than from its diet. All of these metals associated with Pt mining effluent are toxic and can cause detrimental effects in fish, as well as the organisms consuming the fish. However, in the present study, no such effects were found.

In *C. carpio*, the concentrations of Cr, Ni, and Pb were comparable with results found by Vinodhini and Narayanan (2008) in *C. carpio* from ponds in Tamil Nadu, India, while concentrations of Cu, Zn and Cd were comparable with results obtained by Zafarzadeh *et al*. (2018) in the Alagol wetland, Iran. The concentrations of Cd, Cu and Ni were comparable with results found by Bervoets *et al*. (2009) in an exposure study completed with *C. carpio* in different freshwater sites with different types and intensities of pollution in Flanders, Belgium. The concentrations of Cr, Pb and Zn in both muscle and liver tissues from the present study were several fold higher than the concentrations recorded by Bervoets *et al*. (2009). According to Vinodhini and Narayanan (2008), *C. carpio* has the ability to adapt to polluted aquatic environments and are considered good bioindicators in polluted systems. The concentrations of Cu and Pb, as well as Ni, Cu, Cd, and Pb in the muscle tissue of *C. gariepinus* were comparable to results obtained in the impacted Olifants River, South Africa by Coetzee *et al*. (2002) and Jooste *et al*. (2015), respectively. Concentrations of Cr were in the same range as results obtained by Musa *et al*. (2017) in the Nyl River system, which is also impacted by various mining activities, urban effluent, as well as runoff from informal settlements. In the present study, the concentrations of As were six-fold higher than the concentrations recorded by Jooste *et al*. (2015).

The concentrations of Ni, Cu, Cd, and Pb in the muscle tissue of *O. mossambicus* were comparable to results obtained in similar studies of metal bioaccumulation in *O. mossambicus* and other *Oreochromis* species (Al-Kahtani, 2009; Yilmaz, 2009; Jabeen and Chaudhry, 2010; Addo-Bediako *et al*., 2014; Moyo and Rapatsa, 2019) from various river systems in Africa. The concentrations of Cr and Zn in the present study were much lower than the concentrations recorded in the Olifants River, which are also impacted by intensive mining activities (Addo-Bediako *et al*., 2014), whereas the concentrations of As were five-fold higher in the present study. A study completed by Cheung *et al*. (2008) in the intensive industrialised and urbanised Pearl River, China, found that *O. mossambicus* have the ability to accumulate exceptionally high concentrations of As and Pb in their muscle tissue. In the present study, most metals were higher in *O. mossambicus* compared to the other fish species, which indicate that metals do not biomagnify, but rather bioaccumulate in lower functional feeding groups. This was also found to be true in freshwater macroinvertebrates from the Hex River (as seen in Chapter 5), as well as from the Scheldt estuary between Belgium and the Netherlands (Van Ael *et al*., 2017).

6.4.2 Human health risk assessment

Many local communities near the Hex River catchment rely on fish from the river, as well as the impoundments for nutritional protein supplements. The bioaccumulation results indicate that *C. carpio*, *C. gariepinus,* and *O. mossambicus* are integrating metals into their muscle and liver tissue. According to the human health risk assessment, the concentrations of As within these fish species are not acceptable for safe human consumption. Although only the concentrations of As exceeds safe human consumption levels, it is important to keep in mind that a mixture of these

metals occurs within these fish species, potentially posing an even greater risk to people who consume these fish. Adults of 60 kg consuming a 150 g portion of muscle from these fish species twice a week, may be at the risk of exposing themselves to severe health risks. Individuals who are more susceptible to metal health risks include pregnant women, lactating mothers, as well as children (Javed and Usmani, 2019).

Arsenic ingestion not only poses cancer (skin, bladder, and lungs) risks but also poses numerous non-cancer risks such as diabetes, pulmonary and cardiovascular diseases. Also, adverse pregnancy outcomes and infant mortality are associated with As (Quansah *et al*., 2015; WHO, 2018). The risk associated with consuming *C. carpio*, *C. gariepinus* and *O. mossambicus* from Bospoort Dam are respectively 20, 90 and 10% greater than for Olifantsnek Dam. Although fishes from Olifantsnek Dam also pose a health risk to humans, the probability of the risk is minimal due to the strict catch and release recreational fishing protocol implemented at this impoundment. On the other hand, the risk posed by consuming fish from Bospoort Dam is paramount, due to fishers who sell fish to local communities. With the rapid growth of informal settlements in the proximity of the Hex River (Ololade *et al*., 2008) and an increase in rural populations living in poverty, an increase in the demand for cheap nutritional protein supplements is impending, which will result in an increase in the frequency in consumption of fish from Bospoort Dam.

Although no HQ values could be calculated for Pt, it potentially still poses a risk to human health. A study completed by Colombo *et al*. (2008) found that Pt exposure can occur in humans via ingestion and inhalation. Platinum uptake through digestion accounted for 17%, while through inhalation it was 36%. They also concluded that Pt bioavailability slightly increased from the stomach towards the intestine as Pt-chloride form complexes with stomach fluids (Colombo *et al*., 2008). Schmid *et al*. (2007) completed a study where human bronchial epithelial cells were exposed to Pt salts to examine the effects thereof on cellular viability and found that the LC_{50} for Pt(II) and Pt(IV) were in the same range as Cd(II) and Cr(IV). Platinum salts have also been reported to be associated with an increase in incidents of dermatitis, asthma, urticaria, and rhinoconjunctivitis among workers in refineries and catalyst production facilities (Cristaudo *et al*., 2005; Watsky, 2007). It was demonstrated by Gagon and Patel (2007) that chicken embryos accumulated Pt at a higher rate in the brain compared to the liver, due to the lack of a fully developed blood-brain barrier. Humans also do not have a fully developed blood-brain barrier at birth, and Pt can potentially accumulate in the brain of new-borns, which can induce neuro-developmental effects in babies (Wiseman and Zereini, 2009). Humans living in the vicinity of the intensive Pt mining activities in the Rustenburg area are not only exposed to Pt from fish or water consumption but are also exposed to Pt via inhalation of dust from waste dumps and ore transport, as well as emissions of Pt smelters and automobile catalytic converters.

According to Addo-Bediako *et al*. (2014) and Jooste *et al*. (2015), the indigenous *C. gariepinus* and *O. mossambicus* are the preferred target species among rural communities and are more intensely targeted by subsistence fishers in the Olifants River, South Africa. These fish species collected, also posed a human health risk where Co, Cr, and Sb, as well as Cr and Sb, exceeded the HQ value of 1 in *O. mossambicus* and *C. gariepinus*, respectively. However, at Bospoort Dam, subsistence fishers are also actively targeting *C. carpio* from the banks of the impoundment with rod and reel. Nonetheless, all fish species are caught by the increased illegal use of gill nets within this impoundment, which is a common occurrence across South Africa (Weyl *et al*., 2007). This subsistence fishing provides an income, as well as a protein source to the communities and is becoming a vital part of rural livelihoods (McCafferty *et al*., 2012). Granted that the present study only completed a basic human health risk assessment, it provides valuable information and indicates a high risk of adverse health effects for people consuming fish from the Hex River. The present study also provides an important evaluation and understanding of the human health risks which are associated with metal exposure via freshwater fish consumption by the surrounding communities of the Hex River.

6.5 Conclusion

Metal concentrations within the Bushveld Igneous Complex are naturally higher than normal upper continental crust concentrations. However, anthropogenic activities *i.e.* intensive Pt mining, as well as urban and industrial effluent in the Hex River system contribute to even higher metal concentrations than the already elevated background concentrations. Fish species occurring within the Hex River system accumulate high metal concentrations. Fishes from Bospoort Dam (impacted impoundment) accumulated significantly higher concentrations of Cr, Ni, As and Pt, (which are all associated with Pt mining activities), than fishes from Olifantsnek Dam (reference impoundment). The concentrations of Pt within fish were 13-fold higher than Pt concentrations in fish collected from urban systems in other regions, indicating that Pt deriving from mining activities is more bioavailable than Pt deriving from auto-catalysts. These high metal concentrations in the fish tissue pose a risk, to other organisms that consume them, as well as to humans. From the human health risk assessment, concentrations of As exceeded the hazard quotients of 1, in all three fish species from both impoundments, indicating a high probability of adverse human health effects. No HQ values could be calculated for Pb and Pt as no reference dose values are available for these metals. Although only As exceeded the HQ, Pt also poses a potential health risk to humans consuming fish and inhaling dust from mining activities and emissions from Pt smelters and automobile catalytic converters. Even though the present study completed a basic human health risk assessment, it provided valuable information on the understanding of the human health risks associated with metal concentrations via fish consumption. The data allow us to support the hypotheses that fish collected from the impacted impoundment will accumulate higher metal

concentrations than the reference impoundment, and will, therefore, pose a higher human health risk.

CHAPTER 7: THE ROLE OF PARASITES IN MONITORING ENVIRONMENTAL POLLUTION

(Under review as Erasmus, J.H., Wepener, V., Nachev, M., Zimmermann, S., Malherbe, W., Sures, B., Smit, N.J. The role of fish helminth parasites in monitoring metal pollution in aquatic ecosystems: a case study in the world's most productive platinum mining region. *Parasitology Research*, submitted 7 February 2020).

7.1 Introduction

The use of fishes as indicators of aquatic ecosystem health is well established (Adams and Ryon, 1994; Whitfield and Elliot, 2002; Moiseenko *et al*., 2008; Authman *et al*., 2013; 2015). Freshwater fish can accumulate metals either via the ingestion of contaminated food or through the gills and skin (Sfakianakis *et al*., 2015). Consequently, metal concentrations in fish tissues reflect past exposure, as well as the current situation before toxicity can affect the ecological equilibrium of populations in the aquatic environment (Retief *et al*., 2006; Birungi *et al*., 2007; Authman *et al*., 2015). Certain fish parasite taxa have the ability to accumulate metals, orders of magnitude higher than their hosts, and can be used to reliably detect elements with a naturally low abundance in the environment (Sures, 2004; Sures *et al*., 2017). In addition, due to the fact that they lack a digestive system, several parasite taxa, such as acanthocephalans and cestodes, also indicate which metals are biologically available. Therefore, if metals are accumulated by these parasites, the respective metals had to cross the parasites' tegument and are thus biologically available.

According to Sures *et al*. (2017), among the four major endohelminth taxa (Acanthocephala, Cestoda, Digenea, and Nematoda), cestodes are the most diverse group of parasites that can be used as potential bioindicators of metal pollution, followed by nematodes. In addition, cestodes and acanthocephalans also have the highest accumulation capacity. These parasites are able to accumulate various elements, particularly non-essential elements, at exceptionally high concentrations (summarised in Sures, 2004; Sures *et al*., 2017). Numerous laboratory and field studies demonstrated that fish infected with acanthocephalans and cestodes had lower metal concentrations in their tissues than uninfected fish (Gabrashanska and Nedeva 1996; Sures and Siddall 1999; Turcekova and Hanzelova 1999; Sures *et al*., 2017).

Biochemical or molecular level responses, commonly referred to as biomarkers, can be used as initial warning signs to indicate the presence of pollutants in ecosystems and to elucidate adverse effects on organisms (Forbes *et al*., 2006). Biomarkers can be divided into biomarkers of exposure, which indicate the presence of specific pollutants in systems (*e.g.* metallothionein as biomarkers for metals (Le *et al*., 2016)) and biomarkers of effects, such as oxidative stress, proteins involved in pollutant metabolism and excretion, as well as energy budgets (Wepener *et al*., 2011; Sures *et al*., 2017). However, both types of biomarkers do not reflect a specific

response to one stressor and are rather induced by a variety of stressors, including parasites (Sures, 2008). According to Sures *et al*. (2017), parasites intensely interact with pollutant-induced biomarker responses of their associated hosts, by means of influencing their physiology in various ways. Hence, Marcogliese and Pietrock (2011) recommend assessing biomarker responses in infected fish, with variation in intensity, as well as from both reference and polluted conditions.

Studies on platinum group element (PGE) accumulation in parasite-host systems are limited, with only three studies published on acanthocephalans exposed to automobile catalytic converter material (Sures *et al*., 2003; 2005; Zimmermann *et al*., 2005) and a single field study focussing on cestodes and nematodes (Eira *et al*., 2009). The aims of the present study were to (1) determine the metal accumulation in helminth parasite species compared to their hosts from a reference and Pt mining impacted impoundment, (2) assess whether there is a difference between bioaccumulation of metals in tissues of infected and uninfected hosts, and (3) analyse the effect of parasite infection and metal exposure on biomarker responses. The hypotheses stated for this chapter are that (1) parasites and their associated hosts will have higher metal concentrations at the PGE mining impacted site compared to the reference site; (2) infected fish will have lower metal concentrations in their tissues than uninfected fish, and (3) biomarker responses in infected fish will be more pronounced compared to uninfected fish.

7.2 Materials and Methods

7.2.1 Field sampling

Fish were sampled from Olifantsnek Dam and Bospoort Dam during November 2017 and 2018 (refer to Chapter 3 for site description and Chapter 6 for sampling description). Fishes were dissected and screened for endoparasites and the parasites were collected for identification following the procedures described by Scholz *et al*. (2018). Endoparasites from fish with heavy infections were collected for metal analysis and stored in pre-cleaned polyethylene containers and frozen at -20 °C until further analysis. Muscle and liver samples for metal analyses were collected from each fish and stored in pre-cleaned polyethylene containers and frozen at -20 °C until further analysis (see Chapter 6). Muscle, liver, and brain samples were collected for biomarker analyses and were stored in sterile polyethylene containers with a sufficient volume (w:v) of Henrickson's stabilising buffer (HSB), flash-frozen in liquid nitrogen and stored at -80 °C until further biomarker analyses.

7.2.2 Parasites

Parasites (Cestoda and Nematoda) were sampled according to Scholz *et al*. (2015) and Tavakol *et al*. (2015), respectively. Cestodes were collected from the intestine of *Cyprinus carpio* and gently rinsed with saline, where after the cestodes were fixed in 4% hot formaldehyde solution for morphological identification (Scholz *et al*., 2015). Nematodes were collected from the body cavity of *Clarias gariepinus*, cleaned in saline and were subsequently placed in 70% ethanol that was heated to 70 °C to uncoil, where after the nematodes were fixed in 70% ethanol for identification (Tavakol *et al*., 2015). Species were identified with the aid of Scholz *et al*. (2018). Infection parameters, *i.e.* prevalence (P) and mean intensity (MI), were calculated according to Bush *et al*. (1997).

7.2.3 Sample preparation

Muscle, liver, and parasite samples for metal analyses, were freeze-dried (FreeZone® 6, Labconco). Fish tissue samples were digested according to the methods described in Chapter 6. For the parasite samples, approximately 0.05 g (dry weight) of the cestodes and approximately 0.1 g (dry weight) of the nematodes were digested in the same matrix as the fish samples. All the samples were treated following the same methods described in Chapter 6.

7.2.4 Metal analyses

In all samples, the concentrations of Cr, Ni, Cu, Zn, Cd, Pt, and Pb were determined using the methods described in Chapter 2 using an ICP-MS. The ICP-MS was operated with the same settings and quality control protocols as described in Chapter 2. Detection limits for the fish and parasite samples were determined as three times the standard deviation of the blank measurements (Table 7.1).

Table 7.1: Detection limits (µg/g) of the elements of interest obtained for different tissues. Detection limits for the fish and parasite samples were determined as three times the standard deviation of the blank measurements.

Elements	Detection limits (µg/g)		
	Fish	Cestodes	Nematodes
Cd	0.013	0.003	0.00084
Cr	0.19	0.62	0.24
Cu	0.24	0.10	0.08
Ni	0.08	0.023	0.015
Pb	0.29	0.061	0.013
Pt	0.00094	0.00065	0.00047
Zn	1.59	0.95	0.22

7.2.5 Biomarkers

For biomarker analyses, muscle, liver and brain samples stored at -80 °C, were defrosted on ice. Biomarkers of exposure included acetylcholine esterase activity (AChE) and metallothionein content (MT), while biomarkers of effect included catalase activity (CAT), reduced glutathione content (GSH), malondialdehyde content (MDA), protein carbonyls induction (PC), superoxide dismutase activity (SOD) and cellular energy allocation (CEA). Acetylcholine esterase activity was determined in brain tissue, while liver tissue was analysed for MT, CAT, GSH, MDA, PC, and SOD. The energetics biomarkers (CEA) were analysed in muscle tissue. For CAT, GSH, PC and SOD analyses, approximately 0.5 g liver tissue was placed in general homogenising buffer (0.1 M $KHPO_4$, 1.15% KCl, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride solution, 20% glycerol, pH 7.4) in a 1:10 liver weight/volume ratio and homogenised on ice using an Ultra-Turrax T 25 (Janke and Kunkel, Staufen, Germany). The homogenate was divided into aliquots for the different biomarker analyses. For the other biomarkers, the respective homogenising buffers were used. The activity or content of biomarkers was expressed per mg protein. The protein content was determined in each sample using the method described by Bradford (1976), where 5 µL sample with 245 µL Bradford reagent were incubated for 5 minutes at room temperature and measured in triplicate at 595 nm in 96 well plates (Greiner Bio-one UV-star), with a microplate reader (Molecular Devices SpectraMax iD3).

7.2.5.1 Acetylcholine esterase activity

The AChE activity was determined colorimetrically by measuring the production of thiocholine as a result of acetylthiocoline being hydrolysed according to the methods described in Ellman *et al*. (1961). Approximately 0.1 g brain sample was homogenised on ice with Tris-HCl (0.05 M)/sucrose (0.25 M) buffer (pH 7.4) added at a 1:5 tissue weight/volume ratio. The AChE activity was determined colorimetrically by measuring the increase in absorbance at 412 nm for six minutes at one-minute intervals. The homogenate was centrifuged at 9 210 rpm for 10 minutes at 4 °C (Heal Force Neofuge 13R). For the reaction, 5 µL of the supernatant was incubated with 30 mM s-acetylthiocholine iodide and 10 mM 2,2'-dinitro-5,5'-dithio-dibenzoic acid (Ellman's reagent) in a potassium phosphate buffer (0.09 M $KHPO_4$, pH 7.4) at 37 °C for five minutes. The samples were measured in triplicate within a total volume of 235 µL in 96 well plates with a microplate reader. The AChE activity was calculated as absorbance/min/mg protein.

7.2.5.2 Metallothionein content

Metallothionein content was determined using the partially purified metalloproteins fraction that is obtained by acidic ethanol/chloroform fractionation of tissues following the method of Viarengo *et al*. (1997), with slight modifications (see Atli and Canli, 2008). Approximately 0.2 g liver tissue with 1:5 tissue weight/volume ratio Tris-HCl (0.02 M)/sucrose (0.5 M) homogenising buffer (pH 8.6) with 0.006 mM leupeptine, 0.0005 M phenylmethylsulphonylfluoride (PMSF) and 0.01% b-mercaptoethanol was homogenised on ice. The homogenate was centrifuged at 16 360 rpm for 20 minutes at 4 °C. Ice cold (-20 °C) absolute ethanol (500 µL) and absolute chloroform (40 µL) were added to 500 µL supernatant and vortexed for approximately 15 seconds. The samples were subsequently centrifuged at 7 000 rpm for 10 minutes at 4 °C, where after three volumes of -20 °C absolute ethanol were added and vortexed for approximately 15 seconds. The samples were then incubated at -20 °C for 1-2 hours until a pellet formed. The supernatant was decanted, and the pellet was washed with 1 mL washing buffer (87% absolute ethanol, 1% absolute chloroform, 12% homogenising buffer), vortexed and centrifuged at 3 000 rpm for 20 minutes at 4 °C, repeating the wash step twice. The pellet was air dried and resuspended in 300 µL 5 mM Tris-HCl 1 mM EDTA (pH 7.0). For the reaction, 210 µL 0.43 mM 5,5'Dithio-bis (2-Nitrobenzoic acid) (DTNB) in 0.2 M sodium phosphate buffer (pH 8.0) and 15 µL of the sample were added to 96 well plates and incubated for 15 minutes at room temperature. The samples were measured at 412 nm in triplicate in a total volume of 225 µL with a microplate reader. The MT content was calculated as nM MT/mg protein.

7.2.5.3 Catalase activity

Catalase activity was determined colorimetrically by measuring the decomposition of Complex II, an inactive complex of catalase and H_2O_2 by reacting it with $KMnO_4$ using the method described in Cohen *et al*. (1970). The homogenate used for CAT was centrifuged at 9 450 rpm for 10 minutes at 4 °C. For the reaction, 93 µL 6 mM H_2O_2 was added and mixed with 10 µL of the supernatant and left for three minutes. After three minutes, 19 µL 6 N H_2SO_4 were added to stop the reaction and 130 µL 0.01 N $KMnO_4$ were added immediately after. The samples were measured in triplicate in a total volume of 252 µL at 492 nm in 96 well plates with a microplate reader. The CAT activity was calculated as µM H_2O_2/min/mg protein.

7.2.5.4 Reduced glutathione content

The GSH content was determined by measuring the fluorescence obtained from the reaction of o-phthalaldehyde with histamine resulting from excitation at 350 nm and measuring the fluorescence at 420 nm (Cohn and Lyle, 1966). To precipitate the proteins, 75 µL 25% H_3PO_4 were added to 500 µL of the homogenate and left on ice for 10 minutes. The samples were then centrifuged at 3 000 rpm for 10 minutes at 4 °C. Before samples were added to the 96 well plates, 100 µL supernatant was diluted by 1 mL MilliQ® water. For the reaction, 6 µL of the diluted sample was added to the 96 well plates, with 232 µL sodium phosphate buffer (0.1 M $HNaPO_4$, pH 8.0) and 12 µL 0.1% o-pthalaldehyde (OPT), then incubated for 15 minutes at room temperature. The samples were measured in triplicate on a total volume of 250 µL in 96 well plates with a microplate reader. The GSH content was calculated as µg/g wet weight.

7.2.5.5 Malondialdehyde content

The extent of lipid peroxidation was determined colorimetrically by quantifying MDA content in a thiobarbituric acid (TBA) solution following Ohkawa *et al*. (1979) as modified by Üner *et al*. (2005). Approximately 0.1 g liver sample was homogenised in Tris-HCl (0.05 M)/sucrose (0.25 M) buffer (pH 7.4) at a 1:5 tissue weight/volume ratio on ice. The homogenate was centrifuged at 9 210 rpm for 10 minutes at 4 °C. In a 3 mL screwcap, 25 µL supernatant, 50 µL 8.1% sodium dodecyl sulphate (SDS), 375 µL 20% acetic acid (pH 3.5), 375 µL 0.8% thiobarbituric acid (TBA) and 175 µL MilliQ® water were added and incubated in a water bath for 30 minutes at 95 °C. Samples were left to cool to room temperature. Subsequently, 250 µL MilliQ® water and 1.25 mL n-butanol: pyridine (15:1) were added. Samples were then vortexed and centrifuged at 4 000 rpm for 10 minutes at room temperature. The supernatant (245 µL) was transferred to 96 well plates and absorbance was determined in triplicate at 532 nm. The MDA content was expressed as nM/µg protein.

7.2.5.6 Protein carbonyl induction

The PC induction was determined colorimetrically by derivatizing the carbonyl group with 2,4-dinitrophenylhydrazine to form stable 2,4-dinitrophenyl following the methods described by Parvez and Raisuddin (2005). The sample aliquot was centrifuged at 9 680 rpm for 30 minutes at 4 °C and 500 µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl was added to 500 µL of the supernatant. The sample was incubated for one hour at room temperature and vortexed every 10 minutes. An equal volume (500 µL) 6% trichloroacetic acid was added, to precipitate the proteins and centrifuged at 9 450 rpm for three minutes at 4 °C. The supernatant was discarded and the pellet was washed three times by resuspension in 1 mL 1:1 ethanol: ethyl ether where after the supernatant was discarded. To solubilise the proteins, 400 µL 6 M guanidine hydrochloride in 50% formic acid was added, incubated for 15 minutes at room temperature and

centrifuged at 11 950 rpm for five minutes. The supernatant was transferred to a 96 well plate and measured in triplicate at 366 nm. The PC induction was calculated as nM carbonyl/mg protein

7.2.5.7 Superoxide dismutase activity

The SOD activity was determined by using the adapted methodology of Greenwald (1989), where 4 µL of the aliquot sample was transferred to a 96 well plate and 245 µL Tris Buffer (50 mM)/DTPA (0.1 M) was added. For the reaction 4 µL 24 mM pyrogallol was added to the solution and the samples were measured immediately in triplicate for 10 minutes at one-minute intervals. The SOD activity was expressed as ng SOD/ mg protein.

7.2.5.8 Cellular energy allocation

The CEA was analysed using four different assays (protein content, glycogen content, lipid content, and ETS activity) according to De Coen and Janssen (1997), and modified as Rasouli *et al*. (2014). For the energy available (Ea) assays (protein, glycogen, and lipid) 0.2 g muscle sample was homogenised on ice in MilliQ® water at a 1:5 tissue weight/volume ratio. For the energy consumption (Ec) assay (ETS) 0.2 g muscle sample was homogenised with 1:5 tissue weight/volume ratio ETS homogenising buffer (0.1 M Tris-HCl, 0.2% Triton X-100, 15% polyvinyl pyrrolidone and 153 µM $MgSO_4$, pH 8.5) on ice. Protein content was determined according to Bradford (1976). Glycogen content was determined by adding 200 µL 30% KOH to 250 µL sample and incubated for 10 minutes at 95 °C. Samples were left to cool to room temperature, where after 275 µL 55% ethanol was added, vortexed and centrifuged at 3 900 rpm for 10 minutes at room temperature. The supernatant was discarded and the pellet resuspended in 2 mL MilliQ® water. In a 2 mL Eppendorf, 50 µL sample, 50 µL MilliQ® water, 100 µL 6.5% phenol and 500 µL 85% H_2SO_4 were added and vortexed. Samples were incubated for 30 minutes at room temperature, where after 300 µL was transferred to a 96 well plate and measured in duplicate at 492 nm. For the lipid content, 500 µL chloroform, 500 µL methanol and 250 µL MilliQ® water were added to 250 µL homogenate. Samples were then centrifuged at 5 175 rpm for five minutes at 4 °C and 100 µL of the organic phase (bottom phase) was pipetted into glass tubes. Subsequently, 500 µL H_2SO_4 was added in the glass tubes and covered with foil and incubated at 200 °C for 15 minutes. Samples were left to cool and 1 mL MilliQ® water was added. The samples were measured at 360 nm in triplicate in a total volume of 245 µL in 96 well polyethylene plates with a microplate reader.

For the ETS activity, the homogenates were centrifuged at 5 175 rpm for 10 minutes at 4 °C. In a 96 well plate, 25 µL supernatant, with 75 µL buffered substrate solution (BSS) (0.3% Triton X-100 in 0.13 M Tris-HCl, pH 8.5), and 25 µL NAD(P)H solution (1.7 mM NADH, 250 µM NADPH) were added. To start the reaction 50 µL p-IodoNitroTetrazolium chloride (INT) was added and the samples were measured at 490 nm in triplicate, kinetically for five minutes, with one-minute intervals. The protein-, glycogen- and lipid reserves, as well as the ETS activity, were expressed

in Joules/g sample. The total energy available (Ea) was determined as the total of the protein-, glycogen- and lipid reserves, while the CEA was determined as the total energy available (Ea) subtracted by the energy consumption (Ec).

7.2.6 Data analysis

Statistical significance of the spatial and temporal variation of the metal concentrations in the fish and parasites was determined at $p < 0.05$. Normality and homogeneity of variance were tested using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively (GraphPad Prism v7). For parametric data, a Two-way ANOVA and Tukey's and Sidak's multiple comparison tests were performed. Kruskal-Wallis tests with Dunn's multiple comparison tests were performed on non-parametric data to test for significant differences between species, sites, and surveys. A Redundancy Analyses (RDA) was constructed to assess the association of biomarker responses to metal exposure between uninfected and infected fish and between sites, as well as associations of biomarker responses to metal exposure of infected fish liver and the accumulated parasite concentrations between sites. All of the data used for the RDA was log transformed, $x = \log (y + 1)$, as well as standardised and centred. Parasite-host element accumulation ratios were calculated as the ratio of the average metal concentration in the parasites per quantified metal concentration of the host species ($PHEAR_{cestode} = c_{cestode}/ c_{fish\ liver}$ and $PHEAR_{nematode} = c_{nematode}/ c_{fish\ liver}$; see Pérez-del-Olmo *et al*., 2019). The fish liver was used as host exposure level as it gives good information on the metal bioaccumulation within the host, since nematodes actively feed on the host's blood and tissue, while cestodes occur in the intestine and absorb nutrients through their tegument. Host bioconcentration factors ($BCF = c_{fish\ liver}/c_{water}$) and biota-sediment accumulation factors ($BSAF = c_{fish\ liver}/c_{sediment}$) were calculated as the ratio of the average metal concentration in the fish host per quantified metal concentration of filtered water and sediment samples, respectively.

7.3 Results

7.3.1 Parasite abundance

During the survey in November 2017, 23 non-native Common carp, *Cyprinus carpio* Linnaeus, 1758, were collected from Olifantsnek Dam and 10 from Bospoort Dam. Of these, six fish (48%) from Olifantsnek Dam and seven (70%) from Bospoort Dam were infected with the co-introduced cestode, *Atractolytocestus huronensis* Anthony, 1958. The mean intensity of *A. huronensis* was higher at Olifantsnek Dam (7 ± 4) than at Bospoort Dam (6 ± 3). During the survey in November 2018, indigenous sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), were collected at Olifantsnek Dam (8 specimens) and Bospoort Dam (13 specimens). All of the *C. gariepinus* specimens collected from both impoundments were infected with a nematode from the genus *Contracaecum* Railliet and Henry, 1912, with the mean intensity being higher in

specimens collected from Olifantsnek Dam (90 ± 65) than those collected from Bospoort Dam (21 ± 24).

7.3.2 Metal accumulation in host tissues and parasites

Metal concentrations in tissue samples (muscle and liver) of uninfected and infected *C. carpio*, and the cestode, *A. huronensis*, were compared between the two sampling sites (Olifantsnek Dam, reference site and Bospoort Dam, impacted site) during the November 2017 survey (Fig. 7.1; Appendix F, Table F1). Although not significant, the concentrations of Cr, Ni, Zn, and Pt in fish tissues were higher at Bospoort Dam compared to Olifantsnek Dam, while concentrations of Cu in liver samples from both uninfected and infected fish were significantly higher ($p < 0.0001$) at Olifantsnek Dam. Concentrations of Pt in liver samples of infected fish were significantly higher ($p = 0.0495$) at Bospoort Dam compared to Olifantsnek Dam. In the cestode samples, the concentrations of Cr, Cu, Cd, Pt, and Pb were not significant, higher at Olifantsnek Dam compared to Bospoort Dam, while the concentrations of Zn were higher at Bospoort Dam and only the concentrations of Ni were significantly higher ($p < 0.0001$) at Bospoort Dam. Between the two sites, only the concentrations of Cu ($p < 0.0001$; $F = 22.12$), Ni ($p = 0.0004$; $F = 14.31$), and Pt ($p = 0.013$; $F = 6.53$) were significantly different.

When comparing metal accumulation between uninfected and infected hosts, no significant differences were found. However, the concentrations of Cu, Zn, and Cd in muscle samples of infected hosts were lower compared to uninfected hosts at both impoundments. Concentrations of Cr and Ni in liver samples of infected hosts were lower than those of uninfected hosts at both impoundments, while concentrations of Pt in liver samples of infected hosts were lower than those of uninfected hosts at Olifantsnek Dam. Concentrations of Pb in both muscle and liver samples of infected hosts were lower compared to uninfected hosts at both impoundments. The cestode, *A. huronensis*, accumulated significantly higher concentrations of Cr ($p < 0.0001$; $F = 55.8$) compared to muscle and liver of its associated host at both impoundments, Ni ($p < 0.0001$; $F = 10.89$), and Pt ($p = 0.042$; $F = 2.63$) in the parasite-host system. The concentrations of Ni ($p < 0.0001$; $F = 10.89$), Cu ($p = 0.0002$; $F = 6.41$) and Pt ($p = 0.042$; $F = 2.63$) had a significant interaction between sites and the parasite-host system.

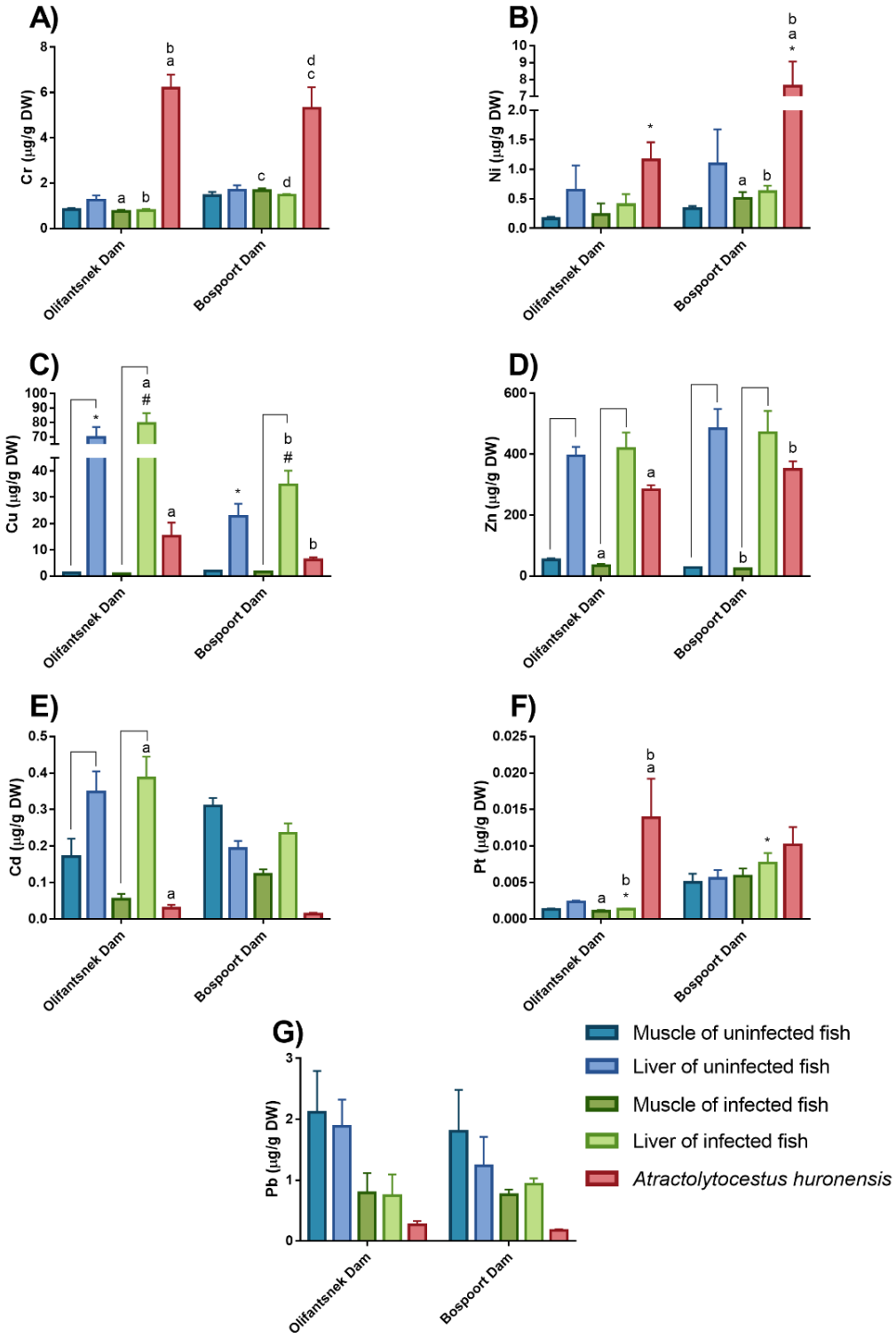

Figure 7.1: Mean concentrations (µg/g DW) of Cr (A), Ni (B), Cu (C), Zn (D), Cd (E), Pt (F) and Pb (G) with standard error of the mean in muscle and liver samples of uninfected and infected *Cyprinus carpio*, as well as the cestode, *Atractolytocestus huronensis*, collected from Olifantsnek Dam (n = 17; n = 6) and Bospoort Dam (n = 3; n = 7) in November 2017. Common alphabetical superscripts indicate significant differences ($p < 0.05$) between muscle and liver of infected fish and its cestode, while common symbolic superscripts indicate significant differences between sites and brackets indicate significant differences between tissues of uninfected and infected fish.

In the second survey in November 2018, similar comparisons were done with the metal concentrations in the muscle and liver samples of the host, *C. gariepinus*, and the nematode, *Contracaecum* sp. (Fig. 7.2; Appendix F, Table F2). However, here, all fish specimens were infected with the nematode and a comparison between uninfected and infected fish was not possible. The comparison between the sampling sites showed that the concentrations of Cr, Zn, Pt, and Pb in fish tissues from Bospoort Dam were higher compared to Olifantsnek Dam, while a reverse trend was found for Ni, Cu, and Cd. Only the concentrations of Pb in the muscle samples ($p = 0.013$) and Pt in the muscle and liver tissues ($p = 0.003$; $p = 0.002$) were significantly higher at Bospoort Dam, while concentrations of Ni and Cd in the liver tissue were significantly higher ($p = 0.0001$; $p = 0.036$) at Olifantsnek Dam. In contrast to the cestodes, the nematodes demonstrated only for Ni ($p = 0.007$, at Bospoort Dam only) and Pt ($p = 0.019$, at Olifantsnek Dam only) significantly higher concentrations than both host tissues. All of the metal concentrations in *Contracaecum* sp. were higher at Bospoort Dam compared to Olifantsnek Dam, albeit not significant. Host and parasite metal accumulation of concentrations of Cr ($p < 0.0001$; $F = 27.97$), Ni ($p = 0.041$; $F = 3.38$), Cu ($p < 0.0001$; $F = 75.41$), Zn ($p < 0.0001$; $F = 88.02$), Cd ($p < 0.0001$; $F = 16.04$), and Pt ($p = 0.004$; $F = 6.13$) were significant, while concentrations of Pt ($p < 0.0001$; $F = 27.90$) and Pb ($p = 0.018$; $F = 6.00$) were significantly different between sites. For Ni and Cd, there was a significant interaction between both the host and parasite accumulation and sites ($p = 0.019$; $F = 4.28$ and $p = 0.0001$; $F = 10.61$).

Between the two different parasite-host systems, both *C. carpio* and *C. gariepinus* accumulated higher concentrations of Cr, Zn, and Pt at Bospoort Dam and higher concentrations of Cu and Cd at Olifantsnek Dam. *Cyprinus carpio* accumulated higher concentrations of Ni at Bospoort Dam and higher concentrations of Pb at Olifantsnek Dam, while *C. gariepinus* accumulated higher concentrations of Pb at Bospoort Dam and higher concentrations of Ni at Olifantsnek Dam. The cestode, *A. huronensis*, accumulated higher concentrations of Cr, Cu, Cd, Pt, and Pb at Olifantsnek Dam and higher concentrations of Ni and Zn at Bospoort Dam, while the nematode, *Contracaecum* sp. accumulated higher concentrations of all the metals analysed during the present study at Bospoort Dam.

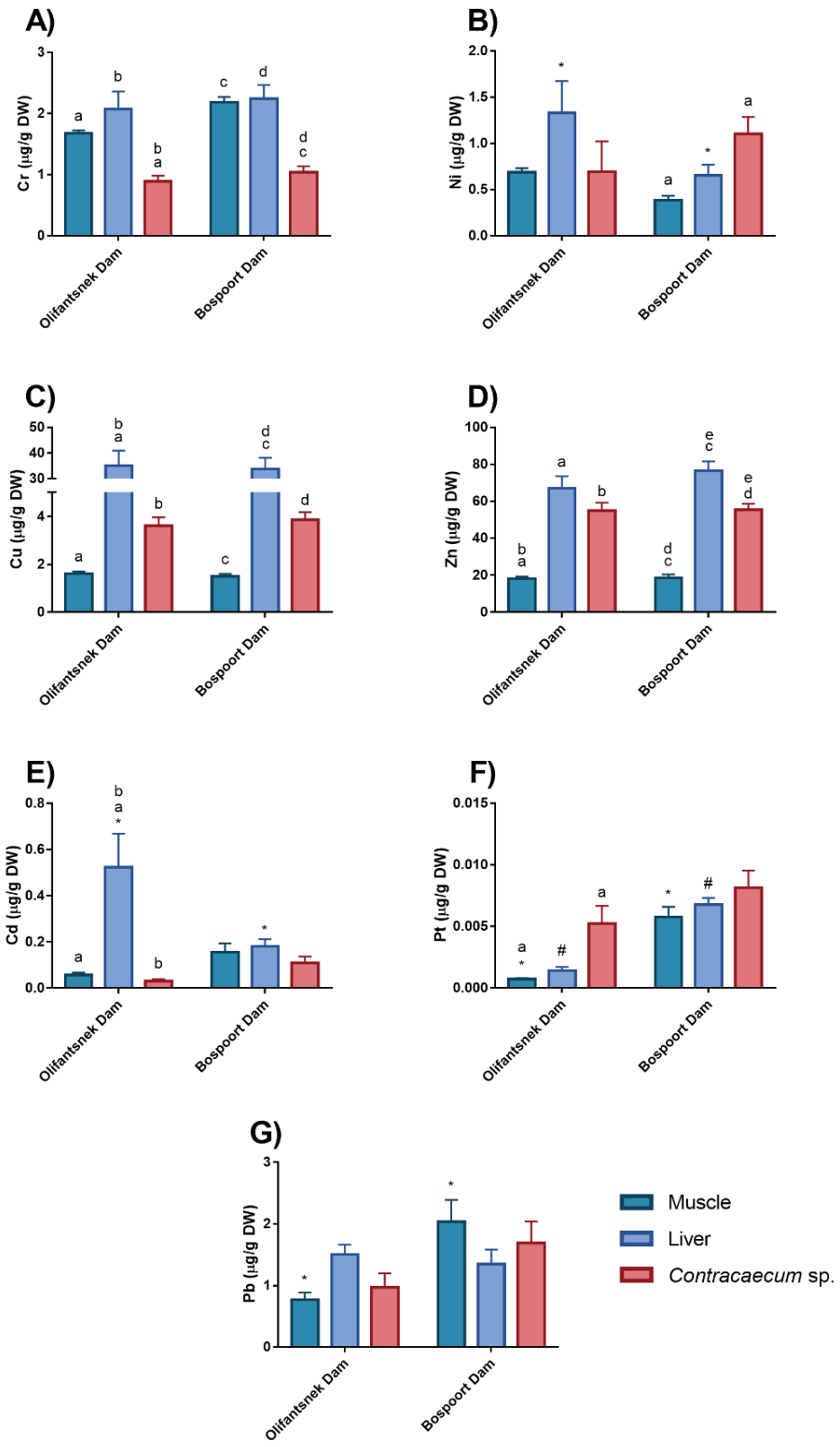

Figure 7.2: Mean concentrations (µg/g DW) of Cr (A), Ni (B), Cu (C), Zn (D), Cd (E), Pt (F) and Pb (G) with standard error of the mean in muscle and liver samples of *Clarias gariepinus*, as well as the nematode, *Contracaecum* sp., collected from Olifantsnek Dam (n = 8) and Bospoort Dam (n = 13) in November 2018. Common alphabetical superscripts indicate significant differences ($p < 0.05$) between *Contracaecum* sp., muscle, and liver, while common symbolic superscripts indicate significant differences between sites.

7.3.3 Bioconcentration factors, biota-sediment accumulation factors, and parasite-host element accumulation ratios

The BCF ranged from 15 (Ni at Bospoort Dam) to 26 737 (Zn at Bospoort Dam) for *C. carpio*, whereas for *C. gariepinus* the BCF ranged between 20 (Ni at Bospoort Dam) and 20 425 (Cd at Olifantsnek Dam) (Table 7.2). The BSAF for *C. carpio* ranged from 0.002 (Cr at Olifantsnek Dam) to 29.1 (Zn at Bospoort Dam), while the BSAF for *C. gariepinus* ranged between 0.005 (Cr at Olifantsnek Dam) and 13.1 (Cd at Olifantsnek Dam). The parasite-host element accumulation ratios ($PHEAR_{cestode}$) ranged from 0.06 (Cd at Bospoort Dam) to 13.7 (Ni at Bospoort Dam), whereas the $PHEAR_{nematode}$ ranged between 0.08 (Cd at Olifantsnek Dam) and 4.5 (Pt at Olifantsnek Dam). The BCF for *C. carpio* and water from Olifantsnek Dam decreased in the order Zn > Cu > Cr > Cd > Pt > Ni > Pb, while the order at Bospoort Dam was Zn > Cr > Cu > Cd > Pb > Pt > Ni. The order of the BCF for *C. gariepinus* and water from Olifantsnek Dam was Cd > Cu > Pb > Cr > Zn > Pt > Ni, whereas at Bospoort Dam the order was Zn > Cd > Cr > Cu > Pb > Pt > Ni. The BSAF for *C. carpio* and sediment at Olifantsnek Dam was Zn > Cu > Cd > Pt > Pb > Ni > Cr, while the order at Bospoort Dam was Zn > Cu > Cd > Pt > Pb > Cr > Ni. The order of the BSAF for *C. gariepinus* and sediment from Olifantsnek Dam was Cd > Zn > Cu > Pb > Pt > Ni > Cr, whereas at Bospoort Dam the order was Cd > Cu > Zn > Pt > Pb > Cr > Ni. For Cr, Ni, Pt, and Pb the PHEAR was higher for the cestode than for the nematode, whereas for Cu, Zn, and Cd no differences between the two parasite taxa occurred. The $PHEAR_{cestode}$ for *A. huronensis* and *C. carpio* from Olifantsnek Dam decreased in the order Pt > Cr > Ni > Pb > Zn > Cu > Cd, while the order at Bospoort Dam was Ni > Cr > Pt > Zn > Pb > Cu > Cd. The order of the $PHEAR_{nematode}$ for *Contracaecum* sp. and *C. gariepinus* from Olifantsnek Dam was Pt > Zn > Ni > Pb > Cr > Cu > Cd, whereas at Bospoort Dam the order was Ni > Pb > Pt > Cd > Zn > Cr > Cu.

Table 7.2: Bioconcentration factors (BCF), biota-sediment accumulation factors (BSAF) and parasite-host element accumulation ratios (PHEAR) calculated as the ratio of the metal concentration in fish host (*Cyprinus carpio*; *Clarias gariepinus*) liver tissue, and parasites (*Atractolytocestus huronensis*; *Contracaecum* sp.), as well as metal concentration of the water and sediment, and their associated host's liver tissue, collected from Olifantsnek Dam and Bospoort Dam.

	Olifantsnek Dam			Bospoort Dam		
	BCF	BSAF	PHEAR	BCF	BSAF	PHEAR
Cyprinus carpio						
Cd	544	1.63	0.08	445	2.40	0.06
Cr	1 122	0.0020	8.24	4 442	0.0083	3.66
Cu	3 868	3.93	0.19	3 911	2.69	0.18
Ni	46	0.0060	7.49	15	0.0077	13.68
Pb	36	0.083	3.37	227	0.19	0.20
Pt	157	0.088	11.74	173	0.48	1.56
Zn	10 016	16.90	0.74	26 737	29.12	0.81
Clarias gariepinus						
Cd	20 425	13.14	0.08	8 261	6.00	0.86
Cr	4 053	0.0048	0.48	7 121	0.033	0.50
Cu	5 025	1.55	0.13	6 965	3.39	0.16
Ni	219	0.024	0.67	20	0.0097	2.26
Pb	4 350	0.41	0.61	5 102	0.46	1.77
Pt	241	0.16	4.53	266	0.93	1.30
Zn	2 885	1.59	0.90	9 591	3.35	0.78

7.3.4 Biological effects in Cyprinus carpio

No significant differences between the sampling sites were found for both biomarkers of exposure, AChE activity and MT content (Fig. 7.3 A – B). However, although without statistical significance, the MT content in the liver was higher in infected *C. carpio* than in uninfected specimens. The results for the biomarkers of effects (Fig. 7.3 C – G) indicated no significant spatial differences, except for GSH content ($p = 0.020$) in infected *C. carpio* and MDA content ($p = 0.036$) in uninfected specimens.

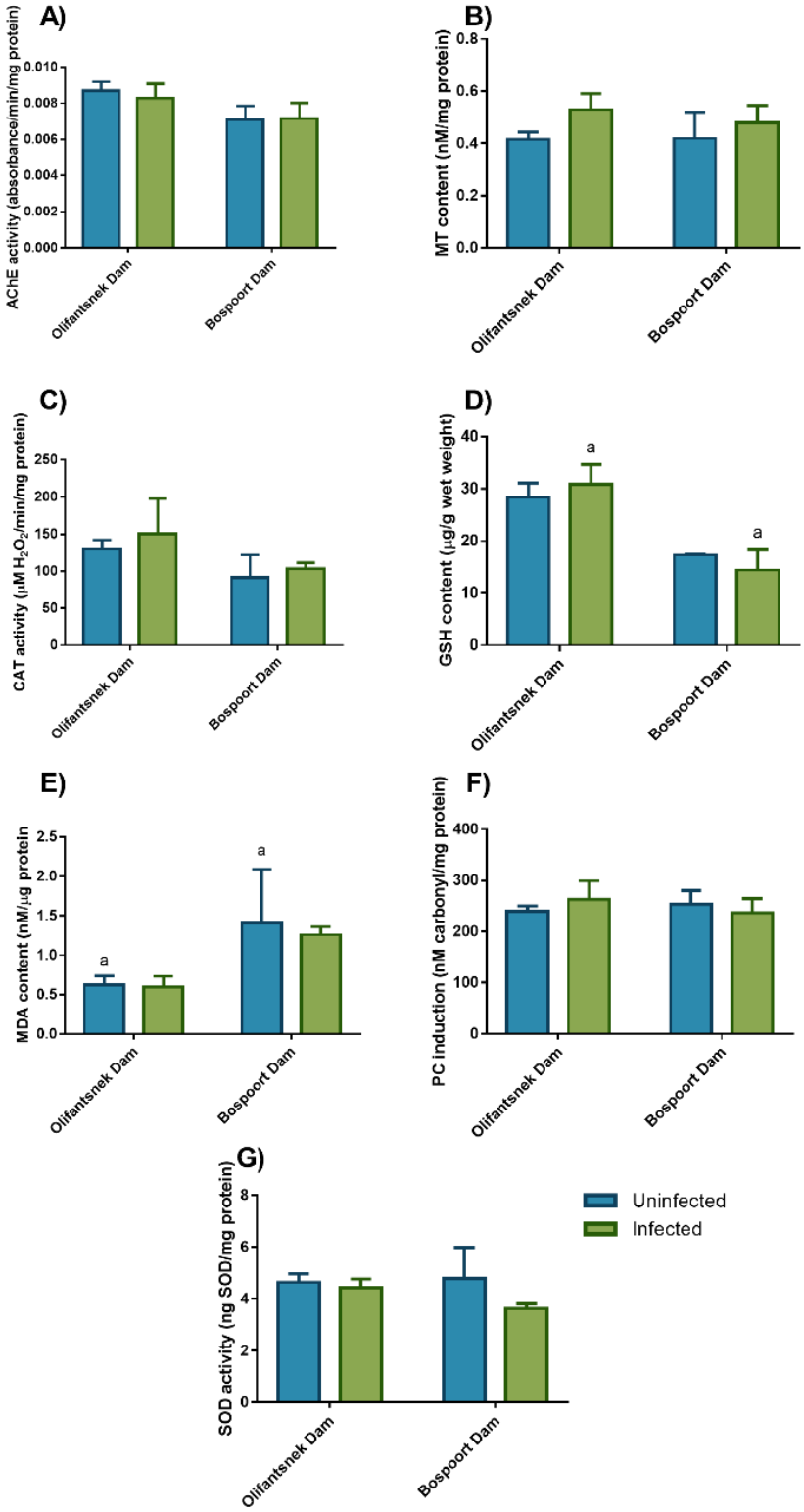

Figure 7.3: Mean with standard error of biomarkers of exposure [A – acetylcholine esterase activity (AChE), B – metallothionein content (MT)] and effect [C – catalase activity (CAT), D – reduced glutathione content (GSH), E – malondialdehyde content (MDA), F – protein carbonyls induction (PC), G – superoxide dismutase (SOD)] from infected and uninfected *Cyprinus carpio* collected in Olifantsnek Dam (n = 17; n = 6) and Bospoort Dam (n = 3; n = 7). All biomarkers were analysed in liver tissue, except AChE, which was analysed in the brain. Common alphabetical superscripts indicate significant differences between sites.

The CEA results indicated significant spatial differences in the cellular protein reserves, total energy reserves (Ea), energy consumption (Ec) and CEA of *C. carpio*, showing higher values at Bospoort Dam than at Olifantsnek Dam (Fig. 7.4 A, D – F). However, statistical significance occurred only for the protein reserves, total energy reserves and CEA, of infected fish. The infected *C. carpio* collected at Olifantsnek Dam had significantly lower (p = 0.03) cellular protein reserves, total energy reserves, and CEA compared to the uninfected fish. No effect of the sampling site and parasitic infection, respectively, revealed in the lipid and glycogen reserves (Fig. 7.4 B, C).

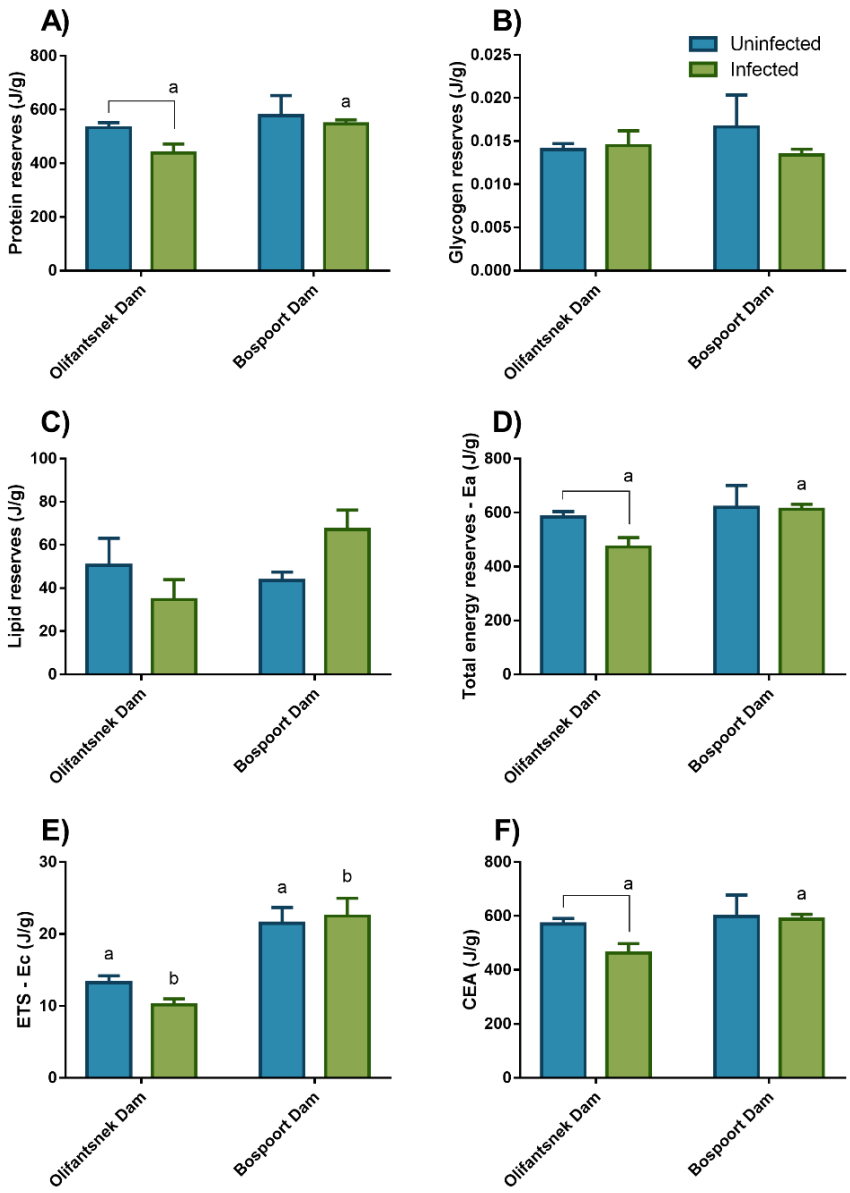

Figure 7.4: Mean with standard error of the different cellular energy reserves [A – protein reserves, B – glycogen reserves, C – lipid reserves, D – total energy reserves (Ea), E – energy consumption (Ec), and F – cellular energy allocation (CEA)] in muscle samples from infected and uninfected *Cyprinus carpio* collected in Olifantsnek Dam (n = 17; n = 6) and Bospoort Dam (n = 3; n = 7). Common alphabetical superscripts indicate significant differences between sites, while brackets indicate significant differences between infected and uninfected fish.

The RDA triplot of biomarker responses, as well as metal bioaccumulation in cestodes and liver samples of uninfected and infected *C. carpio* collected at the two sampling sites in November 2017, indicated clear spatial differences (Fig. 7.5). The Bospoort Dam sampling site was associated with the bioaccumulation of Cr, Ni, and Zn (both cestode and fish liver), Pt (fish liver only), as well as Cu and Pb (cestode only). Additionally, this sampling site (and the above-mentioned metal bioaccumulation in fish liver and cestodes) was associated with the CEA and MDA biomarkers. In contrast, Olifantsnek Dam was associated with the bioaccumulation of Cd (fish liver and cestode), Cu and Pb (fish liver only), as well as with the exposure biomarker AChE and the effect biomarkers CAT, SOD, GSH, and PC. No clear differences were evident between uninfected and infected fish.

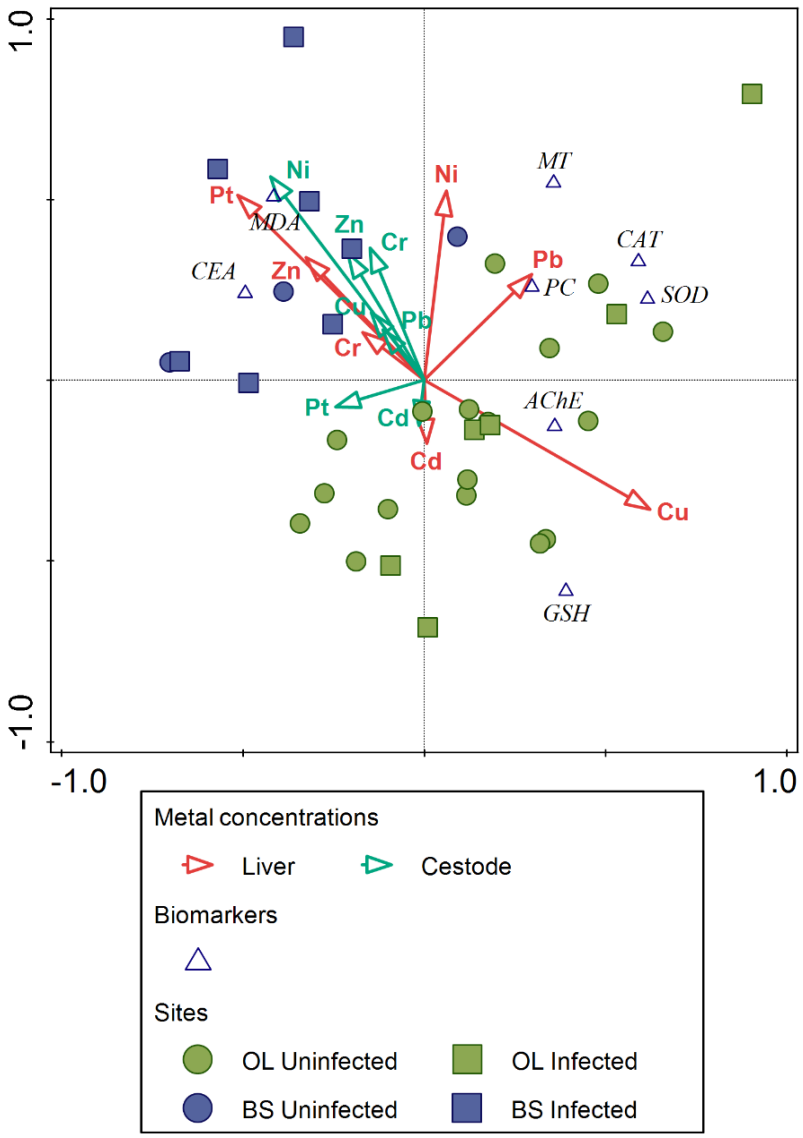

Figure 7.5: RDA triplot of biomarker responses and metal bioaccumulation in the cestode, *Atractolytocestus huronensis*, and the liver of uninfected and infected *Cyprinus carpio* from Olifantsnek Dam (OL) and Bospoort Dam (BS) during November 2017. The triplot describes 35.5% of the variation with 20.5% on the first axis and 15.0% on the second axis. See text for biomarker abbreviations.

7.4 Discussion

7.4.1 Metal accumulation in fish hosts and associated parasites

Metal accumulation in fish collected from the two impoundments in the Hex River catchment indicates that this river system is subjected to Pt mining effluent which contains various metals (Cr, Ni, and Pt). This is in accordance with the results found in Chapter 5, where metal accumulation in macroinvertebrate families, collected from the Hex River, were assessed. An increase in metal concentrations (Cr, Ni, Zn, Pt and Cr, Zn, Pt, Pb) were evident in both fish species, *C. carpio* and *C. gariepinus*, from Olifantsnek Dam and Bospoort Dam. Among the two host species, *C. gariepinus* accumulated the highest concentrations of Cr, Ni, Cu, Cd, and Pb in the muscle tissue, while *C. carpio* accumulated the highest concentrations of Zn and Pt in the muscle tissue. The general accumulation trends in the liver tissue were the same, except for *C. carpio*, which accumulated in addition to Zn and Pt, also high concentrations of Cu. According to Musa *et al*. (2017), due to their feeding habits and close association with the sediments, *C. gariepinus* can be considered as an ideal indicator species for metals in an aquatic system. The concentrations obtained during the present study corresponds with metal concentrations found in similar systems that are affected by anthropogenic influences (Coetzee *et al*., 2002; Crafford and Avenant-Oldewage, 2010; Musa *et al*., 2017).

Considering the parasites, the cestode, *A. huronensis*, accumulated higher concentrations of Cr, Ni, Cu, Pt, and Pb, while the nematode, *Contracaecum* sp. accumulated higher concentrations of Cd and Pb than the host tissues. The phenomenon that parasites have the ability to accumulate these metals in far greater concentrations than their hosts, were also found in other studies for acanthocephalans and Cr, Cu, Cd, Pt, and Pb (Sures and Siddall 1999; Zimmermann *et al*., 2005), for cestodes and Cr, Ni, Cu, Zn, Cd, Pt, and Pb (Tekin-Özan and Kir, 2005; Jirsa *et al*., 2008; Eira *et al*., 2009; Oyoo-Okoth *et al*., 2010; Baruš *et al*., 2012), and for nematodes and Cr, Ni, Cu, Zn, Cd, and Pb (Baruš *et al*., 2007; Akinsanya and Kuton, 2016; Leite *et al*., 2017).

7.4.2 Bioconcentration factors, biota-sediment accumulation factors, and parasite-host element accumulation ratios

The BCF and BSAF of the hosts indicated that the fish accumulated essential metals (Cu and Zn) from the water and sediment that they are exposed to, while the non-essential metals (Ni, Cd, Pt, and Pb) were not readily accumulated. However, *C. gariepinus* from both Olifantsnek Dam and Bospoort Dam accumulated Cd more easily from the environment than Cu or Zn. On the other hand, the cestodes and nematodes collected, accumulated non-essential metals more readily than the essential metals. The results from the present study clearly showed that the cestode, *A. huronensis*, is a good organism to use for the monitoring of Cr, Ni and Pt, while the nematode, *Contracaecum* sp. is one for Ni, Pt, and Pb, as these organisms accumulated these metals effectively from their hosts at both impoundments. This is in accordance with Sures *et al*. (2017),

who concluded that cestodes are pollutant sinks, especially for non-essential elements and have elevated resistance to the toxic effects.

The PHEARs of both the cestode and nematode species were compared with accumulation factors from the literature of different microhabitats within their fish hosts. The bioaccumulation factors calculated for the cestode samples correspond with the bioaccumulation factors calculated by Tekin-Özan and Kir (2005), Jirsa *et al*. (2008), Eira *et al*. (2009), Oyoo-Okoth *et al*. (2010), as well as Baruš *et al*. (2012). The bioaccumulation factors calculated for the nematode samples were all lower than reported by Baruš *et al*. (2007), Akinsanye and Kuton (2016), as well as Leite *et al*. (2017). Regarding the bioaccumulation of PGE by the parasites, the acanthocephalan, *Paratenuisentis ambiguus*, had an accumulation factor of 42 as reported by Zimmermann *et al*. (2005), while the cestode, *A. huronensis*, from the present study had an accumulation factor of 12. On the other hand, the nematode, *Anguillicola crassus*, had an accumulation factor of 0.5 as found by Eira *et al*. (2009), and the nematode, *Contracaecum* sp. from the present study had a maximum accumulation factor of 4.5. The accumulation factors of the parasites analysed in the present study indicate that Pt deriving from PGE mining effluent, are as bioavailable as Pt deriving from auto catalysts, or even more for nematodes, in particular.

The lack of significant differences between infected and uninfected hosts during the present study can be ascribed to the small fish sample size, however, general trends could still be observed between infected and uninfected hosts. Infected *C. carpio* specimens exhibited lower mean metal concentrations (Cr, Ni, Cd, Pt, Pb) than their uninfected conspecifics. This was also found in laboratory experiments for chub (*Leuciscus cephalus*) infected with the acanthocephalan *Pomphorhynchus laevis* (Sures and Siddall, 1999), as well as for wild caught fish infected with cestodes (Gabrashanska and Nedeva, 1996; Turcekova and Hanzelova, 1999). It is, therefore, essential to assess both infected and uninfected fish from aquatic ecosystems to determine metal exposure in a certain system and, to obtain a holistic overview of metal exposure in such a system. If only uninfected fish are assessed in an aquatic system, a potential overestimation of metal concentrations in the system can occur, and *vice versa*.

7.4.3 Effects of metal exposure and parasite infection on biomarker responses in fish

In the present study, biomarker responses of infected and uninfected fish (*C. carpio*), as well as fish from a reference and polluted site were assessed. The biomarkers of exposure, AChE, and MT, indicated that although the metal concentrations in the host's tissues were lower in infected specimens, these individuals were still exposed to metal stress and needed protection strategies to remediate the stressor. The increase of MT concentrations in infected fish from both impoundments contradicts the results found by Baudrimont and colleagues (Baudrimont *et al*., 2006; Baudrimont and de Montaudouin, 2007), who found that MT concentrations decreased in Cd-exposed cockles that were infected by digenean parasites. The biomarkers of effect, however, had no major changes between infected and uninfected specimens, whereas an

increase in MDA concentrations at Bospoort Dam indicates an increase in reactive oxygen species. The CEA concentrations indicated that *C. carpio* collected from Olifantsnek Dam (reference site) had in general less energy available compared to *C. carpio* from Bospoort Dam (impacted site). This increase in energy availability indicates that there are more food sources available in Bospoort Dam and correlates with the increase in nutrients entering this impoundment from the wastewater treatment plant's effluent and raw sewage from the surrounding informal settlements. These upsurge in nutrients create the perfect environment for marginal and water foliage to thrive and, in return, create more food sources for the fish. Although the fish from Bospoort Dam has more energy available, they also consume more energy to protect themselves against stressors caused by an increase in metal exposure.

From the RDA that assessed the association of biomarker responses to metal exposure between uninfected and infected *C. carpio*, as well as between sites, a clear spatial difference was evident between the reference and impacted sites, while there were no clear differences between uninfected and infected fish. However, clear spatial differences were evident for liver metal exposure and parasite exposure. It was also evident that when parasites accumulated higher metal concentrations, a decrease occurred in oxidative stress and AChE inhibition. This can indicate that the parasites can detoxify the fish host from metal concentrations and may have positive effects on the fish.

7.5 Conclusion

The results of the present study provide valuable information on the Pt emissions from mining and production activities in an aquatic system. It also provides information on Pt accumulation in parasite-host systems deriving from PGE mining effluent. The fish and their parasites accumulated higher metal concentrations associated with PGE mining at the impacted site compared to geology borne metals at the reference site. Fish hosts accumulated essential metals more readily from the environment, while the parasites accumulated non-essential metals more easily from their associated hosts. Rather than the fish host species, the use of cestodes and nematodes as accumulation indicators can provide valuable information regarding trace metal pollution. The cestode, *A. huronensis*, proved to be a potential monitoring organism for Cr, Ni, and Pt, while the nematode, *Contracaecum* sp. is a potential organism to monitor Ni, Pt, and Pb. Infected fish were associated with lower metal concentrations (Cr, Ni, Cd, Pt, Pb) compared to uninfected fish, while the parasites had significantly higher metal concentrations (cestode – Cr, Ni, Pt; nematode – Ni, Pt) than their hosts. The parasites demonstrated that the metals and their compounds are biologically available in areas with PGE mining effluent and therefore they can be used as sensitive biological indicators.

CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

8.1 General remarks

With the demand for PGE worldwide still on the rise and mining companies struggling to meet these demands, more mining activities and effective ore processing are needed. As South Africa produces three-quarters of the world's Pt supplies, the pressure to develop more mining activities in the country will increase. However, the full extent of the environmental impacts of the existing Pt mining activities is not understood, making it difficult to effectively implement monitoring and remediation plans. The Hex River is one of the major river systems draining these intensive Pt mining regions in South Africa and is an ideal study area to assess the impacts of PGE mining activities on the aquatic environment. What makes this river ideal is that the upper reaches have no mining impacts with only minor agricultural activities, while the lower reaches are heavily impacted by Pt mining and production activities. Furthermore, the effects of urban and industrial effluent, within this system can also be assessed due to Rustenburg and surrounding urban developments. This system is not only impacted by metal contamination but also experiences additional stressors such as reduced runoff due to drought (as experienced during the November 2017 survey). Increased nutrient inputs from mining and urban effluent and raw sewage spills from informal settlements (encountered during the March 2018 survey) enter the Hex River. Not only does the ecosystem health of the Hex River deteriorate but these stressors can also pose detrimental effects on wildlife, livestock, as well as human communities that utilise the water from this river system. Therefore, the present study contributes to an understanding of the current condition of the Hex River and assess the effects of these stressors on various abiotic and biotic components of the river system. The following conclusions were derived from each of the specific aims set for the present study.

8.2 Assessing ecosystem health of rivers

8.2.1 Water and sediment quality assessment

The Bushveld Igneous Complex is a unique geological formation and is one of the largest layered igneous intrusions and contains some of the richest mineral deposits in the world. This unique complex consists of two platinum-bearing layers that contain 75% of the world's Pt resources. Various metal concentrations in the Bushveld Igneous Complex are several times higher than normal upper continental crust values, resulting in naturally elevated background metal concentrations. Nevertheless, various anthropogenic activities in the Hex River system significantly increase the metal concentrations over the already elevated background concentrations. From these various anthropogenic activities, Pt mining and refining activities were the main sources of Cr, Ni, Cu, As, and Pt contamination, while urban effluent contributed to Zn, Cd, and Pb contamination in the water and sediment of the Hex River. The results indicated that

the metal concentrations in the Hex River increased significantly compared to previous studies completed in the same river between 2014 and 2016. Since mining and production activities will potentially increase, causing the water and sediment quality of the river to further decrease, the results of the present study warrant the call for an extensive monitoring program in the Hex River. In addition, strategies are also required to remediate the quality of the Hex River since the current water and sediment quality of the river already pose risks to the environment and human communities in the catchment.

Interactions between water and sediment metal concentrations in the Hex River were also evident at several sites. A decrease in dissolved concentrations of all the metals with a concomitant increase in sediment levels was evident at sites HX 7, KF, HX 8, DS 1, and HX 9, and *vice versa*. This phenomenon can be ascribed to several factors, including binding of metals to form complexes, adsorption, and precipitation processes. Sediment-bound metals can be resuspended into the water column by physical, chemical or hydrological disturbances. Irrespective of whether the metal concentrations occur in dissolved form within the water column or the sediment, it can become bioavailable to aquatic biota and accumulate via dietary routes or absorption and adsorption processes.

Compared to national and international water and sediment quality guidelines for healthy aquatic ecosystems, the concentrations of Ni, Cu, Zn, and As exceeded the water and sediment guidelines, while the concentrations of Cr exceeded the sediment guidelines. Although no guideline values for Pt exist, concentrations of Pt in water and sediment in the Hex River were 77-fold and four-fold higher, respectively, than concentrations of Pt reported in intensive urban impacted river systems worldwide. These high concentrations are regarded as highly polluted and pose a potential ecosystem health risk. It also poses a potential human health risk to several communities that utilize the water and ecosystem services provided by the Hex River system.

Water and sediment quality variables varied significantly on a temporal and spatial scale. These fluctuations are due to variation in flow patterns, pollutant concentrations, land-use, and water chemistry. Rivers, such as the Hex River, that are prone to drought conditions have an additional stressor as the drought-rewetting process influence metal behaviour. Due to fluctuations in the hydrology of rivers, it is essential to monitor water and sediment quality seasonally, to accurately assess the river health. The results obtained during the present study indicated that there are several interactions between dissolved and sediment metal concentrations and water and sediment quality variables. These physicochemical variables influence the toxicity, bioavailability and uptake of metals. Although the metal concentrations in the Hex River are elevated and exceed several quality guideline values, it is potentially not in a toxic or bioavailable form to aquatic organisms. However, this needed to be assessed and confirmed. Therefore, several biomonitoring approaches were utilized to assess the exposure and effects of metals to the aquatic biota.

The first hypothesis tested for this part of the study, namely that Pt smelters and production activities will be the main contributor to metal pollution in the Hex River, was supported by the data. However, urban and industrial effluent also contributed to the metal contamination in this catchment. The data also supported the hypothesis that the seasonally influenced physicochemical characteristics of water and sediments will influence the behaviour of Pt and associated metals in the aquatic environment.

8.2.2 Aquatic macroinvertebrate assessment

Macroinvertebrate community structures can be influenced by several factors such as water and sediment quality, habitat availability, biological traits (behavioural, physiological characteristics, and life cycle stages), as well as ecological traits (habitat preference, tolerance towards organic enrichment, and biogeographic distribution). For the present study, macroinvertebrate community structures were determined using the traditional taxonomy-based approach, as well as trait-based approach. Clear spatial changes in the community structures were evident. The mining and urban impacted sites had significantly lower species diversity and were dominated by tolerant species, compared to the reference sites, with higher species diversity and more moderately and highly sensitive species. These changes can be ascribed to the fact that the lower reaches of the Hex River are largely organically enriched and the tolerant species were able to thrive, although not to the extent to prohibit the occurrence of moderately sensitive species. Species response curves were constructed to assess the effects of Pt mining effluent on macroinvertebrate species and traits. It was evident that several sensitive species and traits were negatively correlated with total hardness, chlorides, sulphates and metals (Cr, Ni, and Pt) associated with mining effluent. It also indicated that several tolerant species and traits had a significant positive correlation with these pollutants. As these pollutants increased, the density of these tolerant species and traits increased. The results indicated that macroinvertebrate species (*e.g. Physella acuta*, *Tubifex* sp., Chironomidae, Muscidae) and traits (*e.g.* highly tolerant (HT), Decapoda, air breathers using a spiracle (A:S) and breathing tube or other apparatus (A/V)) have adapted or had the ability to overcome these stressors and survive in this impacted system.

Clear temporal changes in the macroinvertebrate community structures were also evident. An extreme change in the community structures was evident during the November 2017 survey brought about by the lower water volume, flow-rate, and increase in pollutants during the drought. Species adapted for these conditions became dominant (*e.g.* Chironomidae, *Tubifex* sp.), while species that cannot tolerate the conditions were supressed (*e.g. Pseudagrion* sp., *Cheumatopsyche thomasseti*, *Hydreana* sp.). However, following the alleviation of the drought conditions, a partial recovery in the macroinvertebrate community structures to the pre-drought structure was evident within four months. This indicates the ecological resilience of the system through the ability of macroinvertebrates to colonise fresh water ecosystems after a drought

event, via aerial dispersal by adult invertebrates, recolonization from upper or lower reaches that were not affected by the drought, or eggs that are resistant to desiccation.

Different macroinvertebrate families were collected for metal bioaccumulation assessment, *i.e.* Lymnaeidae (snails), Baetidae (mayflies), Potamonautidae (crabs), Coenagrionidae (damselflies), Libellulidae (dragonflies), Hydropsychidae (caddisflies), Tubificidae (aquatic earthworms), and Chironomidae (midge larvae). Taxa were selected to represent different trophic levels, different feeding strategies and different habitat preferences. The metal bioaccumulation data indicated that most of the families accumulated higher metal concentrations at the mining impacted site. The families associated with the sediments accumulated the highest metal concentrations, while the families Lymnaeidae, Baetidae, Tubificidae, and Chironomidae emerged as promising biomonitoring indicator organisms in these intensively mining impacted systems. The families from lower trophic levels accumulated higher metal concentrations than the predator families. This was ascribed to the fact that predator families have better developed metal regulating systems and normally have a higher mass to surface ratio than lower trophic level families.

The Tubificidae accumulated concentrations of Pt 55-fold higher than fresh water clams from an intensively urban impacted river. This indicated that the bioavailability of Pt derived from mining activities were similar or even higher than Pt derived from auto-catalysts. The present study demonstrated for the first time that Pt from mining activities was bioavailable to different aquatic macroinvertebrates and provided the crucial data needed on the occurrence of Pt in aquatic biota near Pt production sites.

Although the results also provided valuable information on trait-related responses to Pt exposure, it was found that combined with the traditional taxonomy-based approach information on both the ecosystem structure and function responses can be obtained. Therefore, the hypotheses tested for this part of the study, namely that macroinvertebrate community structures will be significantly altered from the reference conditions due to Pt mining related responses, and that macroinvertebrate traits will be altered as a result of changes in water and sediment quality, were supported by the data. Furthermore, the data supported the hypothesis that macroinvertebrate families from the impacted sites will accumulate higher metal concentrations compared to the reference sites. The hypothesis that predator families will accumulate higher metal concentrations than lower trophic level families was, however, not supported by the data.

8.2.3 Fish assessment

The three economic and ecological important fish species that were assessed during the present study were the non-native common carp (*Cyprinus carpio*), indigenous Sharptooth catfish (*Clarias gariepinus*), and indigenous Mozambique tilapia (*Oreochromis mossambicus*). These fish species are abundant and are targeted by subsistence fishers as a food source in the study area, making it relevant to assess the fish health and potential human health risk posed in the Hex River catchment. With the exception of *O. mossambicus*, the fish collected from Bospoort Dam (impacted impoundment), were generally larger and had higher condition factors compared to Olifantsnek Dam (reference impoundment). This was ascribed to the increased nutrients input to Bospoort Dam and resultant in higher food availability and protective vegetation cover against natural predators.

The fish collected at Bospoort Dam had significantly higher Cr, Ni, As, and Pt concentrations compared to the fish from Olifantsnek Dam. This was indicative of the higher metal bioavailability from water and sediments. The Mozambique tilapia, *O. mossambicus*, had the highest bioconcentration and biota-sediment accumulation factors for Cr, Ni, Cu, As, Pt, and Pb. Concentrations of As were five-fold and six-fold higher in *O. mossambicus* and *C. gariepinus*, respectively, compared to studies completed in other intensively mining impacted rivers of South Africa. The concentrations of Pt were 13-fold higher in *C. carpio* compared to fish collected from an intensively urban impacted river (Danube River, Hungary), indicating that Pt derived from mining activities was more bioavailable than Pt derived from auto-catalyst sources. The present study makes a significant contribution to increasing the knowledge on Pt exposure and bioaccumulation in aquatic biota from systems associated with Pt production sites.

The high metal concentrations in the three fish species studied, pose a risk to the fish and the ecosystem health. Since many local communities in the Hex River catchment rely on fish as a source of protein the metals also pose a risk to human health. During the present study a basic human health risk assessment was completed on the assumption that a person weighing 60 kg, consume a 150 g portion of fish muscle twice a week. Arsenic exceeded the safe hazard quotient (HQ) of 1 for all three fish species from both impoundments. However, the risk associated with consuming the three fish species from Bospoort Dam compared to Olifantsnek Dam was 20, 90 and 10% greater for *C. carpio*, *C. gariepinus,* and *O. mossambicus*, respectively. The risk is the greatest at Bospoort Dam as several subsistence fishers catch fish from Bospoort Dam and sell it to local communities as a food source. Risk from consuming contaminated fish from Olifantsnek Dam is lower since they are not consumed as the impoundment managers applies a strict catch and release policy. The individuals who are the most susceptible to metal health risks are pregnant or breast-feeding mothers and children. While no HQ values could be calculated for Pt since there is no reference dose for Pt, it may potentially pose several human health risks as its bioavailability increases. Previous studies have determined that Pt uptake in humans via

dietary routes only accounted for 17%, while the much greater risk is through inhalation, which accounted for 36%. Therefore, humans living in the Hex River catchment are not only affected by the consumption of contaminated fish but also by inhalation of Pt dust from waste dumps, emissions of Pt smelters and from auto-catalysts. Even though the present study only completed a basic desktop human health risk assessment calculation, it provided valuable information on the risks associated with the consumption of contaminated fish from the Hex River catchment. The hypotheses tested, namely that fishes collected from the impacted impoundment will accumulate higher metal concentrations than the reference impoundment, and will consequently pose a human health risk to persons who consume these fish, were supported by the data.

8.2.4 Environmental parasitology

Parasites are regarded as effective biomonitoring indicator organisms due to their ability to accumulate non-essential metals order of magnitudes higher than their associated hosts. Parasites can, therefore, be used to reliably detect metals with a naturally low abundance in the environment. Several parasitic groups such as cestodes and acanthocephalans lack a digestive system and bioaccumulate metals directly from their surrounding environment thereby indicating which metals are bioavailable. The interaction and metal accumulation between endoparasitic helminths, the cestode, *Atractolytocestus huronensis*, and its associated host *C. carpio*, as well as the nematode, *Contracaecum* sp., and its associated host *C. gariepinus* were assessed. The cestode, *A. huronensis*, accumulated significantly higher Cr, Ni and Pt than its host, while the nematode, *Contracaecum* sp., accumulated significantly higher Ni and Pt than its associated host. The fish hosts accumulated essential metals more easily while the parasites accumulated the non-essential metals. The assessment of parasite-host systems provides more effective and holistic insight into metal contamination in aquatic ecosystems.

The metal bioaccumulation in infected versus uninfected hosts was also assessed to establish whether parasites can have a positive or mutualistic effect on their hosts, and not only negative effects as is mostly assumed. The results indicated that infected hosts from both impoundments accumulated lower metal concentrations (Cr, Ni, Cd, Pt, and Pb; all non-essential metals) compared to uninfected hosts, while the parasites accumulated these metals in higher to significantly higher concentrations than their hosts. It is evident that parasites can protect their hosts, by accumulating potentially toxic metals from the host thereby decreasing the toxic effects thereof on the host. Only three previous studies have been published on acanthocephalans exposed to auto-catalyst materials and one field study focussing on Pt accumulation in cestodes and nematodes from urban systems. The present study, therefore, makes a valuable contribution to Pt bioaccumulation in parasite-host interactions.

The study also assessed the effects of metal exposure and parasite infection using biomarker responses in *C. carpio*. Both the biomarkers of exposure (MT and AChE) indicated that, although the metal concentrations in infected fish were lower, these individuals were still exposed to metal stressors and needed protection strategies to remediate the stressor. However, the biomarkers of effect (CAT, GSH, MDA, PC, SOD, and CEA) had no major changes between infected and uninfected hosts but rather between sites. These results also support the finding that the parasites do not have any negative effects on the hosts but that the responses are rather the result of an increase in exposure concentrations. *Cyprinus carpio* from Bospoort Dam had significantly higher energy reserves which supports the observation in Chapter 6 that the increased nutrients resulted in higher food availability. The energetics of these fish also displayed significantly higher cellular electron transport (energy consumption) activity than fish from Olifantsnek Dam. This is indicative of the greater energy requirements by fish from Bospoort Dam to maintain biochemical / physiological processes to protect themselves against the increased metals and other stressors.

Based on the above, the data support the hypotheses that fish endoparasitic helminths will have higher metal concentrations at the Pt mining impacted site compared to the reference site, and that infected fish will have lower metal concentrations than uninfected fish. The hypothesis that biomarker responses in infected fish will be more pronounced compared to uninfected fish was not supported by the data.

8.3 Final remarks

It can be concluded that the Hex River is impacted mainly by mining activities, although urban and industrial effluent also contribute to metal contamination and nutrient inputs. The metal distribution in water and sediment was not only related to spatial distribution but also influenced by water and sediment physicochemical characteristics associated with seasonal changes. The Pt associated metals and nutrient exposure in the Hex River system resulted in significantly altered macroinvertebrate community between the reference sites in the upper reaches and the lower reaches of the river. The metals were also bioavailable to macroinvertebrates and fish, as well as their associated parasites. Important fish species accumulated high metal concentrations that do not only pose a risk to ecosystem health but also to humans that consume these fish. The present study thus makes an important contribution to the knowledge base on Pt and associated metals in aquatic biota near Pt production activities.

8.4 Recommendations for future studies

Results from the present study showed that metal contamination in the Hex River system has increased over time. It is therefore, vital to implement monitoring programs to assess the stressors in this system in order to develop mitigation and remedial policies and plans. The present study provided valuable baseline data on the ecosystem health and the exposure and effects of Pt and associated metal pollution in the Hex River. However, the present study also identified the gaps in our knowledge and the data required to fully understand the mechanisms of the catchment. Recommendations for future studies to contribute to this information gaps include:

- Studies are needed to assess the speciation and transformation of metals within the Hex River catchment to determine the form of the different metals entering the system and how it is transformed within it. This will provide valuable information on the potential toxicity of metals and will make it easier to assess the ecosystem and associated human health risks.
- While the present study assessed the accumulation and effects in macroinvertebrates, fish and associated parasites, studies that focus on primary producers (phytoplankton, periphyton, and algae) are needed to assess the accumulation and effects of metals at lower trophic levels to establish the exposure via food webs.
- Further studies are needed to assess the accumulation in aquatic (floating and rooted) and marginal vegetation. This is a crucial medium because it is the transitional phase between the aquatic and terrestrial environment. Plants also have good remediation potential and, with proper management, can potentially be used to rehabilitate the Hex River system. The Hex River system represents an ideal case study as the lower reaches of the system are already infested with the water hyacinth (*Eichhornia crassipes*).
- Studies that include stable isotope analyses will enable the establishment of the trophic position of organisms in the food web and construction of food web structures. Once this is achieved in the Hex River, the effects of metals on the alteration of food web structures can be verified.
- Laboratory-based PGE exposure studies are also crucial to determine mechanisms of toxicity related to Pt exposure in PGE mining areas. Such laboratory-based studies can provide valuable information on LC_{50} and EC_{50} concentrations of PGE to endemic species and establish species sensitivity distribution curves. Determining the differences in sensitivities of organisms to PGEs is crucial, as sensitive organisms usually cannot survive in polluted ecosystems, while more tolerant species can be more effective as monitoring organisms.

- Further studies on PGE accumulation in parasites are necessary to determine the accumulation limits of parasites, as well as a bigger sample size is essential. Biomarker responses of the parasites to PGE exposure will establish the biological effects of high metal concentrations.
- The present study provided valuable baseline information on the human health risks associated with the consumption of fish, for future studies to expand on. An extensive human health risk assessment is needed for the Hex River catchment. This assessment should not only determine the risks associated with the consumption of fish but also determine the risks associated with the inhalation of Pt emissions from mining and production activities. Assessing metal concentrations in blood and hair samples will provide valuable information.

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APPENDICES

Appendix A

Table A1: Mean concentrations of metals in water (µg/L; n = 3) with the standard deviation of the mean from sites in the Hex River and tributaries. Sites are grouped into reference sites (HX 1 – HX 3), sites with mining influence (HX 4, HX 5, KF), sites with industrial influence (WV, DS 1), sites with urban influence (HX 7, DS 2) and sites with a combined influence (HX 6, HX 8, HX 9, HX 10, BF, BS).

	Site	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ag	Cd	Pt	Au	Pb
Reference	HX 1	1.3 ± 0.18	0.59 ± 0.31	24.5 ± 16.5	2.2 ± 1.2	14.7 ± 0.54	10.6 ± 2.4	22.8 ± 7.6	1.9 ± 0.15	0.008 ± 0.002	0.030 ± 0.009	0.0040 ± 0.0003	0.65 ± 0.05	0.34 ± 0.07
	OL	5.9 ± 0.14	0.41 ± 0.03	4.7 ± 1.7	0.29 ± 0.04	6.1 ± 0.03	8.1 ± 1.6	11.7 ± 3.1	1.1 ± 0.07	0.006 ± 0.002	0.018 ± 0.004	0.0055 ± 0.0007	0.48 ± 0.04	0.31 ± 0.13
	HX 2	9.2 ± 0.19	0.82 ± 0.08	6.6 ± 3.4	0.48 ± 0.10	4.0 ± 0.78	3.5 ± 1.0	9.2 ± 0.33	1.1 ± 0.02	0.012 ± 0.004	0.017 ± 0.006	0.0042 ± 0.0002	0.44 ± 0.06	0.22 ± 0.09
	HX 3	11.5 ± 0.95	0.37 ± 0.05	4.2 ± 2.2	0.45 ± 0.11	5.4 ± 0.48	3.0 ± 0.25	11.4 ± 2.4	3.7 ± 0.15	0.006 ± 0.001	0.021 ± 0.001	0.0040 ± 0.0010	0.40 ± 0.03	0.47 ± 0.21
Mining	HX4	13.5 ± 0.18	0.36 ± 0.02	81.2 ± 17.1	2.1 ± 0.08	20.1 ± 0.58	7.6 ± 0.16	21.4 ± 9.7	3.4 ± 0.28	0.013 ± 0.004	0.047 ± 0.005	0.197 ± 0.0014	0.93 ± 0.08	0.22 ± 0.07
	HX 5	20.7 ± 0.65	0.42 ± 0.11	8.0 ± 1.5	2.1 ± 0.23	15.8 ± 0.34	8.0 ± 0.67	10.0 ± 3.5	2.6 ± 0.07	0.007 ± 0.001	0.025 ± 0.005	0.091 ± 0.0004	0.62 ± 0.04	0.43 ± 0.08
Industry	WV	4.8 ± 0.10	0.29 ± 0.02	157.1 ± 34.6	1.8 ± 0.08	14.7 ± 0.51	4.6 ± 0.80	10.2 ± 2.0	1.4 ± 0.08	0.005 ± 0.002	0.015 ± 0.003	0.0071 ± 0.0006	0.19 ± 0.01	0.23 ± 0.04
Combined	HX 6	11.3 ± 0.95	0.35 ± 0.05	66.7 ± 5.2	1.3 ± 0.12	18.0 ± 0.11	7.1 ± 1.4	9.6 ± 2.0	2.6 ± 0.09	0.005 ± 0.001	0.028 ± 0.007	0.061 ± 0.0029	0.29 ± 0.01	0.28 ± 0.18
Urban	HX 7	14.1 ± 0.29	0.36 ± 0.08	10.5 ± 5.7	1.8 ± 0.08	21.3 ± 0.51	14.5 ± 0.40	12.7 ± 1.2	3.1 ± 0.05	0.125 ± 0.077	0.12 ± 0.02	0.039 ± 0.0016	0.42 ± 0.07	0.26 ± 0.02

	Site	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ag	Cd	Pt	Au	Pb
Mining	KF	16.8 ± 1.3	0.42 ± 0.01	171.5 ± 3.7	3.8 ± 0.12	726.3 ± 21.6	38.5 ± 2.4	17.8 ± 3.3	12.1 ± 0.32	0.015 ± 0.001	0.11 ± 0.006	0.175 ± 0.0071	0.55 ± 0.10	0.16 ± 0.04
Combined	HX 8	11.6 ± 0.15	0.35 ± 0.05	300.3 ± 27.9	2.2 ± 0.05	131.8 ± 2.0	10.1 ± 0.36	16.8 ± 5.1	4.3 ± 0.01	0.058 ± 0.005	0.050 ± 0.008	0.059 ± 0.0009	0.34 ± 0.09	0.29 ±0.11
Industry	DS 1	9.2 ± 0.52	0.52 ± 0.07	328.8 ± 82.8	5.4 ± 0.73	27.3 ± 1.8	6.7 ± 6.1	21.0 ± 10.3	2.4 ± 0.06	0.012 ± 0.001	0.071 ± 0.04	0.036 ± 0.0076	0.39 ± 0.03	0.42 ± 0.09
Urban	DS 2	16.1 ± 0.60	0.38 ± 0.01	353.5 ± 12.6	3.9 ± 0.09	115.3 ± 2.5	7.1 ± 2.2	18.0 ± 1.4	18.5 ± 1.3	0.036 ± 0.010	0.042 ± 0.02	0.088 ± 0.0052	0.39 ± 0.04	0.31 ± 0.12
Combined	HX 9	9.7 ± 0.20	0.39 ± 0.08	291.9 ± 44.1	2.8 ± 0.08	69.7 ± 2.4	7.3 ± 0.41	11.9 ± 3.0	7.9 ± 0.02	0.012 ± 0.002	0.038 ± 0.009	0.068 ± 0.0027	0.31 ± 0.01	0.34 ± 0.15
	HX 10	8.2 ± 0.28	0.36 ± 0.08	516.5 ± 8.9	2.6 ± 0.14	45.5 ± 0.71	4.1 ± 0.38	10.1 ± 2.8	4.9 ± 0.07	0.016 ± 0.008	0.032 ± 0.008	0.045 ± 0.0012	0.29 ± 0.11	0.28 ± 0.11
	BF	6.7 ± 0.41	0.29 ± 0.05	860.5 ± 49.8	3.3 ± 0.04	16.0 ± 0.14	2.2 ± 0.21	6.1 ± 1.2	3.9 ± 0.09	0.005 ± 0.001	0.021 ± 0.009	0.011 ± 0.0011	0.35 ± 0.08	0.29 ± 0.16
	BS	3.9 ± 0.85	0.29 ± 0.08	315.1 ± 167.4	1.0 ± 0.65	48.8 ± 2.3	2.7 ± 0.71	5.8 ± 0.78	2.8 ± 0.16	0.006 ± 0.002	0.013 ± 0.005	0.027 ± 0.0019	0.17 ± 0.02	0.16 ± 0.01

Table A2: Concentrations of metals in sediment (µg/g DW; n = 3) with the standard deviation of the mean from sites in the Hex River and tributaries. Sites are grouped into reference sites (HX 1 – HX 3), sites with mining influence (HX 4, HX 5, KF), sites with industrial influence (WV, DS 1), sites with urban influence (HX 7, DS 2) and sites with a combined influence (HX 6, HX 8, HX 9, HX 10, BF, BS).

	Site	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ag	Cd	Pt	Au	Pb
Reference	HX 1	133.2 ± 12.3	442.0 ± 95.4	516.1 ± 77.5	22.8 ± 3.5	81.9 ± 12.8	41.3 ± 6.4	79.1 ± 7.8	20.3 ± 0.63	0.28 ± 0.029	0.076 ± 0.011	0.012 ± 0.001	0.12 ± 0.011	10.5 ± 2.1
	OL	101.4 ± 5.3	432.2 ± 55.2	515.1 ± 165.9	13.6 ± 1.6	56.6 ± 6.9	22.5 ± 2.8	42.1 ± 4.0	15.5 ± 0.49	0.16 ± 0.022	0.063 ± 0.010	0.009 ± 0.001	0.14 ± 0.006	6.5 ± 1.4
	HX 2	133.4 ± 15.4	968.1 ± 140.9	635.0 ± 74.6	29.1 ± 3.9	185.8 ± 24.4	40.9 ± 5.3	67.2 ± 10.1	14.6 ± 0.58	0.22 ± 0.032	0.052 ± 0.004	0.018 ± 0.002	0.18 ± 0.028	10.6 ± 1.7
	HX 3	99.4 ± 3.1	753.2 ± 100.1	369.2 ± 165.9	23.2 ± 3.4	112.4 ± 12.9	29.5 ± 1.9	66.4 ± 12.0	15.2 ± 0.76	0.18 ± 0.017	0.11 ± 0.010	0.013 ± 0.002	0.15 ± 0.002	10.7 ± 5.8
Mining	HX4	83.3 ± 6.6	1 203.7 ± 72.3	929.0 ± 99.9	27.7 ± 2.0	168.5 ± 11.0	55.0 ± 9.5	83.2 ± 5.4	19.4 ± 1.0	0.16 ± 0.007	0.11 ± 0.010	0.050 ± 0.014	0.19 ± 0.023	9.0 ± 0.31
	HX 5	73.3 ± 5.4	1 418.1 ± 172.8	687.7 ± 6.8	27.5 ± 4.5	167.8 ± 17.2	40.1 ± 5.8	62.9 ± 8.8	21.2 ± 1.5	0.15 ± 0.013	0.079 ± 0.008	0.042 ± 0.020	0.17 ± 0.020	15.6 ± 4.4
Industry	WV	78.9 ± 9.8	1 316.5 ± 225.3	354.6 ± 22.0	27.5 ± 3.3	166.6 ± 10.1	52.7 ± 3.1	94.2 ± 13.2	15.5 ± 0.60	0.18 ± 0.025	0.099 ± 0.038	0.065 ± 0.045	0.23 ± 0.057	11.1 ± 1.1
Combined	HX 6	72.4 ± 2.9	1 074.6 ± 411.8	1 201.5 ± 624.5	24.2 ± 8.4	152.9 ± 20.5	66.5 ± 28.8	97.0 ± 24.4	19.0 ± 6.3	0.16 ± 0.031	0.072 ± 0.024	0.092 ± 0.066	0.19 ± 0.036	10.6 ± 3.7
Urban	HX 7	65.3 ± 7.1	900.5 ± 154.0	479.5 ± 106.1	23.6 ± 2.5	277.9 ± 134.8	218.2 ± 94.8	130.3 ± 31.5	14.6 ± 1.4	0.57 ± 0.24	0.58 ± 0.31	0.36 ± 0.037	0.37 ± 0.25	28.1 ± 10.9

	Site	V	Cr	Mn	Co	Ni	Cu	Zn	As	Ag	Cd	Pt	Au	Pb
Mining	KF	85.7 ± 3.3	495.0 ± 34.2	621.8 ± 109.1	30.1 ± 3.3	362.0 ± 14.0	32.7 ± 1.2	37.0 ± 1.0	15.7 ± 1.9	0.15 ± 0.012	0.062 ± 0.003	0.015 ± 0.002	0.18 ± 0.033	6.1 ± 0.61
Combined	HX 8	61.8 ± 16.6	834.1 ± 64.0	413.9 ± 103.8	23.9 ± 8.7	179.6 ± 66.9	108.2 ± 45.2	126.4 ± 16.1	16.5 ± 2.0	0.26 ± 0.12	0.31 ± 0.16	0.025 ± 0.005	0.13 ± 0.027	22.0 ± 2.4
Industry	DS 1	38.9 ± 6.1	1 118.8 ± 312.3	230.5 ± 51.1	9.9 ± 2.1	169.8 ± 29.0	28.9 ± 4.5	20.2 ± 5.6	12.6 ± 1.7	0.052 ± 0.009	0.032 ± 0.002	0.29 ± 0.071	0.099 ± 0.016	5.4 ± 0.38
Urban	DS 2	58.3 ± 7.5	497.1 ± 48.7	302.8 ± 85.3	12.9 ± 1.9	126.9 ± 31.2	41.4 ± 13.5	291.3 ± 3.7	16.3 ± 1.6	0.11 ± 0.008	0.13 ± 0.055	0.024 ± 0.009	0.35 ± 0.13	15.5 ± 5.1
Combined	HX 9	59.3 ± 3.8	459.8 ± 97.5	308.7 ± 27.8	16.2 ± 4.5	121.0 ± 46.3	37.3 ± 14.8	91.5 ± 68.8	19.0 ± 4.4	0.12 ± 0.028	0.11 ± 0.053	0.018 ± 0.005	0.15 ± 0.018	10.2 ± 5.3
	HX 10	58.6 ± 3.3	463.6 ± 12.4	475.2 ± 162.3	19.0 ± 3.8	122.6 ± 10.6	38.1 ± 7.4	74.8 ± 14.6	13.9 ± 0.92	0.13 ± 0.015	0.11 ± 0.027	0.096 ± 0.065	0.11 ± 0.020	13.4 ± 7.0
	BF	56.1 ± 6.9	299.5 ± 42.9	441.2 ± 81.3	12.5 ± 1.5	46.5 ± 3.4	12.5 ± 1.0	25.2 ± 3.0	15.4 ± 0.17	0.071 ± 0.008	0.030 ± 0.004	0.006 ± 0.002	0.15 ± 0.081	4.7 ± 0.78
	BS	85.4 ± 26.1	112.4 ± 31.5	1 118.3 ± 495.1	34.9 ± 17.8	132.3 ± 51.4	13.5 ± 5.1	32.5 ± 4.5	15.6 ± 1.7	0.076 ± 0.001	0.061 ± 0.023	0.007 ± 0.004	0.11 ± 0.070	3.6 ± 1.4

Table A3: Water quality guideline values from the Australian and New Zealand's guidelines for fresh water quality (ANZECC) (ANZECC, 2000), European Union's environmental quality standards (EU-EQS) (EU-EQS, 2008), South Africa's target water quality range for aquatic ecosystems (TWQR), with chronic effect value (CEV) and acute effect value (AEV) (DWAF, 1996), and the United States Environmental Protection Agency national recommended water quality criteria for aquatic life (USEPA) (USEPA, 2009), as well as sediment quality guideline values from the Australian and New Zealand's sediment quality guidelines values (ANZECC SQGV) (Simpson *et al*., 2013), Netherlands' sediment quality objective (SQO) (Hin *et al*., 2010) and the United States Environmental Protection Agency sediment quality guideline (USEPA SQG) (USEPA, 2002).

Water quality guidelines values (µg/L)							
	ANZECC	EU-EQS	TWQR	TWQR CEV	TWQR AEV	USEPA CEV	USEPA AEV
Cr	1	-	7	14	200	11	16
Ni	11	20	-	-	-	52	470
Cu	1.4	-	1.4	2.8	12	-	-
Zn	8	-	2	3.6	36	120	120
As	50	-	10	20	130	150	340
Cd	0.20	0.45	0.40	0.80	13	0.72	1.8
Pt	-	-	-	-	-	-	-
Pb	3.4	7.2	1.2	2.4	16	3.2	82
Sediment quality guidelines values (µg/g DW)							
	ANZECC $SQGV_{low}$	ANZECC $SQGV_{high}$	SQO	USEPA SQG			
Cr	80	370	-	80			
Ni	21	52	-	30			
Cu	65	270	36	70			
Zn	200	410	140	120			
As	20	70	2.9	33			
Cd	1.5	10	0.8	5			
Pt	-	-	-	-			
Pb	50	220	85	35			

Table A4: Comparison between metal concentrations in water and sediment from previous studies completed in the Hex River (Somerset *et al*., 2015; Almécija *et al*., 2017), studies completed in the relatively prestine Marico River (Kemp *et al*., 2017), the Olifants River that is impacted by coal mining (Dabrowski and de Klerk, 2013; Gerber *et al*., 2015), a study completed in the Blesbok Spruit that is impacted by gold mining (Roychoudhury and Starke, 2006), as well as studies from rivers impacted by automobile catalytic converter derived Pt (Elbe River, Hoppstock and Alt, 2000; Costa River, Monticelli *et al*., 2010; Ruchter *et al*., 2015) and the present study.

Metal concentrations in water (µg/L)

	Kemp *et al*., 2017	Somerset *et al*., 2015	Dabrowski and de Klerk, 2013	Roychoudhury and Starke, 2006	Hoppstock and Alt, 2000	Monticelli *et al*., 2010	Present study
Cr	5.2 – 6.7	-	-	0.7 – 11.3	-	-	0.29 – 0.82
Ni	4.3 – 10	9 – 22	2 – 63	2.4 – 294	-	-	3.9 – 726
Cu	0.1 – 1.4	3 – 11	1 – 6.5	0.1 – 5.1	-	-	2.2 – 38.5
Zn	0.1 – 8.8	-	1 – 325	8.3 – 273	-	-	5.8 – 22.8
As	0.1 – 1.8	-	0.2 – 1.2	0.2 – 9.9	-	-	1.1 – 18.5
Cd	-	0.05 – 0.09	0.04 – 0.23	0.1 – 1.5	-	-	0.013 – 0.12
Pt	-	0.009 – 0.017	-	-	0.0008	0.0026	0.004 – 0.20
Pb	0.01 – 0.04	0.14 – 0.20	-	0.10 – 7.6	-	-	0.16 – 0.47

Metal concentrations in sediment (µg/g)

	Kemp *et al*., 2017	Somerset *et al*., 2015	Almécija *et al*., 2017	Gerber *et al*., 2015	Roychoudhury and Starke, 2006	Ruchter *et al*., 2015	Present study
Cr	218 – 748	-	732 – 1 520	39.6 – 217.4	62 – 532	-	112 – 1 418
Ni	47.7 – 314	0.34 – 85.1	107 – 399	5.5 – 44.6	16.2 – 438	-	46.5 – 362
Cu	25.3 – 127	26.5 – 64.1	25.0 – 77.3	8.9 – 66.6	12.9 – 362	-	12.5 – 218
Zn	1.56 – 213	-	47.3 – 130	16.5 – 82.9	35.8 – 424	-	20.2 – 228
As	0.1 – 8.8	-	0.9 – 4.9	0.56 – 8.7	2.5 – 127	-	12.6 – 21.2
Cd	0.012 – 0.20	0.041 – 0.28	0.01 – 0.1	0.05 – 0.17	0.1 – 0.4	-	0.030 – 0.57
Pt	-	0.00011 – 0.0007	0.013 – 0.042	-	-	0.085	0.0091 – 0.36
Pb	1.5 – 6.3	1.8 – 4.3	26.6 – 45.1	0.60 – 6.0	7.4 – 38.7	-	3.6 – 28.0

Appendix B

Table B1: Mean concentrations of metals in water (µg/L; n = 3) with standard deviation of the mean, from sites in the Hex River and the two impoundments.

		Cr	Ni	Cu	Zn	As	Cd	Pt	Pb
Site 1	04/17	0.23 ± 0.011	4.3 ± 0.23	3.5 ± 1.1	5.7 ± 1.7	1.5 ± 0.13	0.062 ± 0.014	0.017 ± 0.003	0.29 ± 0.053
	06/17	0.23 ± 0.005	3.2 ± 0.17	4.5 ± 1.7	6.6 ± 2.9	1.5 ± 0.02	0.042 ± 0.003	0.017 ± 0.001	0.30 ± 0.094
	11/17	-	-	-	-	-	-	-	-
	03/18	0.21 ± 0.005	1.8 ± 0.30	12.4 ± 5.8	11.7 ± 4.8	2.1 ± 0.15	0.14 ± 0.011	0.025 ± 0.003	1.4 ± 0.45
Site 2	04/17	0.23 ± 0.006	2.8 ± 0.19	3.0 ± 1.1	4.7 ± 0.73	1.6 ± 0.15	0.052 ± 0.006	0.024 ± 0.005	0.40 ± 0.11
	06/17	0.23 ± 0.013	2.6 ± 0.07	3.1 ± 1.8	5.0 ± 3.5	1.4 ± 0.03	0.0042 ± 0.002	0.015 ± 0.001	0.31 ± 0.059
	11/17	-	-	-	-	-	-	-	-
	03/18	0.21 ± 0.007	3.7 ± 0.17	15.1 ± 1.6	15.3 ± 1.4	2.0 ± 0.04	0.16 ± 0.006	0.020 ± 0.002	1.6 ± 0.16
Olifantsnek Dam	04/17	0.25 ± 0.008	8.0 ± 0.27	5.6 ± 0.70	7.7 ± 6.0	2.3 ± 0.10	0.052 ± 0.008	0.028 ± 0.004	0.55 ± 0.047
	06/17	0.27 ± 0.006	7.7 ± 0.20	6.4 ± 3.2	4.0 ± 1.7	2.3 ± 0.02	0.044 ± 0.008	0.017 ± 0.005	0.33 ± 0.081
	11/17	0.31 ± 0.036	8.7 ± 3.8	27.3 ± 9.4	9.1 ± 0.82	2.2 ± 0.03	0.18 ± 0.10	0.009 ± 0.001	0.27 ± 0.083
	03/18	0.22 ± 0.040	3.1 ± 0.07	9.3 ± 4.5	8.2 ± 3.1	2.2 ± 0.03	0.13 ± 0.004	0.009 ± 0.001	0.94 ± 0.42
	11/18	0.39 ± 0.027	6.1 ± 0.10	7.0 ± 0.37	8.3 ± 1.2	1.1 ± 0.05	0.026 ± 0.005	0.006 ± 0.001	0.35 ± 0.021
Site 4	04/17	0.30 ± 0.005	13.0 ± 0.33	13.2 ± 5.1	8.0 ± 5.7	2.2 ± 0.04	0.066 ± 0.007	0.13 ± 0.009	0.49 ± 0.056
	06/17	0.29 ± 0.010	9.8 ± 0.15	11.8 ± 4.9	8.4 ± 6.0	1.9 ± 0.11	0.048 ± 0.005	0.12 ± 0.003	0.58 ± 0.26
	11/17	0.33 ± 0.015	15.7 ± 0.62	12.7 ± 5.5	7.5 ± 4.6	3.1 ± 0.02	0.17 ± 0.060	0.14 ± 0.007	1.2 ± 0.37
	03/18	0.25 ± 0.039	8.5 ± 0.25	11.0 ± 4.6	12.4 ± 4.8	2.4 ± 0.08	0.15 ± 0.010	0.073 ± 0.002	1.5 ± 0.60
Site 5	04/17	0.25 ± 0.013	58.5 ± 7.4	10.9 ± 0.44	5.3 ± 0.94	2.4 ± 0.27	0.090 ± 0.013	0.071 ± 0.010	0.40 ±0.094
	06/17	0.33 ± 0.007	64.9 ± 2.4	11.1 ± 0.87	10.5 ± 0.16	2.8 ± 0.08	0.089 ± 0.004	0.073 ± 0.003	0.29 ± 0.049
	11/17	0.30 ± 0.028	53.3 ± 16.7	7.5 ± 1.6	13.2 ± 11.4	3.8 ± 0.99	1.17 ± 0.030	0.087 ± 0.023	0.73 ± 0.18
	03/18	0.23 ± 0.009	30.0 ± 1.0	5.1 ± 0.68	5.5 ± 0.45	3.1 ± 0.11	0.14 ± 0.002	0.058 ± 0.004	0.50 ± 0.056
Bospoort Dam	04/17	0.19 ± 0.006	53.4 ± 2.3	13.0 ± 5.6	9.5 ± 4.7	1.9 ± 0.04	0.048 ± 0.001	0.050 ± 0.002	1.0 ± 0.53
	06/17	0.20 ± 0.008	58.8 ± 8.4	7.1 ± 2.3	6.5 ± 2.4	2.0 ± 0.11	0.050 ± 0.004	0.046 ± 0.008	0.48 ± 0.094
	11/17	0.33 ± 0.077	41.9 ± 5.4	8.9 ± 4.7	17.6 ± 7.4	2.5 ± 0.22	0.18 ± 0.054	0.044 ± 0.007	0.41 ± 0.26
	03/18	0.22 ± 0.010	19.8 ± 0.72	4.5 ± 1.9	8.7 ± 5.9	2.3 ± 0.05	0.15 ± 0.004	0.036 ± 0.003	0.55 ± 0.16
	11/18	0.29 ± 0.024	33.5 ± 3.4	4.9 ± 2.7	8.0 ± 2.1	2.6 ± 0.14	0.022 ± 0.007	0.025 ± 0.001	0.23 ± 0.11

Table B2: Significant differences in dissolved metal concentrations within surveys (04/2017; 06/2017; 11/2017; 03/2018; 11/2018) between sites. Dissolved concentrations of Cd had no significant differences between sites during any survey.

		04/17						06/17						11/17				03/18						11/18
		Site 1	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**Site 1**	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**OL**	**Site 4**	**Site 5**	**BS**	**Site 1**	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**BS**
04/17	Site 1	-																						
	Site 2		-																					
	OL	As	As	-			Cr																	
	Site 4	Cr, Cu, As, Pt	Cr, Ni, Cu, As, Pt	Cr, Pt	-	Cr, Pt	Cr, Pt																	
	Site 5	Ni, As, Pt	Ni, As, Pt	Ni, Pt	Ni	-	Cr, As, Pt																	
	BS	Ni, Cu, Pt, Pb	Ni, Cu, Pt, Pb	Ni, Pt	Ni	Pb	-																	
06/17	Site 1							-																
	Site 2								-															
	OL							As	As	-			Cr											
	Site 4							Cr, Pt	Cr, Cu, Pt	Pt	-	Pt	Cr, Pt											
	Site 5							Cr, Ni, As, Pt	Cr, Ni, Cu, As, Pt	Cr, Ni, Pt	Ni, As	-	Cr, As, Pt											
	BS							Ni, Pt	Ni, As, Pt	Ni, Pt	Ni		-											
11/17	OL													-	Cu	Cu	Cu							
	Site 4													As, Pt, Pb	-	Pt	As, Pt, Pb							
	Site 5													Ni, As, Pt	Ni, As	-	Ni, As, Pt							
	BS													Ni, Pt	Ni, Zn		-							

		04/17						06/17						11/17				03/18						11/18
		Site 1	Site 2	OL	Site 4	Site 5	BS	Site 1	Site 2	OL	Site 4	Site 5	BS	OL	Site 4	Site 5	BS	Site 1	Site 2	OL	Site 4	Site 5	BS	BS
03/18	Site 1																	-		Pt		Pb	Pb	
	Site 2																		-	Pb		Cu, Pb	Cu, Pb	
	OL																			-				
	Site 4																	Pt	Pt	Pt, Pb	-	Pt, Pb	Pt, Pb	
	Site 5																	Ni, As, Pt	Ni, As, Pt	Ni, As, Pt	Ni, As	-	Ni, As, Pt	
	BS																	Ni	Ni, Pt	Ni, Pt	Ni		-	
11/18	OL																							Cr, Ni, As, Pt

Table B3: Mean concentrations of metals in sediment (µg/g DW; n = 3) with standard deviation of the mean, from sites in the Hex River and the two impoundments.

		Cr	Ni	Cu	Zn	As	Cd	Pt	Pb
Site 1	04/17	378 ± 24	31.4 ± 10.2	38.7 ± 6.1	35.6 ± 4.9	18.8 ± 1.0	0.077 ± 0.009	0.031 ± 0.002	11.3 ± 0.69
	06/17	321 ± 35	70.3 ± 10.0	37.7 ± 3.3	37.7 ± 3.3	11.7 ± 1.4	0.14 ± 0.057	0.015 ± 0.003	14.0 ± 5.3
	11/17	-	-	-	-	-	-	-	-
	03/18	442 ± 80	108.7 ± 25.1	64.3 ± 6.3	38.0 ± 1.9	17.8 ± 1.2	0.085 ± 0.015	0.015 ± 0.002	13.0 ± 2.4
Site 2	04/17	354 ± 22	46.6 ± 5.3	19.0 ± 1.4	25.2 ± 1.7	14.9 ± 1.1	0.042 ± 0.002	0.020 ± 0.001	7.0 ± 0.67
	06/17	271 ± 9	60.3 ± 4.1	28.7 ± 1.6	28.7 ± 1.6	9.7 ± 0.9	0.14 ± 0.026	0.014 ± 0.002	13.8 ± 2.1
	11/17	-	-	-	-	-	-	-	-
	03/18	506 ± 46	100.1 ± 8.1	49.6 ± 3.9	42.5 ± 3.4	22.4 ± 0.8	0.080 ± 0.011	0.013 ± 0.003	9.7 ± 1.0
Olifantsnek Dam	04/17	380 ± 20	39.5 ± 2.0	12.1 ± 0.6	17.6 ± 0.8	10.5 ± 0.4	0.034 ± 0.003	0.021 ± 0.001	6.4 ± 0.13
	06/17	468 ± 90	82.7 ± 20.8	24.0 ± 2.7	24.0 ± 2.7	8.6 ± 0.9	0.19 ± 0.019	0.015 ± 0.003	12.3 ± 1.6
	11/17	390 ± 65	66.4 ± 12.5	20.2 ± 0.7	20.2 ± 0.7	8.3 ± 1.7	0.10 ± 0.022	0.015 ± 0.003	9.0 ± 1.0
	03/18	380 ± 17	49.5 ± 3.0	19.0 ± 1.0	52.5 ± 11.0	14.5 ± 0.6	0.044 ± 0.003	0.008 ± 0.001	5.8 ± 0.93
	11/18	432 ± 55	56.6 ± 6.9	22.5 ± 2.8	42.1 ± 4.0	15.5 ± 0.5	0.040 ± 0.002	0.009 ± 0.001	6.5 ± 1.4
Site 4	04/17	567 ± 99	73.5 ± 4.1	31.6 ± 1.5	17.9 ± 1.1	10.3 ± 0.7	0.110 ± 0.006	0.084 ± 0.009	7.1 ± 0.24
	06/17	654 ± 170	133.1 ± 12.7	76.0 ± 12.1	76.0 ± 12.1	10.1 ± 0.6	0.35 ± 0.15	0.17 ± 0.086	19.9 ± 8.0
	11/17	567 ± 178	124.7 ± 11.6	72.7 ± 16.0	72.7 ± 16.0	12.4 ± 1.1	0.21 ± 0.068	0.15 ± 0.064	13.2 ± 2.0
	03/18	1 164 ± 456	256.0 ± 105.9	118.7 ± 38.9	103.3 ± 38.3	14.9 ± 3.0	0.19 ± 0.083	0.18 ± 0.064	15.4 ± 1.4
Site 5	04/17	288 ± 24	49.0 ± 5.4	11.0 ± 0.7	15.2 ± 0.7	6.7 ± 0.5	0.066 ± 0.002	0.027 ± 0.011	4.6 ± 0.15
	06/17	446 ± 46	151.2 ± 18.5	36.2 ± 3.0	36.2 ± 3.0	8.8 ± 0.9	0.15 ± 0.031	0.046 ± 0.014	13.3 ± 4.3
	11/17	377 ± 116	130.4 ± 27.4	36.2 ± 11.6	36.2 ± 11.6	14.6 ± 1.2	0.18 ± 0.053	0.029 ± 0.011	9.5 ± 1.5
	03/18	728 ± 115	165.1 ± 50.3	59.6 ± 38.9	52.8 ± 2.9	13.9 ± 1.4	0.14 ± 0.045	0.041 ± 0.020	13.9 ± 3.2
Bospoort Dam	04/17	34 ± 5	20.9 ± 0.8	2.2 ± 0.1	5.5 ± 0.2	7.5 ± 0.3	0.012 ± 0.001	0.009 ± 0.001	2.0 ± 0.55
	06/17	72 ± 4	79.8 ± 16.3	11.4 ± 1.5	11.4 ± 1.5	12.1 ± 1.4	0.15 ± 0.081	0.021 ± 0.008	3.5 ± 1.4
	11/17	74 ± 6	81.2 ± 23.7	13.0 ± 5.1	13.0 ± 5.1	13.6 ± 4.1	0.098 ± 0.023	0.016 ± 0.004	4.9 ± 1.8
	03/18	60 ± 2	56.1 ± 1.2	6.7 ± 0.2	26.6 ± 4.4	13.0 ± 0.3	0.039 ± 0.004	0.005 ± 0.001	1.9 ± 0.34
	11/18	68 ± 3	67.5 ± 7.4	10.1 ± 0.7	22.9 ± 1.7	17.7 ± 2.2	0.030 ± 0.004	0.007 ± 0.001	2.6 ± 0.61

Table B4: Significant differences in sediment metal concentrations within surveys (04/2017; 06/2017; 11/2017; 03/2018; 11/2018) between sites.

		04/17						06/17						11/17				03/18						11/18
		Site 1	Site 2	OL	Site 4	Site 5	BS	Site 1	Site 2	OL	Site 4	Site 5	BS	OL	Site 4	Site 5	BS	Site 1	Site 2	OL	Site 4	Site 5	BS	BS
04/17	Site 1	-	As	Zn, As	Zn, As	Cu, Zn, As, Pb	Cr, Cu, Zn, As, Pb																	
	Site 2		-	As	As	As	Cr, Zn, As																	
	OL			-		As,	Cr,																	
	Site 4		Pt	Pt	-	Cr, As, Pt	Cr, Cu, Pt																	
	Site 5					-	Cr																	
	BS						-																	
06/17	Site 1							-					Zn, Pb											
	Site 2								-				Pb											
	OL									-			Cr, Pb											
	Site 4							Cr, Ni, Cu, Zn, Cd, Pt, Pb	Cr, Ni, Cu, Zn, Cd, Pt, Pb	Cu, Zn, Cd, Pt, Pb	-	Cu, Zn, Cd, Pt, Pb	Cr, Cu, Zn, Cd, Pt, Pb											
	Site 5							Ni	Ni	Ni		-	Cr, Ni, Zn, Pb											
	BS									As		As	-											
11/17	OL													-			Cr							
	Site 4													Cu, Zn, As, Cd, Pt	-	Cu, Zn, Pt	Cr, Cu, Zn, Cd, Pt, Pb							
	Site 5													Ni, As		-	Cr, Zn							
	BS													As			-							

		04/17						06/17						11/17				03/18						11/18
		Site 1	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**Site 1**	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**OL**	**Site 4**	**Site 5**	**BS**	**Site 1**	**Site 2**	**OL**	**Site 4**	**Site 5**	**BS**	**BS**
03/18	Site 1																	-		Ni, Cu, As, Pb		As	Cr, Cu, As, Pb	
	Site 2																	As	-	Cu, As	As	As	Cr, Cu, As, Pb	
	OL																			-			Cr, Zn	
	Site 4																	Cr, Ni, Cu, Zn, Cd, Pt	Cr, Ni, Cu, Zn, Cd, Pt, Pb	Cr, Ni, Cu, Zn, Cd, Pt, Pb	-	Cr, Ni, Cu, Zn, Pt	Cr, Ni, Cu, Zn, Cd, Pt, Pb	
	Site 5																	Cr	Ni	Cr, Ni, Cu, Pb		-	Cr, Ni, Cu, Zn, Pb	
	BS																						-	
11/18	**OL**																							Cr, Zn

Appendix C

Table C1: Abundance of taxa collected at four sites in the Hex River (Sites 1 – 4) during four surveys (04/2017; 06/2017; 11/2017; 03/2018). Sites and surveys are indicated as (Site – Survey). Site 1 during the third survey (S1-3) and Site 2 during the third and fourth surveys (S2-3; S2-4) were dry and no macroinvertebrates were collected.

Taxa	Sites															
	S1-1	S1-2	S1-3	S1-4	S2-1	S2-2	S2-3	S2-4	S3-1	S3-2	S3-3	S3-4	S4-1	S4-2	S4-3	S4-4
Decapoda																
Potamonautes warreni	0	0		0	0	0			4	1	8	6	2	0	0	0
Oligochaeta																
Branchiura sowerbyi	0	1		17	1	0			7	0	18	0	0	0	0	13
Tubifex sp.	34	10		92	6	0			29	52	205	20	85	0	118	185
Hirudinea																
Helobdella stagnalis	0	0		0	0	0			0	3	0	5	0	0	0	0
Marsupiobdella africana	0	0		3	0	0			1	0	0	0	0	0	0	0
Placobdelloides sp.	0	0		0	0	0			0	0	0	0	5	3	2	0
Barbronia sp.	0	0		0	0	0			0	3	0	0	0	0	0	0
Mollusca																
Lymnaea collumella	0	3		8	1	2			0	0	0	0	0	0	0	0
Lymnaea natalensis	0	0		11	0	0			1	0	29	3	0	0	0	0
Burnupia mooiensis	0	0		0	0	2			13	6	11	6	2	0	4	3
Ferrissia cawstoni	0	0		24	0	0			0	0	0	0	0	0	0	0
Gyraulus connollyi	2	0		44	1	1			0	0	0	0	0	0	0	0
Ceratophallus sp.	0	0		16	0	0			0	0	0	0	0	0	0	0
Physella acuta	0	0		0	0	0			0	38	17	2	0	0	37	3
Pisidium costulosum	0	0		19	1	0			1	0	6	0	0	0	0	0
Pisidium langleyanum	0	0		14	0	0			0	0	0	0	0	0	0	0
Pisidium viridarium	0	1		19	0	0			0	0	0	0	0	0	0	0

Taxa	Sites															
	S1-1	**S1-2**	**S1-3**	**S1-4**	**S2-1**	**S2-2**	**S2-3**	**S2-4**	**S3-1**	**S3-2**	**S3-3**	**S3-4**	**S4-1**	**S4-2**	**S4-3**	**S4-4**
Ephemeroptera																
Acanthiops sp.	79	64		8	109	53			58	87	9	41	42	26	0	0
Baetis sp.	39	36		0	84	17			86	118	13	60	0	0	0	0
Cloeon sp.	20	30		0	0	0			46	72	7	33	10	8	9	25
Afroptilum sp.	27	28		0	77	11			41	63	5	31	0	0	0	0
Caenis sp.	13	56		2	20	34			20	4	5	46	0	0	0	0
Odonata																
Lestes sp.	0	0		4	0	0			0	0	0	0	0	0	0	0
Ellattoneura glauca	1	0		0	0	0			0	0	0	0	0	0	0	0
Platycypha caligata	0	0		0	0	9			0	0	0	0	0	0	0	0
Pseudagrion sp.	8	13		15	22	27			65	19	48	14	18	73	28	2
Enallagma glaucum	0	0		0	0	0			0	0	6	0	0	0	0	0
Agriocnemis pinheyi	0	0		0	0	0			0	0	0	4	0	0	0	0
Notogomphus sp.	0	1		4	0	0			0	0	0	0	0	0	0	0
Ceratogomphus sp.	0	0		0	0	0			0	0	0	0	2	0	0	0
Paragomphus sp.	0	0		0	44	0			6	0	1	0	0	0	0	0
Onychogomphus sp.	2	0		0	0	3			0	0	0	1	0	0	0	0
Aeshna sp.	4	1		2	0	0			0	0	4	0	0	1	1	0
Anax sp.	0	0		0	0	0			0	0	0	3	0	0	0	0
Tetrathemis sp.	8	0		1	0	0			0	0	0	4	0	0	0	2
Acisoma sp.	0	0		1	0	0			0	0	0	0	0	0	0	0
Notiothemis sp.	1	0		6	19	11			7	0	1	0	0	0	0	0
Orthetrum sp.	3	2		0	0	0			0	0	0	0	0	0	0	0
Bradinopyga sp.	0	2		0	1	0			0	0	3	0	0	0	0	0
Pantala sp.	0	0		0	0	0			0	0	0	0	2	1	1	0
Trithemis sp.	0	1		4	5	14			8	2	0	2	0	0	0	0
Sympetrum fonscolombii	0	0		0	0	0			0	0	0	1	0	0	0	0

Taxa	Sites															
	S1-1	**S1-2**	**S1-3**	**S1-4**	**S2-1**	**S2-2**	**S2-3**	**S2-4**	**S3-1**	**S3-2**	**S3-3**	**S3-4**	**S4-1**	**S4-2**	**S4-3**	**S4-4**
Hemiptera																
Mesovelia sp.	0	4		0	0	3			1	0	0	0	0	32	0	0
Valleriola moesta	0	2		0	0	0			0	0	0	0	0	0	0	0
Paraphrynovelia sp.	1	0		3	2	0			0	6	4	9	3	2	0	3
Gerris sp.	0	0		1	5	0			0	0	0	0	0	0	0	0
Neogerris sp.	0	0		0	8	2			0	2	5	0	0	8	0	0
Rhagodotarsus sp.	0	0		0	0	0			0	0	0	0	0	18	0	0
Eurymetra sp.	0	0		0	0	2			0	1	0	2	0	0	0	0
Micronecta sp.	0	0		0	10	2			2	5	6	1	76	17	24	7
Sigara sp.	3	1		2	0	0			0	14	1	0	0	4	0	0
Agraptocorixa sp.	1	0		0	0	0			0	0	0	0	0	0	0	0
Anisops sp.	0	0		12	5	0			12	0	0	11	1	10	4	0
Notonecta sp.	2	0		1	0	0			0	0	0	0	0	0	0	0
Enithares sp.	0	1		0	0	0			0	2	1	25	0	0	0	0
Laccotrephes sp.	0	0		0	0	0			0	0	0	0	0	1	0	0
Ranatra sp.	0	0		0	0	0			0	0	0	1	0	1	0	0
Laccocoris sp.	1	0		0	3	0			0	0	8	24	0	0	0	0
Appasus sp.	0	1		0	1	1			15	1	2	5	33	26	38	27
Trichoptera																
Cheumatopsyche afra	1	0		7	0	1			0	0	0	0	0	0	0	0
Cheumatopsyche thomasseti	0	0		11	0	0			5	6	9	50	230	364	1	0
Hydroptila cruciata	0	0		18	0	0			7	0	11	6	1	3	0	0
Leptocerus sp.	0	0		1	0	0			0	0	0	0	0	0	0	0
Ecnomus thomasseti	0	0		4	0	0			0	0	0	7	0	0	0	0
Goera sp.	0	0		0	0	0			0	0	0	3	38	9	8	5
Lepidoptera																
Crambidae	0	1		1	1	0			0	0	0	15	0	0	0	1

Taxa	Sites															
	S1-1	**S1-2**	**S1-3**	**S1-4**	**S2-1**	**S2-2**	**S2-3**	**S2-4**	**S3-1**	**S3-2**	**S3-3**	**S3-4**	**S4-1**	**S4-2**	**S4-3**	**S4-4**
Diptera																
Tipulidae	2	0		0	0	1			0	1	1	0	0	1	0	0
Telmatoscopus sp.	0	4		0	2	2			0	0	0	1	0	0	0	0
Pericoma sp.	1	1		0	0	0			0	0	0	0	0	0	0	0
Bezzia sp.	0	1		0	0	0			3	3	11	7	0	1	0	0
Culicoides sp.	0	0		0	0	1			0	0	3	0	0	0	0	1
Atrichopogon sp.	0	0		0	1	0			0	0	0	0	0	0	0	0
Culicinae	0	0		0	1	0			0	0	0	0	0	0	0	1
Anophelinae	1	0		0	4	3			0	0	0	8	0	2	0	0
Corethrella sp.	0	0		1	0	0			0	0	0	14	0	0	0	18
Simulium adersi	85	0		41	0	0			58	127	17	116	379	64	0	0
Simulium alcocki	0	0		0	7	0			0	0	0	0	0	0	0	0
Simulium hargreavesi	0	0		7	40	0			0	0	0	0	0	0	0	0
Simulium nigritarse	158	81		0	23	70			0	0	0	0	0	0	0	0
Simulium ruficorne	75	0		0	12	17			0	0	0	0	0	0	0	0
Tanypodinae	44	16		0	21	10			22	13	5	9	0	0	0	41
Chironominae	59	101		7	72	221			82	126	30	149	269	217	159	888
Orthocladiinae	6	1		0	11	5			10	10	0	0	0	0	0	19
Tabanidae	0	0		0	0	0			1	8	1	1	1	0	0	0
Athericidae	0	0		0	0	1			0	0	2	0	3	0	2	0
Stratiomyidae	1	7		0	1	0			0	0	0	0	0	0	0	0
Ephydridae	0	0		0	2	0			2	2	0	1	0	1	0	3
Muscidae	1	1		0	1	0			4	19	3	0	2	0	100	4
Coleoptera																
Orectogyrus sp.	0	0		4	0	0			0	0	0	0	0	0	0	0
Dineutus sp.	1	0		0	0	0			0	0	0	0	0	0	0	0
Aulonogyrus sp.	0	0		1	1	10			0	0	6	1	3	6	0	0
Haliplus sp.	3	0		1	0	0			0	0	0	0	0	0	0	0

Taxa	Sites															
	S1-1	**S1-2**	**S1-3**	**S1-4**	**S2-1**	**S2-2**	**S2-3**	**S2-4**	**S3-1**	**S3-2**	**S3-3**	**S3-4**	**S4-1**	**S4-2**	**S4-3**	**S4-4**
Derovatellus sp.	0	0		0	1	0			0	0	0	0	0	0	0	1
Laccophilus sp.	1	1		15	0	4			0	2	0	0	0	4	0	0
Philodytes sp.	0	0		0	7	0			1	3	0	0	0	0	0	0
Africophilus sp.	0	0		0	3	0			0	0	0	0	0	0	0	0
Hydaticus sp.	2	0		0	0	3			0	0	0	0	0	0	0	0
Spercheus senegalensis	0	0		0	0	0			0	0	0	0	0	0	0	3
Amphiops sp.	0	0		0	0	0			1	0	0	0	0	2	0	1
Laccobius sp.	1	0		0	1	0			0	0	0	0	0	0	5	0
Enochrus sp.	1	0		0	2	2			0	0	0	0	2	2	0	2
Ochthebius sp.	0	2		0	0	0			0	0	0	0	0	0	0	0
Limnebius sp.	0	0		1	4	0			1	0	0	0	0	3	0	0
Hydreana sp.	2	13		0	1	17			0	0	0	0	3	0	0	0
Protelmis sp.	0	0		1	0	0			0	0	0	0	0	0	0	0
Leptelmis sp.	1	0		0	0	0			0	0	0	0	0	0	0	0
Lampyridae	0	0		1	0	0			0	0	0	0	0	0	0	0
Rhyssemus sp.	1	0		0	0	0			0	0	0	0	0	0	0	0

Table C2: Abundance of taxa collected at two impoundment sites (Olifantsnek Dam – OL; Bospoort Dam – BS) during three surveys (04/2017; 11/2017; 03/2018). Sites and surveys are indicated as (Site – Survey).

Taxa	Sites					
	OL-1	OL-2	OL-3	BS-1	BS-2	BS-3
Decapoda						
Caridina typus	0	0	7	0	0	0
Oligochaeta						
Branchiura sowerbyi	0	9	8	0	6	8
Tubifex sp.	0	50	15	0	12	41
Hirudinea						
Helobdella stagnalis	0	23	5	3	7	27
Trombidiformes						
Hydrachnidae	0	0	0	0	0	1
Mollusca						
Lymnaea collumella	0	1	0	0	0	0
Lymnaea natalensis	0	0	0	3	0	1
Burnupia mooiensis	21	0	0	9	1	2
Bulinus tropicus	1	0	0	0	0	0
Physella acuta	0	0	0	7	38	8
Melanoides tuberculata	0	0	0	0	1	8
Ephemeroptera						
Cloeon sp.	196	4	18	8	0	12
Povilla adusta	0	0	1	0	0	0
Odonata						
Pseudagrion sp.	0	0	0	18	0	13
Enallagma glaucum	4	0	0	0	0	5
Agriocnemis pinheyi	6	0	0	0	0	0
Onychogomphus sp.	0	0	1	0	0	0
Anax sp.	0	0	0	5	0	0
Bradinopyga sp.	0	0	0	17	0	0
Trithemis sp.	0	0	0	13	1	0
Pantala flavescens	3	0	2	0	0	2
Tholymis sp.	3	0	0	0	0	0
Hemiptera						
Paraphrynovelia sp.	0	0	1	5	0	0
Neogerris sp.	1	0	0	0	0	0
Rhagodotarsus sp.	5	0	0	2	0	0
Tenagogonus sp.	0	0	0	1	0	0
Micronecta sp.	43	9	11	7	297	149
Anisops sp.	9	0	1	0	6	43
Enithares sp.	0	0	0	49	0	85
Appasus sp.	14	0	0	1	1	12

Taxa	Sites					
	OL-1	OL-2	OL-3	BS-1	BS-2	BS-3
Trichoptera						
Ecnomus thomasseti	0	0	2	0	0	0
Diptera						
Psychodidae	0	0	1	0	0	0
Bezzia sp.	0	0	1	0	0	0
Culicinae	0	0	0	1	0	0
Anophelinae	0	0	0	1	0	0
Corethrella sp.	0	0	0	0	0	1
Tanypodinae	2	0	0	0	0	0
Chironominae	131	16	15	6	21	7
Orthocladiinae	0	1	1	0	0	0
Tabanidae	0	0	0	3	0	0
Athericidae	0	0	0	2	0	5
Sciomyzidae	0	0	0	0	0	3
Ephydridae	0	0	0	4	0	0
Coleoptera						
Laccophilus sp.	0	0	0	3	1	0
Berosus sp.	0	0	0	0	2	0
Laccobius sp.	0	0	0	1	0	0
Enochrus sp.	0	0	0	2	0	1
Amphiops sp.	0	0	0	8	0	0
Limnebius sp.	0	0	0	18	0	9
Hydreana sp.	0	0	0	1	0	0
Neochetina sp.	0	0	0	11	0	4

Appendix D

Table D1: Macroinvertebrate families collected from selected sites in the Hex River (Site 1 – 3) and a mine settling pond (Mine), with number of organisms (N), number of replicates analysed (n) and mean dry weight, as well as the moisture content.

Site	Families	N	n	Dry weight (mg)	Moisture content (%)
Site 1	Lymnaeidae	8	1	44.5	72.5
	Baetidae	12	1	18.6	70.2
	Potamonautidae	2	1	95.7	80.7
	Coenagrionidae	13	1	19.5	82.4
	Libellulidae	5	1	79.6	81.4
Site 2	Lymnaeidae	18	2	89.3 ± 7.8	70.8
	Baetidae	70	1	105.3	69.0
	Hydropsychidae	35	1	92.1	65.9
	Tubificidae	48	3	60.4 ± 9.5	80.0
	Potamonautidae	6	5	331.7 ± 109.9	67.6
	Coenagrionidae	32	2	93.9 ± 9.1	78.8
	Libellulidae	5	1	108.3	82.2
Site 3	Lymnaeidae	11	1	55.2	71.1
	Baetidae	84	1	115.8	77.8
	Chironomidae	78	1	71.2	85.2
	Potamonautidae	6	4	187.8 ± 55.3	67.0
	Coenagrionidae	24	1	139.3	82.3
	Libellulidae	3	1	99.9	82.6
Mine	Libellulidae	1	1	77.8	82.1

Table D2: Mean metal concentrations with standard deviation (SD) of the mean in samples collected from selected sites in the Hex River (Site 1 – 3), a mine settling pond (Mine) and urban effluent (Urban).

	Site 1	Site 2	Site 3	Mine	Urban
Water concentrations					
Cr (µg/L)	< LOD	< LOD	< LOD	< LOD	< LOD
Ni (µg/L)	1.83 ± 0.30	8.46 ± 0.25	30.02 ± 1.02	47.36 ± 1.95	115.27 ± 2.45
Cu (µg/L)	21.23 ± 15.84	10.95 ± 4.55	5.13 ± 0.68	6.55 ± 1.06	7.13 ± 2.19
Zn (µg/L)	20.61 ± 15.83	12.44 ± 4.81	5.50 ± 0.45	11.51 ± 6.43	17.99 ± 1.44
Cd (µg/L)	0.140 ± 0.011	0.152 ± 0.010	0.143 ± 0.002	0.158 ± 0.008	0.042 ± 0.015
Pt (µg/L)	0.025 ± 0.003	0.073 ± 0.002	0.058 ± 0.004	0.472 ± 0.007	0.088 ± 0.005
Pb (µg/L)	2.13 ± 1.45	1.47 ± 0.60	0.50 ± 0.06	0.74 ± 0.27	0.31 ± 0.12
Sediment concentrations					
Cr (µg/g DW)	417.6 ± 54.2	1 258.7 ± 511.7	775.8 ± 155.1	1 608.7 ± 150.4	497.1 ± 48.7
Ni (µg/g DW)	108.7 ± 25.1	256.0 ± 105.9	165.1 ± 50.3	198.7 ± 13.7	126.9 ± 31.2
Cu (µg/g DW)	64.3 ± 6.3	118.7 ± 38.9	59.6 ± 38.9	79.1 ± 11.4	41.4 ± 13.5
Zn (µg/g DW)	38.0 ± 1.9	82.9 ± 44.5	83.2 ± 52.7	391.1 ± 103.1	227.6 ± 110.5
Cd (µg/g DW)	0.095 ± 0.024	0.391 ± 0.322	0.236 ± 0.139	0.199 ± 0.117	0.129 ± 0.055
Pt (µg/g DW)	0.015 ± 0.002	0.175 ± 0.064	0.041 ± 0.020	0.608 ± 0.017	0.024 ± 0.009
Pb (µg/g DW)	13.0 ± 2.4	40.8 ± 43.0	13.9 ± 3.2	10.9 ± 2.1	15.5 ± 5.1

< LOD – below the limit of detection.

Table D3: Comparison between previous studies (Somerset *et al*., 2015; Almécija *et al*., 2017) and present study completed in the Hex River.

		Cd	Cr	Cu	Ni	Pb	Pt	Zn
Present study	Water (µg/L)	0.15 ± 0.009	0.28 ± 0.07	12.4 ± 10.9	13.4 ± 12.8	1.4 ± 1.1	0.052 ± 0.021	12.8 ± 10.6
	Sediment (µg/g)	0.24 ± 0.22	817.4 ± 453.7	80.8 ± 39.7	176.6 ± 88.0	22.6 ± 25.6	0.077 ± 0.082	68.0 ± 41.2
Somerset *et al*. (2015)	Water (µg/L)	7.0 ± 1.8	-	6.8 ± 3.3	15.3 ± 6.2	17.8 ± 2.6	0.013 ± 0.004	-
	Sediment (µg/g)	11.1 ± 13.3	-	41.6 ± 16.9	39.3 ± 45.3	2.9 ± 1.1	0.00033 ± 0.00027	
Almécija *et al*. (2017)	Water (µg/L)	-	-	-	-	-	-	-
	Sediment (µg/g)	0.075 ± 0.05	1 211 ± 341.7	41.8 ± 24.0	189.8 ± 139.8	33.5 ± 8.4	0.021 ± 0.009	75.6 ± 38.3

Table D4: Critical body burden of metals for Chironomidae and Tubificidae as calculated by Bervoets *et al*. (2016) as the accumulated concentrations that were related to macroinvertebrate community changes.

	Chironomidae	Tubificidae
Cr (µg/g DW)	10	24
Ni (µg/g DW)	6.5	8.4
Cu (µg/g DW)	57	71
Zn (µg/g DW)	490	930
Cd (µg/g DW)	3.2	28
Pb (µg/g DW)	73	79

Appendix E

Table E1: The mean and standard deviation of metal concentrations (µg/g DW) in the three fish species (*Cyprinus carpio* (n = 33; n = 34), *Clarias gariepinus* (n = 20; n = 16), *Oreochromis mossambicus* (n = 29; n = 20)) collected during the three surveys from Olifantsnek Dam and Bospoort Dam.

Element	Olifantsnek Dam									Bospoort Dam								
	Cyprinus carpio			*Clarias gariepinus*			*Oreochromis mossambicus*			*Cyprinus carpio*			*Clarias gariepinus*			*Oreochromis mossambicus*		
	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018
Muscle																		
Cr	n.s.	0.80 ± 0.29	1.7 ± 0.51	0.93 ± 0.24	1.1 ± 0.39	1.7 ± 0.13	2.3 ± 0.30	3.1 ± 0.56	2.2	1.2 ± 0.35	1.6 ± 0.27	2.2 ± 1.5	0.68 ± 0.23	1.2	2.2 ± 0.30	3.1 ± 0.79	n.s.	2.0 ± 1.0
Ni	n.s.	0.13 ± 0.13	0.97 ± 0.60	0.04 ± 0.01	0.10 ± 0.06	0.69 ± 0.13	0.34 ± 0.17	0.37 ± 0.36	0.86	0.26 ± 0.22	0.46 ± 0.24	0.67 ± 0.30	0.02 ± 0.01	0.06	0.39 ± 0.16	1.1 ± 0.42	n.s.	1.2 ± 0.36
Cu	n.s.	1.1 ± 0.74	1.5 ± 0.33	0.81 ± 0.24	1.2 ± 0.62	1.6 ± 0.25	2.1 ± 0.27	2.5 ± 0.49	1.2	0.90 ± 0.36	1.6 ± 0.27	1.8 ± 0.47	0.84 ± 0.22	1.1	1.5 ± 0.44	2.7 ± 0.44	n.s.	2.1 ± 0.33
Zn	n.s.	48.3 ± 18.9	19.3 ± 4.6	18.3 ± 2.2	22.7 ± 7.9	18.1 ± 3.8	23.1 ± 3.1	25.1 ± 6.6	13.8	19.8 ± 4.6	24.7 ± 3.7	24.5 ± 9.6	18.2 ± 2.5	16.3	18.5 ± 7.0	40.8 ± 4.7	n.s.	23.3 ± 3.2
As	n.s.	2.8 ± 0.35	5.3 ± 0.62	3.7 ± 0.37	1.8 ± 0.68	5.6 ± 0.99	4.0 ± 1.1	9.7 ± 2.6	8.9	3.9 ± 0.51	5.9 ± 0.63	7.1 ± 1.4	3.0 ± 1.4	1.5	9.1 ± 1.0	6.9 ± 2.8	n.s.	5.5 ± 0.83
Cd	n.s.	0.14 ± 0.16	0.07 ± 0.05	0.06 ± 0.05	0.12 ± 0.11	0.06 ± 0.03	0.10 ± 0.09	0.05 ± 0.05	0.84	0.04 ± 0.03	0.24 ± 0.26	0.14 ± 0.09	0.03 ± 0.01	0.04	0.16 ± 0.14	0.05 ± 0.05	n.s.	0.18 ± 0.11
Pt	n.s.	0.0013 ± 0.0005	0.0015 ± 0.0011	0.0021 ± 0.0003	0.0017 ± 0.0006	0.0007 ± 0.0002	0.0022 ± 0.0005	0.0017 ± 0.0002	0.0045	0.0027 ± 0.0004	0.0056 ± 0.0025	0.0077 ± 0.0078	0.0019 ± 0.0006	0.0011	0.0058 ± 0.0030	0.0023 ± 0.0008	n.s.	0.0013 ± 0.0011
Pb	n.s.	0.87 ± 0.87	0.94 ± 0.45	0.88 ± 0.71	1.5 ± 0.85	0.77 ± 0.35	1.4 ± 1.1	0.44 ± 0.34	1.1	0.55 ± 0.55	1.1 ± 0.77	1.4 ± 0.67	0.45 ± 0.35	1.3	2.0 ± 1.3	0.69 ± 0.71	n.s.	1.7 ± 1.1
Liver																		
Cr	n.s.	1.1 ± 0.78	1.7 ± 0.28	0.77 ± 0.21	0.99 ± 0.17	2.1 ± 0.81	2.8 ± 0.59	3.2 ± 1.0	3.9	1.4 ± 0.50	1.5 ± 0.23	2.1 ± 0.58	0.70 ± 0.01	0.92	2.0 ± 0.35	5.5 ± 2.6	n.s.	2.3 ± 0.81
Ni	n.s.	0.59 ± 0.25	0.96 ± 0.37	0.03 ± 0.01	0.24 ± 0.26	1.3 ± 0.97	1.3 ± 0.57	2.0 ± 0.72	4.1	1.3 ± 1.5	0.76 ± 0.57	1.1 ± 0.54	0.43 ± 0.16	0.49	0.65 ± 0.41	9.0 ± 1.7	n.s.	5.8 ± 1.6

Element	Olifantsnek Dam									Bospoort Dam								
	Cyprinus carpio			*Clarias gariepinus*			*Oreochromis mossambicus*			*Cyprinus carpio*			*Clarias gariepinus*			*Oreochromis mossambicus*		
	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018	05/2017	11/2017	11/2018
Cu	n.s.	72.1 ± 27.1	88.9 ± 32.5	25.4 ± 14.5	19.3 ± 9.7	35.0 ± 16.9	117.3 ± 69.9	602.4 ± 260.3	427.8	49.6 ± 23.6	31.2 ± 13.4	67.8 ± 23.1	46.4 ± 2.3	56.7	33.8 ± 15.9	112.7 ± 39.4	n.s.	30.0 ± 17.8
Zn	n.s.	400.4 ± 123.4	314.4 ± 129.0	84.6 ± 29.3	75.6 ± 15.1	67.1 ± 18.3	67.3 ± 10.7	77.4 ± 15.0	79.0	752.9 ± 336.4	474.6 ± 163.9	404.9 ± 137.5	101.4 ± 12.6	111.5	76.5 ± 18.4	96.7 ± 6.7	n.s.	79.7 ± 13.1
As	n.s.	4.0 ± 2.0	5.3 ± 0.87	4.6 ± 0.59	1.6 ± 0.31	5.8 ± 2.0	6.6 ± 1.3	10.6 ± 3.1	15.8	3.3 ± 0.37	5.1 ± 0.88	7.1 ± 1.9	1.2 ± 0.30	0.32	8.9 ± 1.4	16.0 ± 6.1	n.s.	9.1 ± 4.5
Cd	n.s.	0.32 ± 0.12	0.33 ± 0.18	0.11 ± 0.07	0.69 ± 0.33	0.52 ± 0.41	0.14 ± 0.09	0.21 ± 0.11	0.27	0.30 ± 0.29	0.26 ± 0.12	0.16 ± 0.07	0.95 ± 0.32	0.26	0.18 ± 0.11	0.1 ± 0.04	n.s.	0.26 ± 0.17
Pt	n.s.	0.0021 ± 0.0009	0.0011 ± 0.0004	0.0030 ± 0.0007	0.0026 ± 0.0008	0.0014 ± 0.0008	0.0027 ± 0.0008	0.0059 ± 0.011	0.0051	0.0036 ± 0.0005	0.0070 ± 0.0033	0.0081 ± 0.0078	0.0043 ± 0.0014	0.0039	0.0067 ± 0.0021	0.0045 ± 0.0009	n.s.	0.0039 ± 0.0016
Pb	n.s.	1.5 ± 1.5	0.83 ± 0.28	0.03 ± 0.01	1.5 ± 1.0	1.5 ± 0.42	1.7 ± 1.3	0.47 ± 0.30	1.2	0.56 ± 0.42	1.0 ± 0.46	1.2 ± 0.28	0.53 ± 0.46	1.7	1.4 ± 0.85	0.72 ± 0.36	n.s.	2.4 ± 1.4

n.s. – no fish sample collected during the survey.

Appendix F

Table F1: Mean metal concentration (µg/g DW) with standard deviation (SD) of the mean in *Cyprinus carpio* and *Atractolytocestus huronensis* collected from Olifantsnek Dam (n = 6) and Bospoort Dam (n = 7).

Element	Olifantsnek Dam					Bospoort Dam				
	Uninfected		Infected		*Atractolytocestus huronensis*	Uninfected		Infected		*Atractolytocestus huronensis*
	Muscle	Liver	Muscle	Liver		Muscle	Liver	Muscle	Liver	
Cr	0.82 ± 0.31	1.24 ± 0.88	0.74 ± 0.20	0.79 ± 0.18	6.18 ± 1.48	1.46 ± 0.28	1.70 ± 0.35	1.67 ± 0.26	1.47 ± 0.15	5.29 ± 2.50
Ni	0.16 ± 0.13	0.24 ± 0.21	0.23 ± 0.43	0.40 ± 0.36	1.16 ± 0.73	0.34 ± 0.08	1.09 ± 1.01	0.51 ± 0.28	0.62 ± 0.27	7.61 ± 3.84
Cu	1.19 ± 0.84	69.6 ± 29.8	0.79 ± 0.20	79.3 ± 17.4	15.1 ± 12.7	1.81 ± 0.28	22.7 ± 8.35	1.49 ± 0.22	34.8 ± 14.0	6.12 ± 2.68
Zn	53.5 ± 17.7	394.2 ± 124.0	33.7 ± 14.8	417.7 ± 131.5	282.6 ± 37.9	27.7 ± 1.46	484.3 ± 111.7	23.4 ± 3.59	470.4 ± 189.9	349.6 ± 72.2
Cd	0.17 ± 0.19	0.35 ± 0.24	0.05 ± 0.04	0.39 ± 0.14	0.03 ± 0.02	0.31 ± 0.03	0.19 ± 0.03	0.12 ± 0.03	0.24 ± 0.07	0.01 ± 0.01
Pt	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.014 ± 0.013	0.005 ± 0.002	0.006 ± 0.002	0.006 ± 0.003	0.008 ± 0.004	0.010 ± 0.006
Pb	2.11 ± 2.56	1.88 ± 1.58	0.79 ± 0.72	0.75 ± 0.85	0.26 ± 0.16	1.80 ± 1.18	1.23 ± 0.82	0.76 ± 0.22	0.93 ± 0.25	0.18 ± 0.04

Table F2: Mean metal concentration (µg/g DW) with standard deviation (SD) of the mean in *Clarias gariepinus* and *Contracaecum* sp. collected from Olifantsnek Dam (n = 8) and Bospoort Dam (n = 13).

Element	Olifantsnek Dam			Bospoort Dam		
	Muscle	Liver	*Contracaecum* sp.	Muscle	Liver	*Contracaecum* sp.
Cr	1.68 ± 0.13	2.07 ± 0.81	0.89 ± 0.26	2.18 ± 0.30	2.24 ± 0.83	1.04 ± 0.34
Ni	0.69 ± 0.13	1.33 ± 0.97	0.69 ± 0.87	0.39 ± 0.16	0.65 ± 0.41	1.10 ± 0.66
Cu	1.62 ± 0.25	35.0 ± 16.9	3.61 ± 1.01	1.49 ± 0.44	33.8 ± 15.9	3.87 ± 1.13
Zn	18.1 ± 3.79	67.1 ± 18.3	55.0 ± 12.0	18.5 ± 7.01	76.5 ± 18.4	55.6 ± 11.4
Cd	0.06 ± 0.03	0.52 ± 0.41	0.03 ± 0.02	0.16 ± 0.14	0.18 ± 0.11	0.11 ± 0.09
Pt	0.0007 ± 0.0002	0.0014 ± 0.0008	0.0052 ± 0.0041	0.0058 ± 0.0030	0.0067 ± 0.0021	0.0081 ± 0.0051
Pb	0.77 ± 0.35	1.50 ± 0.42	0.97 ± 0.64	2.04 ± 1.28	1.35 ± 0.85	1.69 ± 1.21