

**Fish, water and sediment quality as indicators of land-use change in the
Steelpoort River (Olifants River system, Limpopo Province)**

by

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DISSERTATION

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DECLARATION

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

.....

Ms ET Makushu

.....

Date

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- Prof Jooste and Prof Addo-Bediako for their guidance throughout this study.
- Flemish Interuniversity Council (VLIR-UOS) for financial support.
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DEDICATION

- I would like to dedicate this research to my parents for always being there for me and granting me the opportunity to further my studies.

ABSTRACT

This study aimed to determine the effects of water and sediment quality on fish and the effects of land-use on the water and sediment quality of the Steelpoort River system. To achieve these aims, a few objectives had to be met. The water and sediment quality, the level of metal bioaccumulation of two fish species, the level of acetylcholine esterase inhibition in fish brain tissue, as well as changes in land-use over time. The study adds value in the scientific knowledge of the Steelpoort catchment given that there's little information about it. It is thus a good baseline, which gives direction for further studies conducted within this catchment.

The Steelpoort River, is a tributary of the Olifants River system. The Olifants River (Mpumalanga and Limpopo provinces) is a river stressed by anthropogenic activities with the Steelpoort River being a major sub-catchment. The Steelpoort catchment is filled with mines, agricultural activities, and other developments, such as, the construction of a new dam (De Hoop Dam opened 2014), residential areas and malls. The mines within the catchment are mostly platinum and chrome mines.

Sampling was conducted for high and low flow at five sampling sites: Two upstream sites, 1 and 2, and three downstream sites 3, 4, and 5. Site 1 is the reference site for the study as this section (ecoregion) was described as "least threatened". It was hypothesised that high concentrations of metals and nutrients would be present in the water and sediment, and that the metal would be present in fish tissue and would have an effect on human health, that the biomarker AChE would show signs of inhibition within brain tissue, and lastly that land-use characteristics would have an effect on the water and sediment quality, and inhibition of AChE.

Water and sediment samples were collected quarterly over a period of a year at five sites, whereas fish samples (*Chiloglanis pretoriae* and *Labeobarbus marequensis*) were only collected bi-annually at three sites. The physico-chemical, nutrients, cations and ions, and metals and metalloids were determined. The two fish species were used for metal bioaccumulation and AChE analyses. Human health risk and bio-concentration factor (BCF) analyses were conducted. Using the land-cover details obtained from South African National Biodiversity Institute (SANBI),

Department of Environmental Affairs (DEA) and The Council for Scientific and Industrial Research (CSIR), land-use characteristics were determined and evaluated.

The *in-situ* physico-chemical parameters assessed in this study were found to be within acceptable limits of the Target Water Quality Range (TWQR) at all the sites. The Electrical Conductivity (EC) and Total Dissolved Solids (TDS) parameters were highest at Site 5 and lowest at Site 1, this could be due to Site 5 being the last site before the Steelpoort River connects to the Olifants River, hence this site receives all the ions and solutes from upstream; and the lowest value at Site 1 could be attributed to this site having the least number of land-use effects. For nutrients, the highest total nitrogen concentration was recorded at Site 5 and the highest phosphate concentration at Site 1. This river is mesotrophic according to both the total inorganic nitrogen and phosphorus levels. Metals in the water column that were above DWAF guidelines for Aquatic Ecosystems were Al, Ba, Mn and Zn. In the sediment the following metals were above the Canadian sediment quality guidelines: Cd, Cu, Cr and Zn, which can pose a threat to the health of fish through biomagnification through the food chain.

Both *C. pretoriae* and *L. marequensis* had bioaccumulated metals from the river system. The concentrations of metals in the muscle tissue of *C. pretoriae* were higher and this could be due to the fact that unskinned samples were used in the case of *C. pretoriae*. A variety of metals were present in both fish species but Al, Fe, Sr and Zn were present in higher concentrations. Concentrations of metals were highest in samples from Site 5. The human health risk assessment revealed that Pb could cause health related impacts to the humans who consume a single 150 g fish meal once a week in *C. pretoriae*; and that Co could cause potential risks in the near future. The BCF values revealed that there were more bioconcentrated metals in *C. pretoriae* than in *L. marequensis* and this could also be due to different diets.

The AChE activity was higher for *L. marequensis* during high flow at all sites and higher at Site 1 during low flow but lower at Site 5 and equivalent at Site 2 than the AChE activity recorded for *C. pretoriae*. This could be due to the ability of *L. marequensis* to expel pesticides from their system before the pesticides affect brain activity by decreasing the function of AChE. Both fish species had more AChE activity during low flow for sites 1 and 2 than during high flow. The high flow AChE

activity was higher for Site 5 in both fish species. The results confirm the hypotheses were that metals would be present in fish tissue and would have an effect on human health; and that the biomarker AChE would show signs of inhibition within brain tissue.

The agricultural activity has decreased over time within the catchment, from 2000 to 2014 and this could be due to a decrease of fertile land. There has also been a decrease in the mining activity from 2000 to 2009 and then an increase during 2014, where the decrease could be due to the closure of mines at the end of their life span; and the increase in 2014 could be due to the opening of new mines. Residential areas have increased from 2000 through to 2014. The availability of water decreased from 2000 to 2009, and then increased drastically in 2014. The drastic increase is due to the multiple dams that have been built over time in order to provide water resources to the increased population, and also for mining and irrigation purposes.

The water quality showed an increase in the NO_3 , SO_4 and PO_4 ; meanwhile the EC has decreased over time. The decrease in EC means that there are fewer ions in the river. The increase in phosphates and nitrates could mean there is a lot of fertilizers and pesticides being used in agriculture even with the decrease in agricultural land-use over the years. The pesticides can also be due to the increase in residential areas, where pesticides are also used to get rid of pests.

Table of Contents

DECLARATION	i
ACKNOWLEDGEMENTS	ii
DEDICATION	iii
ABSTRACT	iv
LIST OF FIGURES.....	ix
LIST OF TABLES	x
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW	1
1.1 INTRODUCTION	1
1.1.1 Background	1
1.1.2 Problem statement	3
1.1.3 Research question	3
1.1.4 Hypotheses	3
1.1.5 The aims of the study	4
1.1.6 The objectives of the study	4
1.2 SAMPLING SITES.....	4
1.2.1 Selection	4
1.2.2 Site description	5
1.2.3 Sampling frequency	9
1.3 LITERATURE REVIEW	9
1.3.1 Water quality	9
1.3.2 Sediment quality	10
1.3.3 Land-use effects on water and sediment quality	11
1.3.4 Bioaccumulation	12
1.3.5 Acetylcholinesterase as a biomarker of pesticide pollution	13
1.3.6 Impacts of dams on river systems	14
CHAPTER 2: EFFECTS OF LAND-USE CHANGE.....	15
2.1 INTRODUCTION	15
2.1.1 Aims and objectives of the chapter.....	16
2.2 METHODS AND MATERIALS.....	16
2.3 RESULTS AND DISCUSSION	17
2.4 CONCLUSIONS.....	24
CHAPTER 3: WATER AND SEDIMENT QUALITY	24

3.1 INTRODUCTION	25
3.1.1 Water pollution	25
3.1.2 Sediment quality	26
3.1.3 Aim and Objectives of the chapter	27
3.2 METHODS AND MATERIALS.....	27
3.2.1 Sampling.....	27
3.2.2 Analysis.....	28
3.3 RESULTS AND DISCUSSION	28
3.3 CONCLUSIONS.....	59
CHAPTER 4: FISH METAL BIOACCUMULATION AND ACETYLCHOLINE ESTERASE INHIBITION	61
4.1 INTRODUCTION	61
4.1.1 Metal bioaccumulation.....	61
4.1.2 Human health risk	61
4.1.3 Biomarker of response: AChE.....	63
4.1.4 Objectives of the chapter	63
4.2 METHODS AND MATERIALS.....	64
4.2.1 Sampling.....	66
4.2.2 Analysis.....	66
4.3 RESULTS AND DISCUSSION	69
4.3.1 Fish metal bioaccumulation	69
4.3.3 Bio-concentration Factor (BCF)	83
4.3.4 Acetylcholine esterase inhibition.....	85
4.4 CONCLUSIONS.....	86
CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS	88
5.1 WATER AND SEDIMENT QUALITY	88
5.2 FISH METAL BIOACCUMULATION AND ACETYLCHOLINE ESTERASE INHIBITION	89
5.3 EFFECTS OF LAND-USE CHANGE.....	90
5.4 RECOMMENDATIONS	90
REFERENCES	91
APPENDIX 1: Water and Sediment quality results	101
APPENDIX 2: Metal bioaccumulation results	107

LIST OF FIGURES

Figure 1.1: Steelpoort River system with sampling sites indicated by a star.	5
Figure 1.2: A picture of Site 1 located upstream (E' 29.8471; S'25.101)	6
Figure 1.3: A picture of Site 4 located upstream (E' 29.9614; S' 24.953)	7
Figure 1.4: A picture of Site 3 located downstream (E' 30.0172; S' 24.894)	7
Figure 1.5: A picture of Site 4 located downstream (E' 30.2009; S' 24.718)	8
Figure 1.6: A picture of Site 5 located downstream (E' 30.3020; S' 24.659)	9
Figure 2.1: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2000.	18
Figure 2.2: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2009.	19
Figure 2.3: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2014.	20
Figure 2.4: Graphic presentation of historical data of selected water quality parameters of the Steelpoort River (1990-2014).	23
Figure 3.1: Selected metals (mg/L) present in the water of the Steelpoort River for high flow and low flow.	40
Figure 3.2: Graphic representation of selected metals (mg/kg) present in the sediment of the Steelpoort River.	49
Figure 4.1: The shortspine suckermouth or shortspine rock catlet <i>Chiloglanis pretoriae</i> (Image from www.google.co.za)	64
Figure 4.2: The Lowveld largescale yellowfish <i>Labeobarbus marequensis</i> (Image from www.google.co.za)	65
Figure 4.3: Results for AChE activity within the brain of fish in the Steelpoort River	85
Figure 4.4: Results for AChE activity within the brain of fish in the Steelpoort River	85

LIST OF TABLES

Table 2.1: Percentages of the land-use characteristics and vegetation within the catchment of the Steelpoort River for the years 2000, 2009 and 2014.	21
Table 3.1: Major effects of water quality variables (DWAF 1996).	26
Table 3.2: Water quality parameters in the Steelpoort River during high flow.	29
Table 3.3: Water quality parameters in the Steelpoort River during low flow.	29
Table 3.4: Nutrients present in the water of the Steelpoort River during high flow.	30
Table 3.5: Nutrients present in the water of the Steelpoort River during low flow.	30
Table 3.6: The effects of Inorganic Nitrogen on aquatic ecosystems (Dallas & Day 2004)	37
Table 3.7: The effects of inorganic phosphorus on aquatic ecosystems (Dallas & Day 2004)	39
Table 3.8: Metals concentrations in the sediments in the Steelpoort River during high flow.	47
Table 3.9: Metals concentrations in the sediments in the Steelpoort River during low flow.	48
Table 4.1: Health effects on humans of selected metals	62
Table 4.2: The concentrations of metals in the muscle tissue of metals in the muscle tissue of <i>Chiloglanis pretoriae</i> for low flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.	70
Table 4.3: The concentrations of metals in the muscle tissue of metals in the muscle tissue of <i>Chiloglanis pretoriae</i> for high flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.	70
Table 4.4: The concentrations of metals in the muscle tissue of metals in the muscle tissue of <i>Labeobarbus marequensis</i> for low flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.	71

Table 4.5: The concentrations of metals in the muscle tissue of metals in the muscle tissue of *Labeobarbus marequensis* for high flow (mg/kg dry weight), at the Steelpoort River. 71

Table 4.6: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Chiloglanis pretoriae* during high flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg. 75

Table 4.7: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Chiloglanis pretoriae* during low flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg. 76

Table 4.8: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Labeobarbus marequensis* during high flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg. 78

Table 4.9: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Labeobarbus marequensis* during low flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg. 79

Table 4.10: The bio-concentration factor values calculated between mean metal concentrations in water compared to muscle tissue levels of the two fish species from the Steelpoort River. (LBM- *Labeobarbus marequensis*, CPRE- *Chiloglanis pretoriae*) (b/d- below detection). 83

Table 4.11: The bio-concentration factor values calculated between mean metal concentrations in sediment compared to muscle tissue levels of *Labeobarbus marequensis* and *C. pretoriae* at the Steelpoort River (b/d- below detection). 84

Outlay of dissertation

Chapter 1: This chapter sets the tone for the dissertation, by introducing the theme and reviewing the literature on freshwater ecosystems. A research question followed by hypotheses, aims and objectives are included.

Chapter 2: Water and sediment quality methodology and results are presented with analyses.

Chapter 3: In this chapter, biomarkers; fish metal bioaccumulation and acetylcholine esterase inhibition in fish due to pesticide infestation methodology and results are presented with analyses.

Chapter 4: This chapter lays out the determined land-use changes within the catchment; this is presented through the methodology and analysed results.

Chapter 5: This chapter outlays recommendations based on final conclusions.

EndNote X7 reference manual software was utilised for references and *African Zoology* as a reference style.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 Background

Water is essential for the survival of every living creature in the world (Yu *et al.* 2012). In comparison to the rest of the world, Africa has a high variable water distribution with water supplies unequally distributed in both geographical extent and time (Ankley *et al.* 1994). The southern and northern parts of Africa are subjected to prolonged droughts, receiving considerably less rainfall annually than the equatorial regions (di Toro *et al.* 2005). With expanding populations resulting in rapidly increasing water demands, it was predicted that many African countries could exceed their surface water resources by 2015 (Ankley *et al.* 1994). In addition, climate change models predict a decrease in surface water availability in southern Africa over the next century (US-EPA 2002, de Wit & Stankiewicz 2006).

Human activities are detrimentally impacting the water quality in freshwater ecosystems through freshwater habitat degradation, changes in land-use, unsustainable water abstraction, species introduction and extinctions and pollution e.g. sewage, fertilizers, pesticides and metals (e.g. metals from acid mine drainage) (Davies & Day 1998). Water quality includes physical, chemical and Biological characteristics of water such as pH, dissolved oxygen, turbidity, salinity, conductivity, reduction/oxidation potential, ionic concentrations, nutrients (nitrogen and phosphorus), toxic organic compounds such as pesticides and inorganic compounds such as metals and pathogens (e.g. *E. coli*) (Dallas & Day 2004). Changes in land-use in a catchment can lead to deterioration in water quality by increasing inputs of nutrients, pollutants, sediments and organic matter (Townsend *et al.* 2004).

Acid mine drainage (AMD) mobilises metals (e.g. Fe, Cr, Co, V, Sb) and metalloids (e.g. Al, As, Se, Te), hereafter metals, from the soil and sediment. These metals may be precipitated to the river sediment (Chapman & Wang 2000, di Toro *et al.* 2001). Sediments in rivers act as a sink for metals, particularly in impoundments; these sediments may also release metals back to the water column or be ingested by aquatic organisms living in, or feeding on organisms living in, or near the sediment.

Metals accumulating in freshwater fish may result in long-term human health impairment in communities consuming fish from local impoundments (de Lange *et al.* 2012). Pesticides are difficult to quantify in the environment because they are at such dilute concentrations and degrade rapidly (Fulton & Key 2001). Some pesticides made up of carbamates or organophosphates result in the inhibition of neurotransmitters, such as acetylcholinesterase, and they can be measured in the brain tissue of fish (Fulton & Key 2001).

In South Africa, the Olifants River System in Mpumalanga and Limpopo provinces has been subjected to numerous anthropogenic stressors including extensive agriculture, industrial and mining pollution, untreated sewage and AMD (Ashton & Dabrowski 2011). The Steelpoort River is a major sub-catchment of the Olifants River, and has been ecologically poorly documented, yet has been subjected to major changes in land-use specifically increased mining activities, increased human population density (Ashton & Dabrowski 2011) and the construction of De Hoop Dam. The state of the Steelpoort River was described as Fair to Unacceptable in the State of Rivers Report, conducted in 1999 (Ballance *et al.* 2001).

Study area

The Olifants River (in Mpumalanga and Limpopo provinces) is a river stressed by anthropogenic activities with the Steelpoort River being its major sub-catchment (Figure 1.1). Major changes have taken place in the Steelpoort sub-catchment over the last 20 years including the construction of De Hoop Dam opened 2014, increased mining activity, and increased human population density (Ashton & Dabrowski 2011).

Catchment description

Upper catchment

Site 1 is within this catchment (Figure 1.2). The soil has high clay content with the geology being the Rustenburg Layered Suite, which is part of the Bushveld Igneous Complex. The eastern side of this area is dominated by gabbro and norite whilst the west is dominated by gabbro and diorite. In 2002 it was reported that 30% of this area was being cultivated and a wide area was mined for vanadium and gabbro using strip mining. The mean daily temperatures range from 2.8°C in winter to

24.9°C in summer and the daily climate temperatures are different at various localities with the higher temperatures on plains and lower on higher lying plateaus. Rainfall in summer is in the form of thunderstorms and ranges from 720 mm in the east to 600 mm in the west.

Middle catchment

This is where Site 2 (Figure 1.3) and Site 3 (Figure 1.4) are situated. The geology is characterised as Rustenberg Layered Suite of the Bushveld igneous complex with ultramafic rocks. The rocks in this area consist mainly of pyroxenite, gabbro, norite, and anorthosite, they are characterised by intrusions of bronzitite, magnetite, harzburgite, diorite, and dunite. In low lying areas glenrosa and mispah soils are most common, with rocky and clayey properties.

Lower catchment

This is where site 4 (Figure 1.5) and 5 (Figure 1.6) are situated. The geology is the Rustenburg Layered Suite on the eastern lobe of the Bushveld Igneous Complex, characterised by mafic and ultramafic intrusive rocks. This suite is dominated by “norite, gabbro, anorthosite, and pyroxenite, with localised protrusions of magnetite, chromitite, serpentinitised harburgite, olivine diorite, shale, dolomite and quartzite”.

1.1.2 Problem statement

The Steelpoort River has been subjected to major changes in recent years including the construction of the new dam, increased mining activity, and increased human population density, thus the current state of the Steelpoort River is unknown.

1.1.3 Research question

Is there a connection between land-use change and water and sediment quality, bioaccumulation of metals in fish, and the inhibition of acetylcholine esterase in fish brain?

1.1.4 Hypotheses

- I. Water and sediment quality would be compromised.
- II. Unacceptable concentration of some of the metals and nutrients would be present in fish tissue and can have a potential impact on human health.
- III. The biomarker AChE would show signs of being inhibited.
- IV. Land-use characteristics would have an effect on the water quality.

1.1.5 The aims of the study

- i. To determine the effects of water and sediment quality on fish of the Steelpoort River system,
- ii. To determine the effects of land-use on the water and sediment quality of the Steelpoort River system.

1.1.6 The objectives of the study

- i. To determine the water and sediment quality of the Steelpoort River at five sites during low flow and high flow.
- ii. To determine the level of metal bioaccumulation of two fish species at three sites.
- iii. To determine the effects of pesticides on two fish species of the Steelpoort River by evaluating the acetylcholine esterase inhibition in brain tissue.
- iv. To determine the land-use change and its impacts on water and sediment quality in the Steelpoort River.

1.2 SAMPLING SITES

1.2.1 Selection

Five sites were selected for this study; upstream from the dam, Site 1, just below the dam, Site 2, and downstream there are sites 3, 4, and 5. The sites are easily accessible, thus sampling is unchallenging. Site 1 is the reference site for the study as this region was described as “least threatened” by Walter *et al.* (2011) while the remainder of the sites all fall under regions described as “critically endangered”. The land-use characteristics in close proximity to each site vary, and they are an overall representation of all the land-use characteristics within the catchment.

1.2.2 Site description

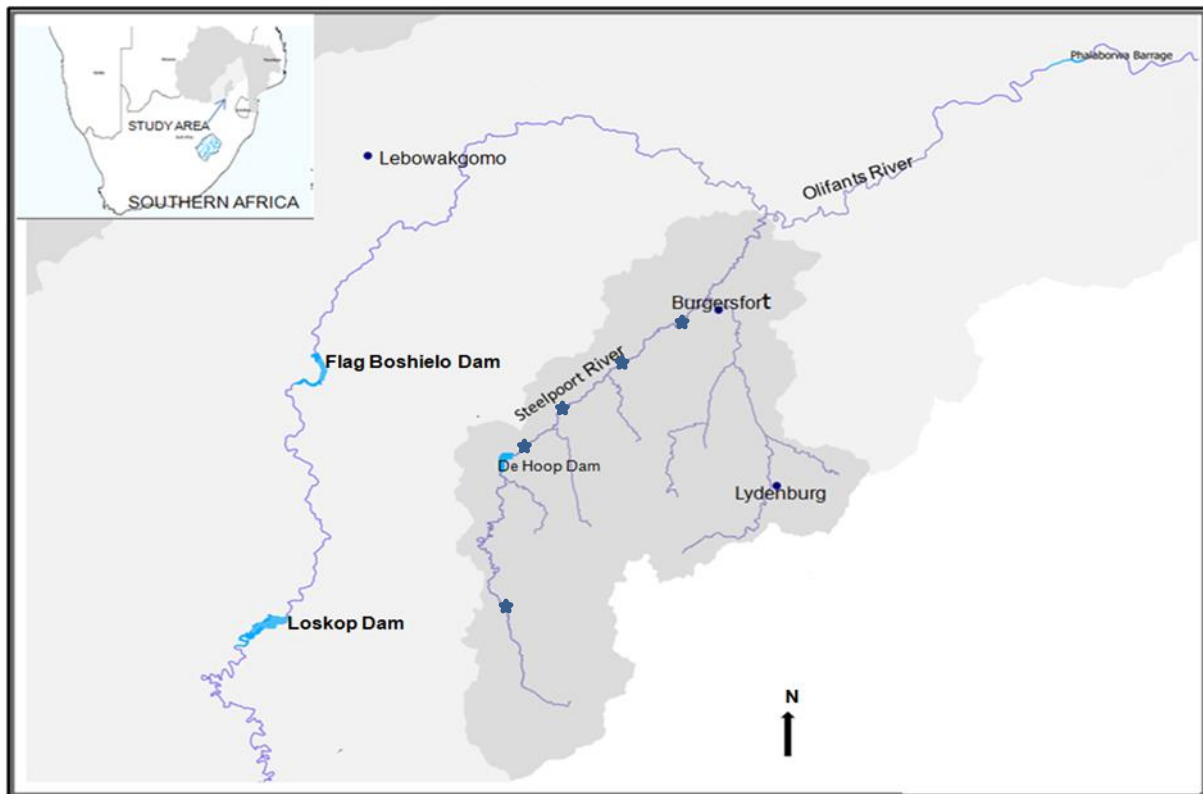


Figure 1.1: Steelpoort River system with sampling sites indicated by a star.

The soil at this site (Figure 1.2) is mostly clay with some parts being sandy and this allows for big trees to thrive. The water is shallow with a slow flow of water. The catchment surrounding the site is mostly used for agriculture; there are no mining activities within this catchment. This is the reference site because it is the least impacted site by land-use activities. It is the only site situated above the dam, thus it is unaffected by dam activities.



Figure 1.2: A picture of Site 1 located upstream at the Steelpoort River (E' 29.8471; S'25.101)

This site (Figure 1.3) is situated right below the dam, thus the primary area affected by dam activities. Selection of this Site was motivated by the effects of the dam. The soil at this site is sandy, allowing for big trees to grow and create plenty of shade. The water has a steady gradient flow.



Figure 1.3: A picture of Site 2 located upstream at the Steelpoort River (E' 29.9614; S' 24.953)

This site (Figure 1.4) is situated in an area experiencing illegal fishing, water pollution due to cars and clothing being washed in the river. Agricultural and residential areas are the main land-use characteristics within the catchment in close proximity to this site. The soil is sandy, with big trees that create shade. There is a steady gradient of water flow and the water is shallow.



Figure 1.4: A picture of Site 3 located downstream at the Steelpoort River (E' 30.0172; S' 24.894)

This site (Figure 1.5) is situated in close proximity to a bridge and is therefore easily accessible. Mining and agricultural land-use characteristics are within this catchment. This site has sandy soil with big trees growing. The water has a slow flowing gradient and is mostly shallow.



Figure 1.5: A picture of Site 4 located downstream at the Steelpoort River (E' 30.2009; S' 24.718)

This is the final site (Figure 1.6) and is affected by residential areas and mining activities. It is close to the junction where the Steelpoort River sub-catchment connects to the Olifants River. The soil is sandy, with big trees. The water is neither deep nor shallow and has a fast flowing water gradient.



Figure 1.6: A picture of Site 5 located downstream at the Steelpoort River (E' 30.3020; S' 24.659)

1.2.3 Sampling frequency

Sampling was conducted quarterly in 2014; during February, May, August and October. Sampling was done for Sites 1 to 5 in the months mentioned above. Data for sampling done for February and May were pooled to become high flow and those for August and October were pooled to become low flow. The high flow and low flow description is to validate the seasonality of the river.

1.3 LITERATURE REVIEW

1.3.1 Water quality

Rivers are confined and uni-directional systems that act as drains for the landscape. Any activities within the catchment of a river are manifested in the river and its ecosystems (Dallas and Day 2004). When a catchment is subjected to activities such as agriculture and mining, the impacts of pollutions of these activities will result in

low pH, high metal concentrations and high salinity which will have serious ecological impacts (de Lange *et al.* 2005).

Globally there are water quality guidelines for human use, maintenance of the integrity of the aquatic ecosystems and other users like agriculture, industrial and recreation. The South African water quality guidelines are a series of seven volumes that have been compiled by the Department of Water Affairs in line with international standards (DWA 1996). The physico-chemical characteristics of water are important in determining the suitability of water for human use and the survival of aquatic organisms, particularly pH, dissolved oxygen saturation, and temperature (DWA 1996, Dallas & Day 2004). Dallas & Day (2004) summarised the impacts of changes in the physico-chemical characteristics of water on aquatic biota as follows: changes in pH influence the toxicity of many dissolved compounds, particularly aluminium; increased nutrient inputs increase productivity of algae and aquatic macrophytes, but may decrease oxygen concentrations during the night. Nutrients (inorganic nitrogen and phosphorus) are essential for aquatic biota but when present in excess can cause eutrophication (Davies & Day 1998). Toxic constituents seldom occur in un-impacted aquatic ecosystems in high concentrations, and include inorganic elements like metals, ammonia and fluoride and organic compounds like pesticides (DWA 1996).

1.3.2 Sediment quality

Sediments are integral to the functioning of aquatic ecosystems as they serve as a reservoir of nutrients and essential metals (Burton 2002). Metals, nutrients, pathogens and organic chemicals tend to adsorb onto both inorganic and organic particles that settle in depositional areas. Sediments may accumulate excessive quantities of contaminants that directly and indirectly disrupt the ecosystem, causing significant contamination or loss of aquatic biota (Burton 2002). The water column and sediment are in direct contact in aquatic systems. This leads to an exchange of constituents, including metals, between these media. Distribution of a metal between water column and sediment is dependent on the physical (movement of water, i.e. fluid transport, and movement of particulate matter, i.e., sediment transport) and the chemical characteristics (controls the speciation of the metal and its sorption to particulate matter in both the water column and the sediment) of the system

(Chapman & Wang 2000, di Toro *et al.* 2001). Concentrations of metals in sediments usually exceed those of overlying water by three to five orders of magnitude. With such high concentrations, the bioavailability of even minute fractions of metals in the sediment assumes considerable importance. The composition of sediments is complex and variable and it is often difficult to assess the bioavailability of sediment bound metals. In water and sediments, the most important factors in determining the bioavailability of metals are water hardness, alkalinity, pH, Redox potential, and the composition of and concentration of ions, particulate matter and organic carbon (di Toro *et al.* 2001).

1.3.3 Land-use effects on water and sediment quality

Surface mining methods e.g. strip mining, show changes in topography and surface drainage and also increase the potential for soil erosion, subsidence, long-term compaction and reduced agricultural capacity. Disturbance and disruption of the natural groundwater regime occurs and this has the potential to pollute both ground and surface water. In addition, changes in topsoil characteristics increase acidity and salt content, changes in vegetation cover, development of nutrient deficiencies, surface desiccation and all these have the potential for production of atmospheric dust and other pollution (Ankley *et al.* 1996).

Acid mine drainage (AMD) is one of the most recognised consequences of mining to freshwaters worldwide and is commonly linked with coal, pyritic sulphide, copper, lead, zinc and silver mining operations (Watson *et al.* 2012). Streams affected by AMD typically have low pH, high concentration of dissolved metals (Fe and Al), and substrate coated with metal hydroxide precipitate (Watson *et al.* 2012). Changes in land-use are known to influence water and sediment quality, river-flow, sediment transport and riparian zones, resulting in declines in aquatic biota (Richards *et al.* 1996, Johnson *et al.* 1997). Agricultural land-use degrades streams by increasing non-point inputs of pollutants, impacting riparian and stream channel habitat, and altering flows increasing inputs of sediments, nutrients, and pesticides (Allan 2004). Streams draining agricultural lands support fewer sensitive insect and fish taxa than streams draining forested catchments (Allan 2004). Streams in highly agricultural landscapes tend to have poor habitat quality, reflected in declines in habitat indexes and bank stability, as well as greater deposition of sediments on and within the streambed (Allan 2004). Appropriate management programmes can improve stream

conditions within the constraints of the existing land-use and agricultural activities (Allan 2004). With the increase in human population, pollution has also increased (Woodborne *et al.* 2012). Sewage run-off can be a visual nuisance together with littering. Sewage and other chemicals that human beings discard into the river system can cause lack of oxygen due to eutrophication (Van den Heever & Frey 1994).

1.3.4 Bioaccumulation

The contamination of water bodies by heavy metals may have detrimental effects on the ecological balance of the recipient water body and also have biological effects on the aquatic biota (Luoma 1989, Wang & Fisher 1999). Metals can accumulate in animals and plants, this is called metal bioaccumulation; which is the ability of an organism to accumulate substances in their tissues through ingestion of food (Dallas & Day 2004). Amongst the variety of the biota, fishes are the ones that can never escape the effects of the heavy metals; this is why fishes are mostly used to evaluate the state of the aquatic ecosystem (Olaifa *et al.* 2004, Croteau *et al.* 2005, Jackson *et al.* 2005). As the pollutants (e.g. metals) build up in the food chain, fishes get the highest dose through a process known as biomagnification (McGeer *et al.* 2004). The rate of accumulation of metals (e.g. arsenic, cadmium, chromium, lead, manganese and mercury) in aquatic biota depends on various factors; the capability of the organisms to be able to digest and excrete metals and finally the concentration of the metals in the water body (The Fish Tissue Advisory Committee 1992). The ability of metals to accumulate in tissues of aquatic species also depends on a number of factors present in water e.g. abiotic factors (pH, temperature) and biotic factors (organism's age and size) (He *et al.* 1998). The toxicity of metals to aquatic organisms depends on the availability of the metal (and its valence state) and is influenced by water hardness, pH, dissolved oxygen, temperature, salinity, interactions with other metal salts and suspended solids (Crafford & Avenant-Oldewage 2010).

Studies have shown that when heavy metals are present in excess, they can alter the physiological and biochemical activities in both fish tissues and brain tissues (Horowitz 1985, Lee *et al.* 2000, Heath *et al.* 2004). A study done by Coetzee *et al.* (2002) at the upper Olifants River, determined the metal bioaccumulation of *Clarias gariepinus* and *Labeo umbratus*, found that Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe

accumulated in the muscle, skin, gills and liver, with the highest concentration on the gills and liver and AI as a toxic agent of AMD. Elevated concentration of AI has detrimental effects on the aquatic biota and humans that consume contaminated fish as seen at Lake Loskop during the late summer of 2008 (Oberholster *et al.* 2012).

1.3.5 Acetylcholinesterase as a biomarker of pesticide pollution

Non-point-source agricultural pollution, mainly pesticide contamination of runoff or spray drift, is regarded as the greatest threat to aquatic systems in rural areas (Thiere & Schulz 2004). Organophosphorus (OPs) carbamate, and synthetic pyrethroid compounds are the most commonly used insecticides. Because of their rapid degradation in the environment, OPs pesticides have become one of the most widely-used insecticides. However, these compounds generally lack target specificity and have high acute toxicity toward many non-target vertebrate and invertebrate species; this is why many terrestrial and aquatic organisms are at risk of being exposed to these compounds in the environment (Fulton & Key 2001). The uses of OPs vary; in agriculture they are used to control insects on fruits, vegetables, grain crops, and stored seeds (Ahlf *et al.* 2009). The most commonly used insecticides in agriculture are the OPs chlorpyrifos and terbufos (Du Laing *et al.* 2009). For household uses the OPs diazinon and chlorpyrifos are common in controlling termites, houseflies, and cockroaches as well as the protection of plants (Aspelin & Grube 1999). Chlorpyrifos and malathion are the OPs insecticides commonly used for commercial purposes, government and in industries. Cholinesterases are divided into two broad classes; acetylcholinesterases (AChEs) and butyrylcholinesterases (BChEs) (Van Den Berg *et al.* 1999). In fishes, the neurotransmitter AChE is predominant in muscle and brain tissue, while liver and plasma contain mostly BChEs, a catalyst in the hydrolysis of acetylcholine into choline and acetic acid at cholinergic synaptic sites (Brungs *et al.* 1978). Once bound, OPs compounds are considered irreversible inhibitors, as recovery usually depends on new enzyme synthesis (Fulton & Key 2001). Responses organisms demonstrate with the inhibition of AChE by OPs and carbamate pesticides are of a broad spectrum. The responses are dependent on the type of pesticide, exposure time, dose and route, water quality and species of fish (Burton 2010).

1.3.6 Impacts of dams on river systems

Rivers possess a delicate ecology which is dependent on a regular cycle of disturbance within certain tolerances (Dudgeon *et al.* 2006). Plant and animal communities that inhabit the river and river margins have evolved and adapted to the river's unique pattern of flood and drought, slow and fast current, however; dams disrupt this ecology (Arthington *et al.* 2010). If the dam is allowed to release water from its reservoir, it will often do so only once in a while, rather than in frequent, small floods as are seen in nature. This leads to scouring and armouring of the riverbed (Arthington *et al.* 2010). The higher energy of the sudden floods picks up and removes smaller sediments like silt, sand, and gravel, as well as aquatic plants and animals, leafy debris, and large woody debris (Arthington *et al.* 2010). Complex sets of habitats are erased (Arthington *et al.* 2010).

CHAPTER 2: EFFECTS OF LAND-USE CHANGE

2.1 INTRODUCTION

The Steelpoort River is on the eastern side of the middle section of the Olifants River catchment (Stimie *et al.* 2001). There are various water users within the Steelpoort River, and a large percentage of mines at its lower end. In the past, the Steelpoort

River basically shared the border between the former Lebowa and the Transvaal Province of the old Republic of South Africa. The boundary is the border between Mpumalanga and Limpopo Province (Stimie *et al.* 2001).

This area is mostly populated on the north bank of the Steelpoort River in the southern district of the Limpopo province, with an average population density of 117 persons/km²; it is Limpopo's most populated district, about three times the average density of the province, which is 41 persons/km² (DWAF 1995). There is competition and potential conflict between irrigation and mining at the Steelpoort River catchment (DWAF 1995). There is a good potential for storage to secure water supply to mines, irrigation and domestic water users (DWAF 1995).

The uses of water are mainly, large scale irrigation, community irrigation, industry, mining, domestic use, livestock, aquaculture, forestry and recreation (Small & Stimie 2000). The surface and groundwater resources are being threatened by increasing level of contaminants from industrial, agricultural, mining and residential sources (Small & Stimie 2000). There is a large chrome smelter and the service industries are concentrated around the mining communities (Small & Stimie 2000). There are chrome, granite, magnetite, alluvial gold, coal, vanadium and platinum mines as well as mines for construction of materials like bricks, stone and sand. There are about 50 mines in total (Small & Stimie 2000). Agricultural co-operatives are stationed within this sub-basin and exports of agricultural and citrus products are dispatched from the Steelpoort area (Small & Stimie 2000).

There were initial impacts discussed in the survey done in 2005 before De Hoop Dam was built (ORWRDP 2005). The Dam would affect 15 private landowners, of which two are Communal Property Associations and the balance, individual commercial farmers (ORWRDP 2005). Mitigating this impact is complicated due to

the fact that the farms will all be divided by the dam and road realignment into two or more segments (ORWRDP 2005).

The dam would reduce the annual flows in the Steelpoort River by approximately 63% (ORWRDP 2005). It would also have a net positive impact on the downstream aquatic ecology, by improving base flow conditions (ORWRDP 2005). This positive impact would be further enhanced by the option of releasing water into the Steelpoort River, thus using the river as a natural conveyance, up to a proposed abstraction weir at Steelpoort town (ORWRDP 2005). The project area is relatively poorly serviced in terms of health facilities, sanitation systems, domestic water supplies, and other essential services; so it was a concern that the potential influx of people during construction of the dam could have a negative impact on the already limited services (ORWRDP 2005).

2.1.1 Aims and objectives of the chapter

To determine land-use change and its effects on the water and sediment quality of the Steelpoort River system. To achieve the aim, the land-use would be determined for the years 2000, 2009 and 2014 in order to detect changes over time. The findings will be compared to the water quality data present for all these years.

2.2 METHODS AND MATERIALS

Data Collection

Characteristics of the study area were determined within the land-cover maps provided by various organisations. The 2000 land-cover details were provided by ARC and CSIR, the 2009 by SANBI and lastly the 2014 by DEA. Historical water quality data for River Health Project was obtained from the DWA surface water quality data-base

Data analysis

In ArcGIS Spatial Analyst using the land-cover details, land-use characteristics were determined and evaluated. The changes in land-use were calculated in order to see the differences over time. Graphs were created to determine the changes over time pertaining to the water quality. The relationship between the changes in land-use and changes in water quality were evaluated.

2.3 RESULTS AND DISCUSSION

The results showing the characteristics of the land-use are represented (Figure 2.1, Figure 2.2, Figure 2.3 and Figure 2.4) and discussed according to their variations. Mining, agriculture and residential areas are the land-use characteristics seen within this catchment. These results are coupled with historical water quality data to deduce their effects on water quality.

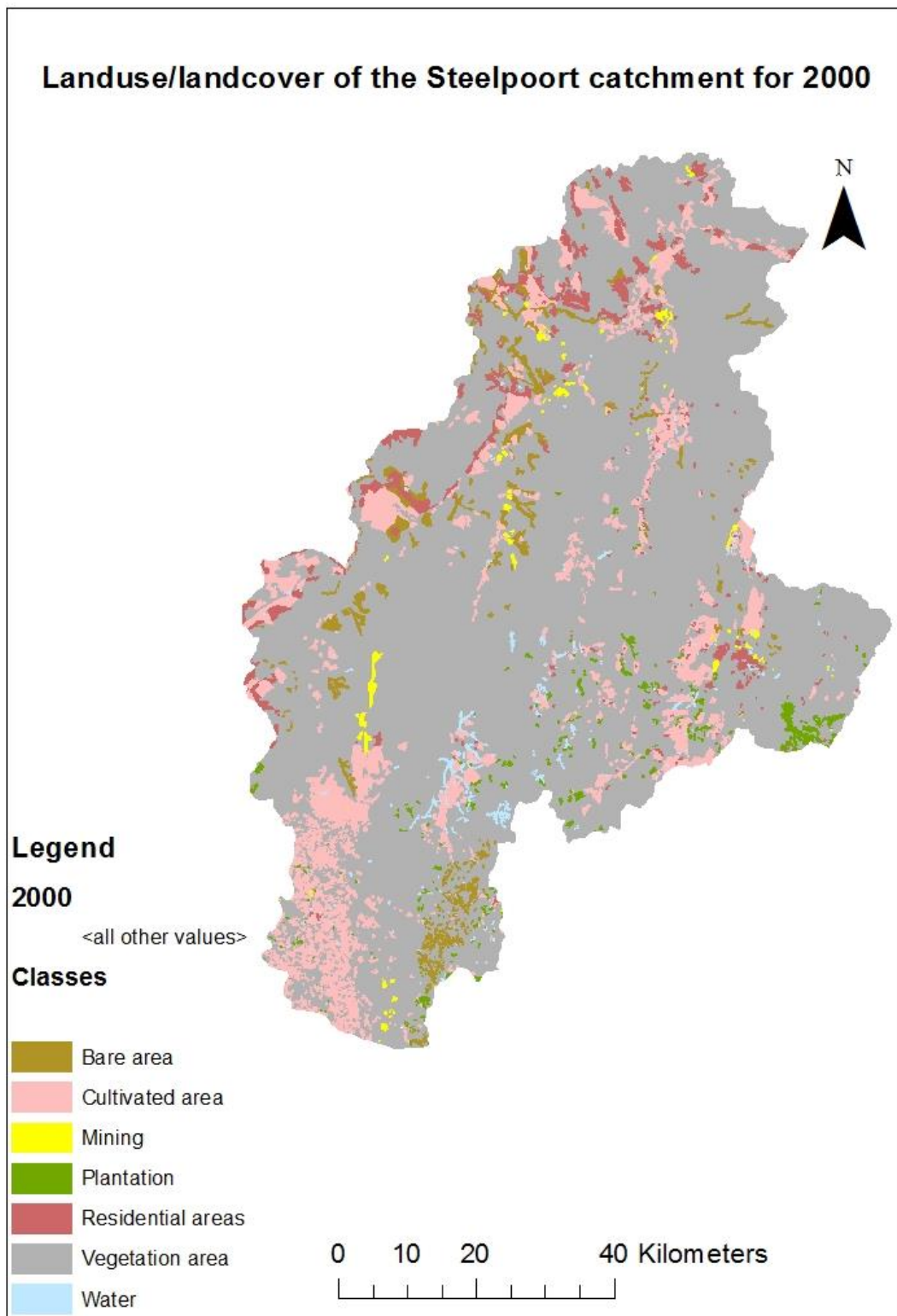


Figure 2.1: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2000.

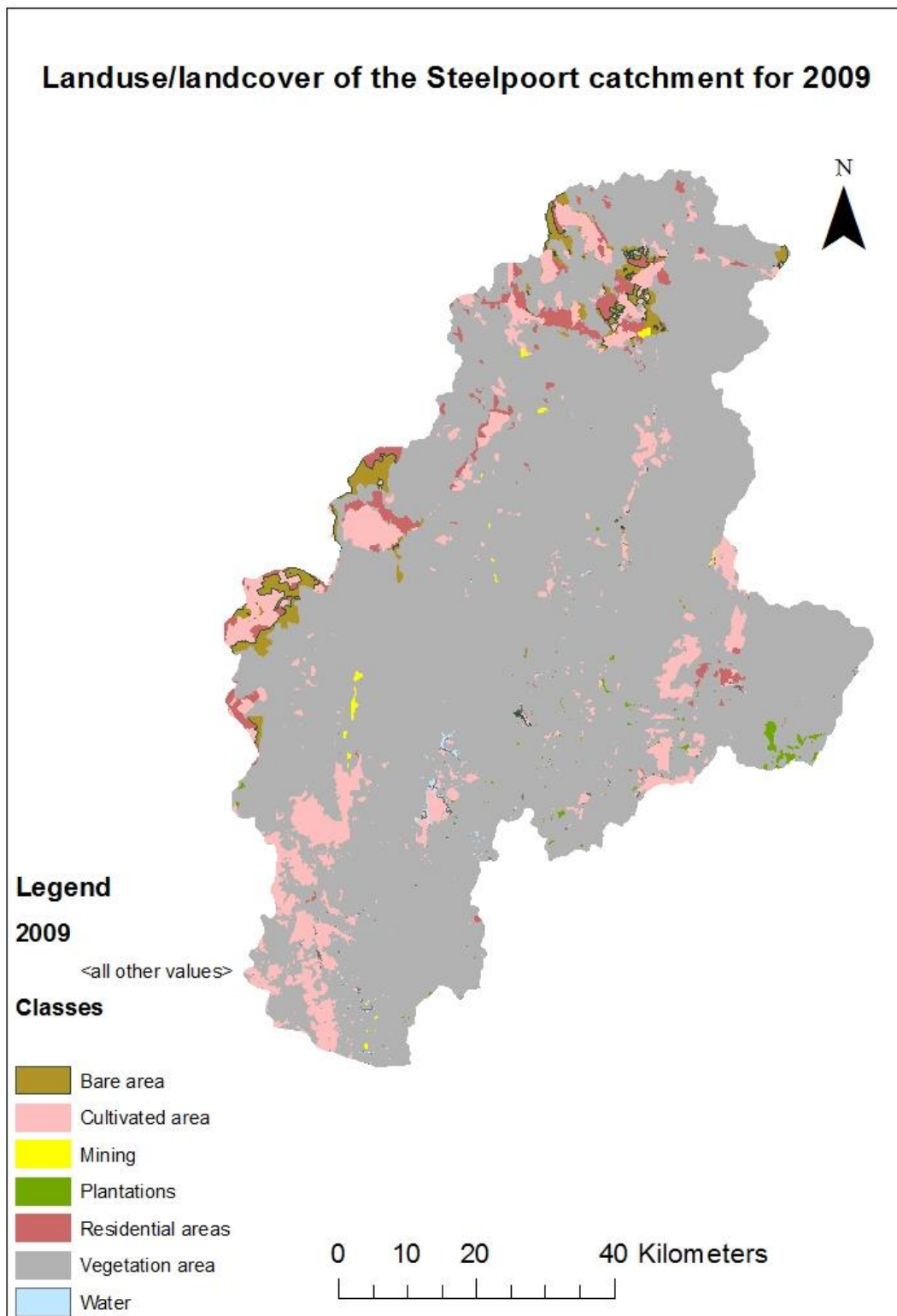


Figure 2.2: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2009.

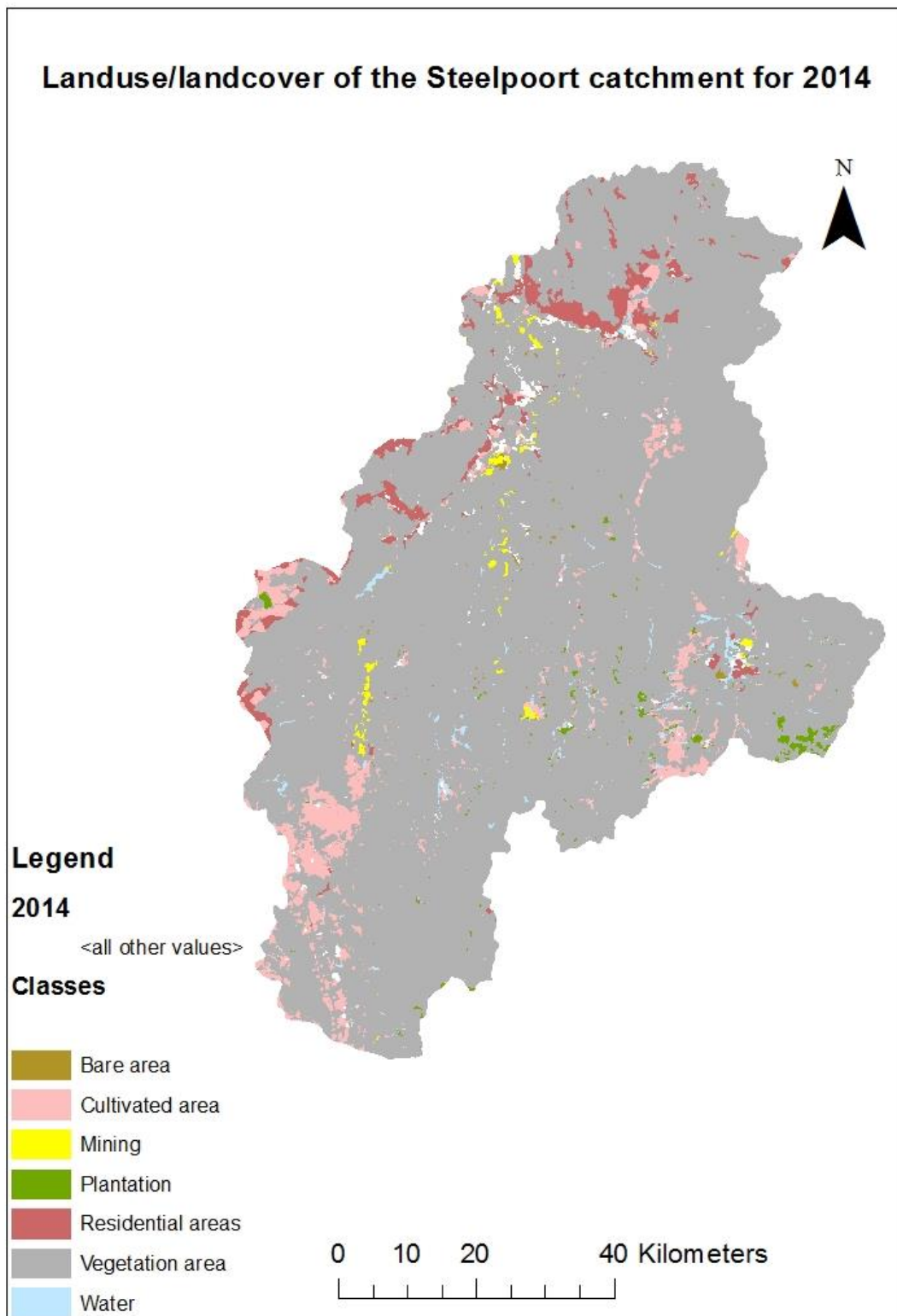


Figure 2.3: Presentation of the land-use characteristics within the catchment of the Steelpoort River during the year 2014.

Table 2.1: Percentages of the land-use characteristics and vegetation within the catchment of the Steelpoort River for the years 2000, 2009 and 2014.

Catchment characteristics	2000	2009	2014
Bare area (%)	2	0.2	0.8
Cultivated area (%)	9.4	8.9	7.4
Mining (%)	0.4	0.03	0.1
Plantation (%)	0.7	0.2	1.1
Residential area (%)	2.5	2.6	3.3
Vegetation area (%)	84.4	87.9	85.2
Water (%)	0.5	0.2	2

Only the land-use and not land-cover characteristics will be discussed because they are anthropogenic. The land-use characteristics are cultivated area, mining, plantations, residential area and water (Figure 2.1) (Figure 2.2) (Figure 2.3). The cultivated areas and plantations are within the agriculture category, the water refers to manmade dams and not any natural systems. The highest land-use characteristic is agriculture throughout all the years. During the year 2000 the agriculture was 10.1% which is higher than the 9.1% recorded in 2009 and even higher than the 8.5% recorded in 2014 (Table 2.1). This means that the agricultural activity has decreased over time within the catchment and this can be due to a decrease of fertile land. There was a decrease in the mining activity from 2000 to 2009 and then an increase during 2014. During the year 2000, the mining activity was 0.4%, in 2009 it was 0.03%, and in 2014 it was 0.1% (Table 2.1). The decrease from 2000 to 2009 could be due to the closure of mines at the end of their life span (Watson *et al.* 2012); and the increase in 2014 could be due to the opening of new mines. Residential areas have increased from 2000 at 2.5% to 2.6% in 2009 and even higher during 2014 at 3.3% (Table 2.1). The water was at 0.5% in 2000 and then decreased to 0.2% in 2009 and then increased drastically to 2% in 2014 (Table 2.1). The drastic increase may be due to the multiple dams that have been built over time (Richards *et al.* 1996) in order to provide water resources to the increased population, and also for irrigation purposes.

The water quality shows an increase in the NO_3 , SO_4 and PO_4 ; whilst the EC has decreased over time (Figure 2.4). The decrease in EC means that there are fewer ions in the river, thus decreasing conductivity. The increase in phosphates and nitrates show that there has been an increase in the use of pesticides and fertilizers (Davies & Day 1998). Pesticides can also come from the residential areas, where people make use of them. Sulphates are not toxic, but in excess form sulphuric acid and can have devastating effects on aquatic ecosystem; and can be due to water seeping from mines, where sulphate levels can be extremely high as mentioned in Chapter 3.

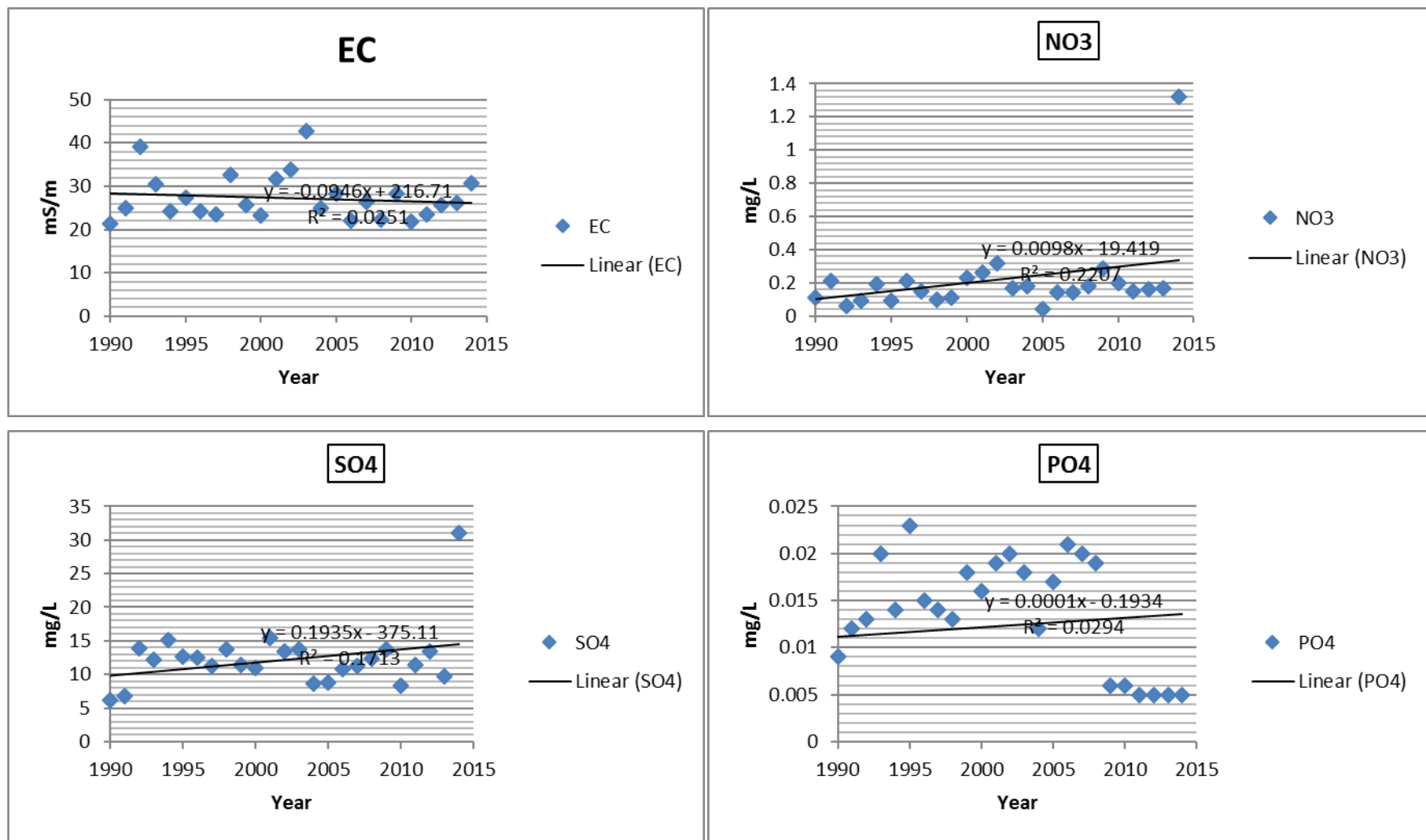


Figure 2.4: Graphic presentation of historical data of selected water quality parameters of the Steelpoort River (1990-2014).

2.4 CONCLUSIONS

The agricultural activity has decreased over time within the catchment, from 2000 to 2014 (Table 2.1). There was a decrease in the mining activity from 2000 to 2009 and then an increase during 2014 (Table 2.1). Residential areas have increased from 2000 through to 2014 (Table 2.1). The water from 2000 to 2009 decreased then increased in 2014 (Table 2.1). The drastic increase is due to the multiple dams that have been built over time, including the De Hoop Dam which was completed in 2014, in order to provide water resources to the increased population, and also for irrigation purposes.

The measurements of NO_3 , SO_4 and PO_4 increased; meanwhile the EC had decreased over time (Figure 2.4). The increase in phosphates and nitrates could mean there is a lot of fertilizers and pesticides within the agriculture. The increase in sulphates is not detrimental since it is an essential ion in protein but can be detrimental in excess when there is water seeping from mines, where sulphate levels can be extremely high.

The hypothesis of this chapter was that the land-use characteristics will have an effect on the water quality. The hypothesis was supported by the results within the chapter.

CHAPTER 3: WATER AND SEDIMENT QUALITY

3.1 INTRODUCTION

Water quality differs in various parts of the world, from continent to continent, and from region to region (Dallas & Day 2004). These differences are attributable to biotic composition, geomorphology, geology and soils, and climate (Dallas & Day 2004). The geomorphology of the landscape determines the gradient of rivers, hence the amount of turbulence and thus the amount of gases dissolved in the water; also determines the degree of erosion, hence turbidity and the quantity of suspended material in the water (Dallas & Day 2004). The geology of an area is a factor because of the variations in the chemical compositions of the rocks and soils that contribute different quantities and different proportions of ions, including nutrients to the water when it flows or percolates through them (Dallas & Day 2004). Catchment vegetation, too, may produce organic compounds that, when leached into water, affect pH and inhibit microbial activity (Dallas & Day 2004).

3.1.1 Water pollution

Globally, inland waters have been adversely impacted by constant increases in domestic, agricultural, mining and industrial water abstraction and the release of contaminants containing metals and pesticides (Suedel *et al.* 1994). Although most metals naturally occur in the biogeochemical cycle, many are released into inland waters as industrial, mining, agricultural and domestic effluents, many of which may have deleterious effects (Dallas & Day 2004). Chemical contamination poses a serious threat to the survival of aquatic organisms.

Table 3.1: Major effects of water quality variables (DWAF 1996).

Water Quality Variables	Major Effects
Physical factors	
Temperature	Determines metabolic rate Determines availability of nutrients and toxins Determines oxygen saturation level Changes provide cues for breeding, migration, etc.
Turbidity and suspended solids	Turbidity determines degree of penetration of light, hence vision, photosynthesis. Suspended solids reduce penetration of light, smother and clog surfaces (e.g. gills) and absorb nutrients, toxins, etc.
Chemical factors	
pH	Determines ionic balance Affects chemical species and therefore availability Affects gill functioning
Conductivity, salinity, TDS, individual ions	Affect osmotic, ionic and water balance
Dissolved oxygen	Required for aerobic respiration
Organic enrichment	Reduces oxygen concentration Increases nutrient levels
Nutrient enrichment	Not toxic <i>per se</i> : cause eutrophication and thus affect community structure
Biocides	Usually target specific groups (e.g. molluscs, insects, plants) and thus alter community structure
Trace metals	Many are essential at low concentrations Some mutagenic, teratogenic, carcinogenic Some metabolic inhibitors

3.1.2 Sediment quality

Sediment quality has been assessed by making comparisons between concentrations of contaminants, using numerical values of sediment quality guidelines (SQGs) (Hamilton 2004, Demirak *et al.* 2006). Based on these comparisons, hazardous potential risks of groups of sediment-bound contaminants can be estimated (Hamilton 2004, Demirak *et al.* 2006). An important aspect in the risks caused by sediment-bound chemicals is the degree of exposure encountered by sediment-dwelling organisms (Timmermans *et al.* 1989). Often only a fraction of

the contaminants bound to sediments are biologically available, in part because desorption can be slow. Thus, actual exposure levels are lower than would be expected on the basis of the total concentrations of compounds in sediment (Timmermans *et al.* 1989).

3.1.3 Aim and Objectives of the chapter

To assess the water and sediment quality in the Steelpoort River at five sites during low flow and high flow.

To achieve the aim, the in-situ physico-chemical parameters, nutrients, metals and metalloids in the water column were determined and discussed (for the sediment quality only metals and metalloids) according to the available water and sediment quality guidelines.

3.2 METHODS AND MATERIALS

3.2.1 Sampling

Water samples were collected seasonally over a period of a year. During high flow, which is the rainy season and low flow, which is after the rainy season. At each of the five sites a handheld YSI 556 Multi Probe System meter was used to determine the *in situ* physico-chemical parameters: temperature, pH, electrical conductivity (mS/m) and dissolved oxygen (mg/L and % saturation). A Mettler Toledo SevenGo Conductivity meter was used to measure TDS (Total Dissolved Solids) in mg/L and salinity in parts per thousand (‰).

At all the sampling sites, acid pre-treated polypropylene sampling bottles (1000, 500 and 250 ml) were used to collect water samples for analyses. Sediment samples were collected using a Friedlinger mud grab and transferred to 250 ml polypropylene sampling bottles.

The water samples were refrigerated immediately in a battery operated fridge and kept refrigerated at (4°C) in the laboratory for chemical analysis. The sediment samples were frozen to prevent bacteriological and chemical activities prior to analysis.

3.2.2 Analysis

Water and sediment samples were submitted to a SANAS accredited laboratory for metal analyses using the Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). The sediment samples were dried for up to 96 hours at 60°C in a drying oven, fully digested with nitric and hydrochloric acid (4:1), filtered and the solution analysed for metals using ICP-MS according to Bervoets & Blust (2003). At the Department of Biodiversity, inorganic nitrogen and phosphate (NH_4 , NO_2 , NO_3 and PO_4), some anions (SO_4 , Cl, and F), turbidity and total water hardness were analysed using a Merck Pharo 100 Spectroquant and Merck™ cell/reagent test kits.

3.3 RESULTS AND DISCUSSION

Water quality

In this section, the water quality constituents are presented for Sites 1 to 5 for high flow and low flow in Table 3.2, the nutrients in Table 3.3 and metals in Figures 3.1 and 3.2. The results were compared to the South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems (DWAF 1996) and the Canadian Council of Ministers of the Environment: Water Quality Guidelines (CCME 2012) in order to determine if they were within or above acceptable water quality ranges.

Table 3.2: Water quality parameters in the Steelpoort River during high flow.

Water Quality Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd
Water temperature	19.3	23.15	23.45	21.35	22.25	21.9	1.67
Dissolved oxygen (mg/L O ₂)	10.52	8.36	7.83	7.93	8.7	8.67	1.09
Dissolved oxygen (%)	115.25	95.9	93.5	92.92	98.35	99.18	9.24
pH	8.58	8.4	8.88	8.71	8.79	8.67	0.19
Conductivity (EC) mS/m	169.35	190.85	237.53	287.25	316.98	240.39	62.39
TDS mg/L	82.95	88.8	111.2	138.6	154.7	115.25	31.07
Salinity (‰)	0.08	0.09	0.11	0.14	0.17	0.11	0.04
Water Hardness (mg/L CaCO ₃)*	19.5	16.5	21.5	28	36.5	24.4	7.97
Turbidity NTU	12.5	3	9.5	23	21	13.8	8.27
Calcium (mg/L)	26.5	44	47.5	68.5	49	47.1	14.96
Magnesium (mg/L)	14.5	9.5	13	15.5	20.5	14.6	4.01
Potassium (mg/L)	1.8	2	2.18	1.81	2.3	2.02	0.22
Sodium (mg/L)	8.5	12.5	14	20	18	14.6	4.55

Table 3.3: Water quality parameters in the Steelpoort River during low flow.

Water Quality Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd
Water temperature	20.45	15.75	15.35	13.15	18	16.54	2.78
Dissolved oxygen (mg/L O ₂)	10.79	12.35	9.3	10.52	9.14	10.42	1.3
Dissolved oxygen (%)	132.15	135.9	111.15	113.35	111.3	120.77	12.2
pH	8.84	8.95	9.1	9	9.3	9.0	0.18
Conductivity (EC) mS/m	263.08	192.58	344.5	493.5	576	373.93	159.04
TDS mg/L	125.2	92.8	163.45	242	275	179.69	77.05
Salinity (‰)	0.12	0.09	0.16	0.23	0.265	0.173	0.07
Water Hardness (mg/L CaCO ₃)*	37	24.5	41	49.5	55	41.4	11.79
Turbidity NTU	11.5	3.5	4	15.5	11.5	9.2	5.24
Calcium (mg/L)	26.15	20.3	25.65	31.8	30.35	26.85	4.52
Magnesium (mg/L)	12.8	9	13.25	22.75	33.15	18.19	9.78
Potassium (mg/L)	1.8	2.35	1.95	1.9	1.85	1.97	0.22
Sodium (mg/L)	13.5	10.25	32.75	50.8	52.3	31.92	19.88

Table 3.4: Nutrients present in the water of the Steelpoort River during high flow.

Water Quality Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd
Nitrate (mg/L NO ₃ -N)	0.99	1.13	0.86	1.30	2.18	1.29	0.52
Nitrite (mg/L NO ₂ -N)	0.06	0.03	0.03	0.06	0.03	0.04	0.01
Ammonium (mg/L NH ₄ -N)	0.17	0.09	0.07	0.16	0.07	0.11	0.05
Total Nitrogen	1.27	1.25	0.96	1.51	2.28	1.46	0.5
Ortho-Phosphate (mg/L PO ₄)	<0.05	<0.05	<0.05	<0.05	<0.05		
Phosphorus (mg/L P)	1.64	2.61	2.96	0.8	0.66	1.73	1.04

Table 3.5: Nutrients present in the water of the Steelpoort River during low flow.

Water Quality Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd
Nitrate (mg/L NO ₃ -N)	1.12	0.55	1.1	1.6	2.5	1.37	0.73
Nitrite (mg/L NO ₂ -N)	0.02	0.02	0.03	0.04	0.03	0.03	0.01
Ammonium (mg/L NH ₄ -N)	0.02	0.03	0.03	0.22	0.05	0.07	0.09
Total Nitrogen	0.6	0.58	0.6	1.87	2.56	1.24	0.92
Ortho-Phosphate (mg/L PO ₄)	<0.05	0.05	0.05	0.07	<0.05	0.06	0.01
Phosphorus (mg/L P)	0	0	0	0.15	0	0.03	0.07

Water temperature

In this study, the water temperature ranged from 13.15 (Table 3.3) to 23.45 °C (Table 3.2). The lowest temperatures were during low flow (Table 3.3) and the highest during high flow (Table 3.2). The highest values were recorded at Site 3 during high flow and the lowest at Site 3 as well during low flow. A statistically non-significant p value between sites was calculated ($p > 0.05$) for both high flow and low flow.

Temperature may be defined as the condition of a body that determines the transfer of heat to, or from, other bodies (DWAF 1996). The solubility of gasses such as, H, N, CO and O decrease with increasing temperature (DWAF 1996). Temperature plays an important role in water by affecting the rates of chemical reactions and therefore also the metabolic rates of organisms (DWAF 1996). Temperature is therefore one of the major factors controlling the distribution of aquatic organisms. Thermal characteristics of running waters are dependent on various features of the region and catchment area, including, the latitude and altitude of the river, hydrological factors such as the source of water, the relative contribution of ground

water, and the rate of flow or discharge, climatic factors such as air temperature, cloud cover, wind speed, vapour pressure and precipitation events, and structural characteristics of the river and catchment area, including topographic features, vegetation cover, channel form, water volume, depth and turbidity (DWAF 1996).

Dissolved Oxygen (DO)

The highest DO concentration was 10.52 mg/L O₂ at Site 1 and the lowest 7.83 mg/L O₂ at Site 3 (Table 3.2); these concentrations were obtained during high flow. A statistically non-significant p value between sites was calculated ($p > 0.05$) for both high flow and low flow.

Gaseous oxygen from the atmosphere dissolves in water and is also generated during photosynthesis by aquatic plants and phytoplankton, it is, however, moderately soluble in water (DWAF 1996). Equilibrium solubility, termed the saturation solubility, varies non-linearly with temperature, salinity and atmospheric pressure, and with other site-specific chemical and physical factors. The maintenance of adequate DO concentrations is critical for the survival and functioning of the aquatic biota because it is required for the respiration of all aerobic organisms (DWAF 1996). Therefore, the DO concentration provides a useful measure of the health of an aquatic ecosystem (DWAF 1996).

pH

The pH ranged from 8.58-8.88 during high flow (Table 3.2) and from 8.84-9.3 during low flow (Table 3.3). The highest values were obtained during low flow (Table 3.3).

The pH value is a measure of the hydrogen ion activity in a water sample (DWAF 1996). The pH is affected by factors such as temperature, the concentrations of inorganic and organic ions, and biological activity (DWAF 1996). The pH may also affect the availability and toxicity of constituents such as trace metals, non-metallic ions such as ammonium, and essential elements such as selenium (DWAF 1996). Industrial activities generally cause acidification rather than alkalisation of rivers (DWAF 1996). Elevated pH values can be caused by increased biological activity in eutrophic systems (DWAF 1996). The pH values may fluctuate widely from below 6 to above 10 over a 24-hour period as a result of changing rates of photosynthesis and respiration (DWAF 1996). A change in pH from that normally encountered in unimpacted streams may have severe effects upon the biota (DWAF 1996). Impacts

of indirect pH changes include changes in the availability of toxic substances such as aluminium and ammonia (DWAF 1996).

Electrical Conductivity (EC)

The highest EC was 316.98 mS/m at Site 5 and the lowest 169.35 mS/m at Site 1 (Table 3.2). The highest and lowest values were obtained during high flow. There was a statistically non-significant difference between sites ($p>0.05$) during high flow and low flow.

Conductivity is a measurement that estimates the amount of total dissolved ions in the water. It is basically the measurement of the ability of the water to conduct electricity. Electrical conductivity impacts directly on the osmotic pressure of water; it is thus a limiting factor for aquatic organisms (Dallas & Day 2004).

Total Dissolved Solids (TDS)

The highest TDS concentration was 275 mg/L at Site 5 (Table 3.3) and the lowest 82.95 mg/L at Site 1 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). A statistically non-significant difference between sites was recorded ($p>0.05$) during high flow and low flow.

The TDS is a measure of the quantity of all compounds dissolved in water that carry an electrical charge (DWAF 1996). Since most dissolved substances in water carry an electrical charge, the TDS concentration is usually, used as an estimate of the concentration of total dissolved solids in the water (DWAF 1996). The TDS concentration is directly proportional to the electrical conductivity (EC) of water (DWAF 1996). Natural waters contain varying quantities of TDS as a consequence of the dissolution of minerals in rocks, soils and decomposing plant material, the TDS concentrations of natural waters therefore being dependent at least in part on the characteristics of the geological formations which the water has been in contact with (DWAF 1996). The TDS concentration also depends on physical processes such as evaporation and rainfall (DWAF 1996).

Salinity

The highest salinity concentration was 0.27 at Site 5 (Table 3.3) and the lowest 0.08‰ at Site 1 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). A statistically non-significant

difference between sites was recorded ($p>0.05$) during high flow and not during low flow.

Salinity is the measurement of the saltiness of the water, it is usually measured by TDS (Heath *et al.* 2010). In natural systems, it is influenced by the geological characteristics, dissolution of salts from land surface, soil, and aquifer material by the rising groundwater (Chapman 1996). Different species have different salinity toleration levels, which is dependent on their physiology (Dallas & Day 2004).

Water hardness

The highest water hardness was 55 (mg/L CaCO₃) at Site 5 (Table 3.3) and the lowest 16.5 (mg/L CaCO₃) at Site 2 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). There was a statistically significant difference between sites ($p<0.05$) during both high flow and low flow. The water hardness values indicated that all the Sites had soft water because the values are <60 mg/L (Dallas & Day 2004).

Water hardness is the measure of the amount of dissolved calcium and magnesium in the water (Warren & Haack 2001). Hardness is caused by compounds of calcium and magnesium, and by a variety of other metals (Warren & Haack 2001). Water hardness classifications depict that 0 to 60 mg/L as calcium carbonate is classified as soft; 61 to 120 mg/L as moderately hard; 121 to 180 mg/L as hard; and more than 180 mg/L as very hard.

Turbidity

The highest turbidity value was 23 NTU at Site 4 (Table 3.2) and the lowest 3 NTU at Site 2 (Table 3.2). The highest and lowest values were obtained during high flow (Table 3.2). There was a statistically non-significant difference between sites ($p>0.05$) during both high flow and low flow. Site 4 being the most turbid and Site 2 being the least turbid may be due to Site 2 is after De Hoop Dam so clearer water flows from it which explains why it's the least turbid.

Turbidity is the water quality characteristic most obvious to the casual observer, because its immediate visual effect is to decrease the clarity of water (Dallas & Day 2004). This factor, together with water colour, leads to impeded light penetration, an effect that may have far-reaching ecological consequences (Dallas & Day 2004). Turbidity is defined as an expression of the optical property that causes light to be

scattered and absorbed rather than transmitted in straight lines through the sample (Dallas & Day 2004). Suspended matter such as clay, silt, finely divided organic and inorganic matter, plankton and other microscopic organisms causes the scattering of light, whilst the absorption of light is caused by soluble coloured organic compounds (Dallas & Day 2004).

Calcium (Ca)

The highest Ca concentration was 68.5 mg/L at Site 4 (Table 3.2) and the lowest was 20.3 mg/L at Site 2 (Table 3.3). The highest concentration was during high flow whilst the lowest was during low flow. There was a statistically significant difference between sites ($p < 0.05$) during low flow but not during high flow ($p > 0.05$).

Calcium is one of the major elements essential for living organisms, found in structural material such as, bones, teeth, mollusc shells and crustacean (e.g. crab) exoskeletons, it is vital for muscle contraction, nervous activity, energy metabolism and a great variety of other biochemical interactions (DWAF 1996). Calcium ions are often the major cations in inland waters (DWAF 1996). Although it is known that calcium is a vital element, very little is known about the actual effects of changes in its concentration on aquatic biotas (DWAF 1996). It is clear, mainly from empirical evidence that waters low in calcium may be unable to support molluscs and crustaceans, both of which require calcium for the construction of shells and exoskeletons (DWAF 1996).

Magnesium (Mg)

The highest Mg concentration was 33.15 mg/L at Site (Table 3.3) and the lowest was 9 mg/L at Site 2 (Table 3.3). The highest and lowest concentrations were both obtained during low flow. There was a statistically significant difference between sites ($p < 0.05$) during low flow but not during high flow ($p > 0.05$).

Magnesium is an essential element, being found in chlorophyll and in a variety of enzymes and being involved in the processes of muscle contraction and the transmission of nervous impulses (Dallas & Day 2004). Since it is usually found in relatively high concentrations, it is unlikely to act as a limiting nutrient or a toxin (Dallas & Day 2004). Very little is known about its effects on aquatic organisms (Dallas & Day 2004).

Potassium (K)

The highest K concentration was 2.35 mg/L at Site 2 (Table 3.3) and the lowest was 1.8 mg/L at Site 1 (Table 3.2) (Table 3.3). The highest concentration was during low flow whilst the lowest was during both high flow and low flow. There was a statistically non-significant difference between sites ($p>0.05$) during high flow and low flow.

Potassium, like sodium, is involved in ionic balance in all organisms, and in the transmission of nervous impulses and in muscle contraction in animals (DWAF 1996). Since it occurs in much lower concentrations than sodium does, potassium can sometimes act as a nutrient, the lack of which limits plant growth (DWAF 1996). It has been shown that potassium concentration tends to correlate most closely with invertebrate community structure in multivariate analyses of a number of streams (Dallas & Day 2004). Because of the mathematical techniques employed, and the type of chemical data included, the significance of such results is questionable (Dallas & Day 2004). The results do suggest, however, that potassium may act as a limiting nutrient for animal communities as well as for plants (Dallas & Day 2004).

Sodium (Na)

The highest Na concentration was 52.3 mg/L at Site 5 (Table 3.3) and the lowest was 8.5 mg/L at Site 1 (Table 3.2). The highest concentration was during low flow whilst the lowest was during both high flow and low flow. There was a statistically non-significant difference between sites ($p>0.05$) during high flow and low flow.

Sodium is ubiquitous in natural waters and is the major cation in sea water and in many South African inland waters (Dallas & Day 2004). It is the major cation involved in ionic, osmotic and water balance in all organisms and is also involved in the transmission of nervous impulses and in muscle contraction (Dallas & Day 2004). Sodium is probably the least toxic metal cation and its effects on aquatic systems are almost entirely as a major contributor to TDS (Dallas & Day 2004).

Nutrients: Inorganic nitrogen

Nitrate and Nitrite

The highest nitrate concentration was 2.5 mg/L at Site 5 and the lowest 0.55 mg/L at Site 2 (Table 3.5). The highest nitrite concentration was 0.06 mg/L at Site 1 and 4 (Table 3.4) and the lowest 0.02 mg/L at Site 1 and 2 (Table 3.5). There were statistically non-significant differences between sites for nitrate and nitrite ($p > 0.05$) during both high flow and low flow.

Nitrite nitrogen (NO_2) is the inorganic intermediate, and nitrate nitrogen (NO_3) the end product, of the oxidation of organic nitrogen and ammonia (DWAF 1996). Nitrate is the more stable than nitrite and is usually far more abundant in the aquatic environment (DWAF 1996). In view of their co-occurrence and rapid inter-conversion, nitrite and nitrate are usually measured and considered together (DWAF 1996). Inter-conversions between the different forms of inorganic nitrogen are part of the nitrogen cycle in aquatic ecosystems. Inorganic nitrogen is primarily of concern due to its stimulatory effect on aquatic plant growth and algae (DWAF 1996). Site-specific conditions, especially the availability of phosphorus, are critically important in modifying the influence of inorganic nitrogen on eutrophication (DWAF 1996).

Ammonium

The highest NH_4^+ concentration was 0.17 mg/L at Site 1 (Table 3.4) and the lowest was 0.02 mg/L at Site 1 (Table 3.5). The highest concentration was during high flow whilst the lowest was during both low flow and low flow. There was a statistically significant difference between sites ($p < 0.05$) during low flow but not during and high flow ($p > 0.05$).

Ammonium ion (NH_4^+) is a reduced form of inorganic nitrogen derived mostly from aerobic and anaerobic decomposition of organic materials and exists either as ions, or can be adsorbed onto suspended organic and inorganic material (DWAF 1996). It has little or no toxicity to aquatic biota, but however contributes to eutrophication (DWAF 1996). Occurring in commercial fertilizers, following application of fertilizer, if the concentration of such compounds exceeds the immediate requirements of the plant, transport *via* the atmosphere or irrigation waters can carry these nitrogen compounds into aquatic systems (DWAF 1996). At low to medium pH values, the ammonium ion dominates, but as pH increases ammonia is formed, the latter being

considerably more toxic to aquatic organisms (DWAF 1996). Prior exposure or acclimation to ammonia increases the tolerance of fish to ammonia and enables them to withstand concentrations that would otherwise be acutely lethal (DWAF 1996).

Total nitrogen

The sum of nitrate, nitrite and ammonium was calculated to get the total nitrogen. The highest total nitrogen concentration was 2.56 mg/L at Site 5 (Table 3.5) and the lowest 0.6 mg/L at Site 1 (Table 3.5). The mean calculated for all the sites during high flow was 1.46 mg/L and 1.24 mg/L during low flow, thus the status of the river is mesotrophic at all sites (Table 3.6).

Table 3.6: The effects of inorganic nitrogen on aquatic ecosystems (Dallas & Day 2004)

Nitrogen (mg/L)	Effects
<0.5	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms.
0.5-2.5	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
2.5-10	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems, nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
>10	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

Nutrients: Phosphorus

Phosphate

The highest phosphate concentration was 2.96 mg/L at Site 3 (Table 3.4) and the lowest 0 mg/L PO₄ at Sites 1, 2, 3 and 5 (Table 3.5). Concentrations for PO₄ were higher during high flow. There was a non-statistically significant difference between sites for inorganic phosphorus (p>0.05) for both high flow and low flow. The PO₄ mean during high flow was 1.73 mg/L and 0.03 mg/L during low flow, classifying the water of the Steelpoort River as mesotrophic at all sites (Table 3.4) (Table 3.5).

Phosphorus can occur in numerous organic and inorganic forms, and may be present in waters as dissolved and particulate species (DWAF 1996). Elemental phosphorus does not occur in the natural environment (DWAF 1996).

Orthophosphates, polyphosphates, metaphosphates, pyrophosphates and organically bound phosphates are found in natural waters (DWAF 1996). Phosphorus is an essential macronutrient, and is accumulated by a variety of living organisms; playing a major role in the building of nucleic acids and in the storage and use of energy in cells (DWAF 1996). In un-impacted waters it is readily utilised by plants and converted into cell structures by photosynthetic action and is considered to be the principle nutrient controlling the degree of eutrophication in aquatic ecosystems (DWAF 1996).

Natural sources of phosphorus include the weathering of rocks and the subsequent leaching of phosphate salts into surface waters, in addition to the decomposition of organic matter (DWAF 1996). Spatial variation is high and is related to the characteristics of regional geology (DWAF 1996). Elevated levels of phosphorus may result from point-source discharges such as domestic and industrial effluents, and from diffuse non-point sources in which the phosphorus load is generated by surface and subsurface drainage (DWAF 1996). Non-point sources include atmospheric precipitation, urban runoff, and drainage from agricultural land, in particular from land on which fertilizers have been applied (DWAF 1996). The most significant effect of elevated phosphorus concentrations is its stimulation of the growth of aquatic plants (DWAF 1996). Both phosphorus and nitrogen limit plant growth, and of these, phosphorus is likely to be more limiting in fresh water (DWAF 1996). The effect is dependent on the form of phosphorus present in the water, since not all forms are available for uptake by plants (DWAF 1996). Other factors, such as water temperature, light and the availability of other nutrients, also play an important role in limiting plant growth (DWAF 1996).

Table 3.7: The effects of inorganic phosphorus on aquatic ecosystems (Dallas & Day 2004)

Phosphorus (mg/L)	Effects
<0.005	Oligotrophic conditions; usually moderate levels of species diversity; usually low productivity systems with rapid nutrient cycling; no nuisance growth of aquatic plants or the presence of blue-green algal blooms.
0.005-0.025	Mesotrophic conditions; usually high levels of species diversity; usually productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms seldom toxic.
0.025-0.250	Eutrophic conditions; usually low levels of species diversity; usually highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae; algal blooms may include species which are toxic to man, livestock and wildlife.
>0.250	Hypertrophic conditions; usually very low levels of species diversity; usually very highly productive systems; nuisance growth of aquatic plants and blooms of blue-green algae, often including species which are toxic to man, livestock and wildlife.

Metals and metalloids

This section shows the metal concentrations detected within the water column. Metals that were not detected are not presented. Results were compared to the South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems (DWA 1996), the British Columbia Environmental Protection Division: Water Quality Guidelines (BC-EPD 2006), the Canadian Council of Ministers of the Environment: Water Quality Guidelines- Aquatic Life (CCME 2012) and the United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life (US-EPA 2012).

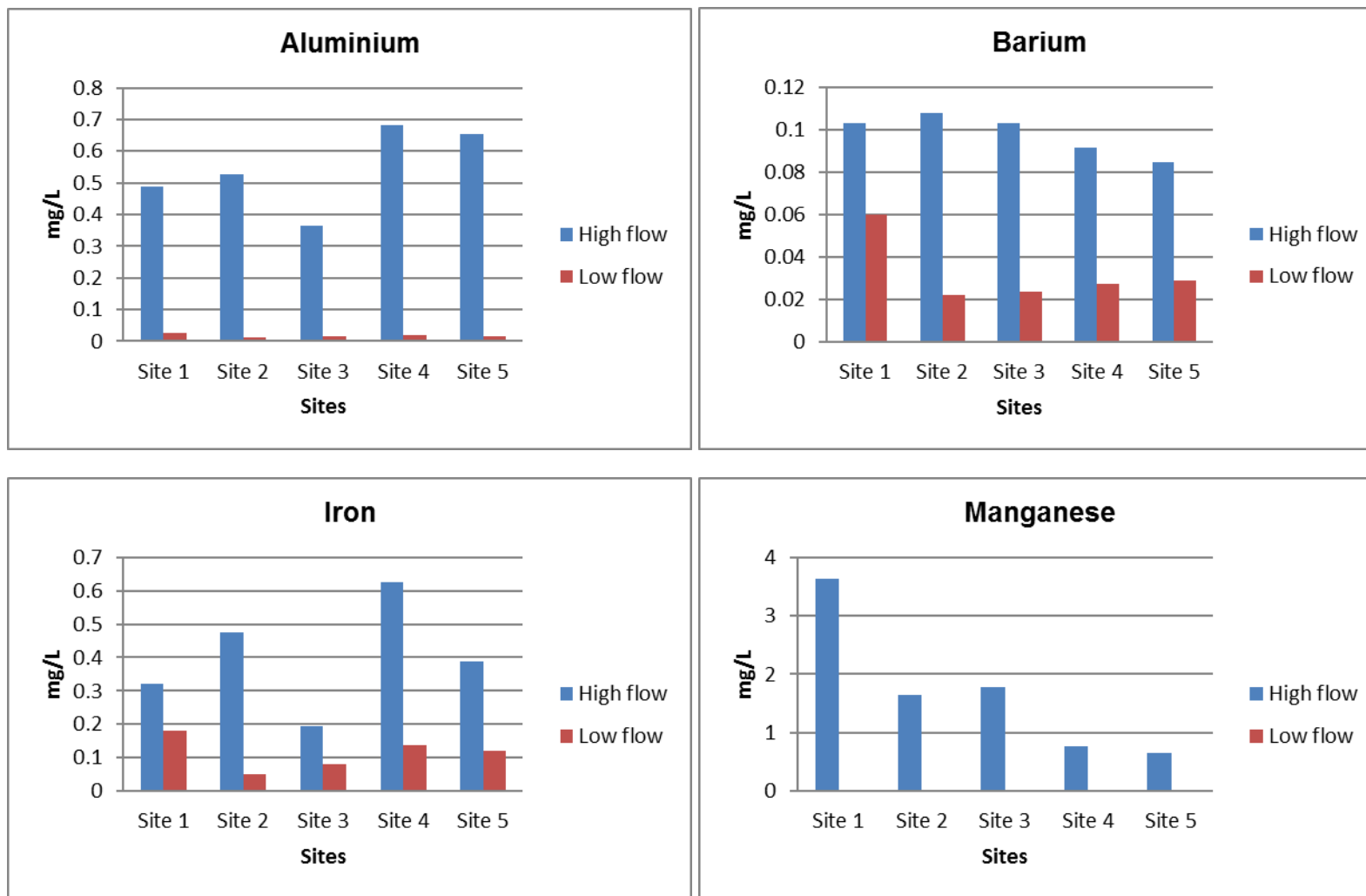


Figure 3.1: Selected metals (mg/L) present in the water of the Steelport River for high flow and low flow.

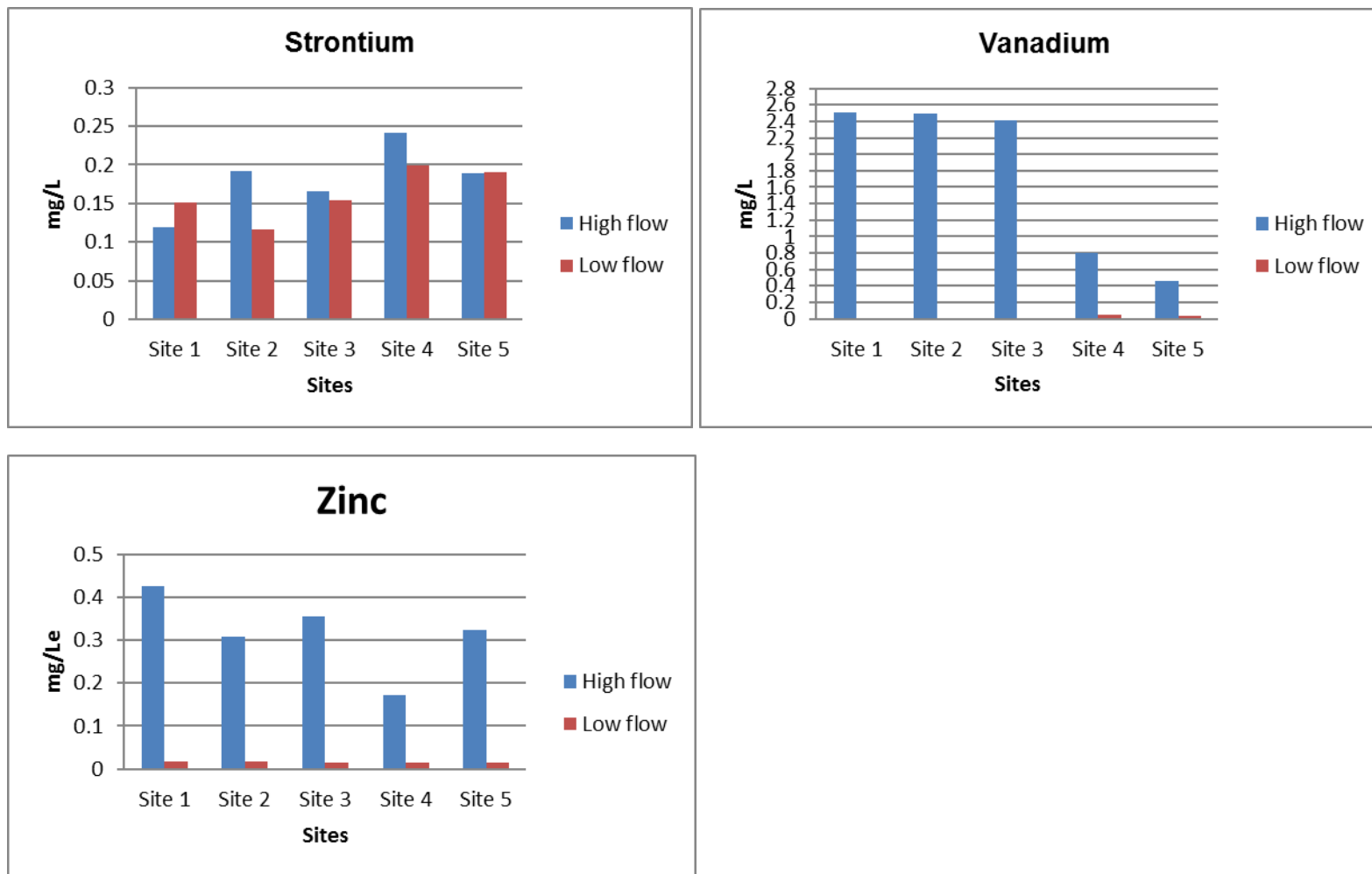


Figure 3.1 (cont.): Selected metals (mg/L) present in the water of the Steelpoort River for high flow and low flow.

Aluminium (Al)

In this study, the Al concentrations ranged from 0.011 to 0.68 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. All the recorded concentrations were above for DWAF (1996) and CCME (2012) guidelines.

Aluminium is the third most abundant metal in the earth's crust and it is widely distributed, naturally occurring in air, soil and water (DWAF 1996, ATSDR 2008). It is very reactive and found in combination with other elements, mostly oxygen, silicon, and fluorine. These chemical compounds are commonly found in soil, minerals, rocks (especially igneous rocks), and clays (ATSDR 2008). High levels in the environment can be caused by the mining and processing of aluminium ores or the production of aluminium metal, alloys and compounds (ATSDR 2008). Small amounts of aluminium are released into the environment from coal-fired power plants and incinerators (ATSDR 2008). It cannot be destroyed in the environment; it can only change its form or become attached or separated from particles (ATSDR 2008). Aluminium is used to make beverage cans, pots and pans, airplanes, siding and roofing, and foil (ATSDR 2008). It is found in consumer products including: antacids, astringents, buffered aspirin, food additives, antiperspirants, and cosmetics (ATSDR 2008). The concentration of aluminium in natural waters (e.g., ponds, lakes, streams) is generally below 0.1 mg/L (ATSDR 2008). It is described as a non-critical element, though there is growing concern over the effects of elevated concentrations of aluminium in the environment, primarily that mobilized as a result of acid mine drainage and acid precipitation (DWAF 1996).

Barium (Ba)

In this study, the Ba concentrations ranged from 0.06 to 0.108 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 1 and the highest during low flow at Site 2. All the recorded concentrations were below guidelines.

These compounds are solids, existing as powders or crystals, and they do not burn well (ATSDR 2007). Two forms of barium, barium sulphate and barium carbonate, are often found in nature as underground ore deposits (ATSDR 2007). Barium is sometimes found naturally in drinking water and food (ATSDR 2007). Because certain barium compounds (barium sulphate and barium carbonate) do not mix well

with water, the amount of barium usually found in drinking water is small. Barium sulphate ore is mined and used in several industries, mostly by the oil and gas industries to make drilling mud; it is also used to make paints, bricks, tiles, glass, rubber, and other barium compounds (ATSDR 2007). Doctors use it to perform medical tests and take x-ray photographs of the stomach and intestines (ATSDR 2007). The length of time that barium will last in air, land, water, or sediments following release of barium into these media depends on the form of barium released (ATSDR 2007). Barium compounds that do not dissolve well in water, such as barium sulphate and barium carbonate, can persist for a long time in the environment (ATSDR 2007).

Iron (Fe)

In this study, the Fe concentrations ranged from 0.04 to 0.63 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. Sites 1, 2, 4 and 5 concentrations were above CCME (2012) guidelines during high flow.

Iron is the fourth most abundant element in the earth's crust and may be present in natural waters in varying quantities depending on the geology of the area and other chemical properties of the water body (DWAF 1996). The two common states of iron in water are the reduced (ferrous, Fe^{2+}) and the oxidised (ferric, Fe^{3+}) states (DWAF 1996). Most iron in oxygenated waters occurs as ferric hydroxide; ferric salts are insoluble in oxygenated waters, and hence iron concentrations are usually low in the water column (DWAF 1996). In reducing waters, the ferrous form, which is more soluble, may persist and, in the absence of sulphide and carbonate anions, high concentrations of ferrous iron may be found (DWAF 1996). The toxicity of iron depends on whether it is in the ferrous or ferric state, and in suspension or solution. Although iron has toxic properties at high concentrations, inhibiting various enzymes, it is not easily absorbed through the gastro-intestinal tract of vertebrates (DWAF 1996).

Manganese (Mn)

In this study, the Mn concentrations ranged from 0.002 to 3.64 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 5 and the highest during low flow at Site 1. All the concentrations recorded during high flow were above DWAF

(1996) guidelines whilst only Sites 1, 2 and 3 were above and CCME (2012) guidelines.

Manganese is the eighth most abundant metal in nature, and occurs in a number of ores. Soils, sediments and metamorphic and sedimentary rocks are significant natural sources of manganese (DWAF 1996). Industrial discharges also account for elevated concentrations of manganese in receiving waters. It's an essential micronutrient for plants and animals (DWAF 1996). In aquatic ecosystems, it does not occur naturally as a metal but is found in various salts and minerals, frequently in association with iron compounds (DWAF 1996). When it is not present in sufficient quantities, photosynthetic productivity may be limited and plants may exhibit chlorosis or failure of leaves to develop properly (DWAF 1996). A deficiency in manganese in vertebrates leads to skeletal deformities and reduced reproductive capabilities (DWAF 1996). High concentrations of manganese are toxic, and may lead to disturbances in various metabolic pathways such as the inhibition of the formation of dopamine (DWAF 1996). The concentration of dissolved manganese is influenced by changes in redox potential, dissolved oxygen, pH and organic matter (DWAF 1996).

Strontium (Sr)

In this study, the Sr concentrations ranged from 0.12 to 0.24 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. All the concentrations recorded were below the guidelines.

Strontium is a natural and commonly occurring element, in its pure form, it is a hard, white-coloured metal, but this form is not found in the environment, in the environment it is usually found in the form of minerals (ATSDR 2004). There are two types of strontium compounds, those that dissolve in water and those that do not (ATSDR 2004). Rocks, soil, dust, coal, oil, surface and underground water, air, plants, and animals all contain varying amounts of strontium. Strontium compounds, such as strontium carbonate, are used in making ceramics and glass products, pyrotechnics, paint pigments, fluorescent lights, medicines, and other products (ATSDR 2004).

Vanadium (V)

In this study, the V concentrations ranged from 0.002 to 2.506 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 3 and the highest during low flow at Site 1. There are no guidelines for vanadium.

Vanadium is a naturally occurring element. It is widely distributed in the earth's crust at an average concentration of approximately 100 mg/kg (ATSDR 2009). Depending on its form, vanadium can be a grey-white metal or light grey or white lustrous powder (ATSDR 2009). Vanadium is used in producing rust-resistant, spring, and high-speed tool steels (ATSDR 2009). Most foods have naturally occurring low concentrations of vanadium (ATSDR 2009). Seafood generally contains higher concentrations of vanadium than meat from land animals (ATSDR 2009). It may also be found in various commercial nutritional supplements and multivitamins (ATSDR 2009). Forms of vanadium such as metavanadate or vanadyl sulphate in experimental treatment of diabetes have been reported to cause nausea, mild diarrhoea, and stomach cramps (ATSDR 2009). It occurs naturally in soil, water, and air (ATSDR 2009). Releases of vanadium to the environment are mainly associated with industrial sources, especially oil refineries and power plants using vanadium rich fuel oil and coal (ATSDR 2009).

Zinc (Zn)

In this study, the Zn concentrations ranged from 0.0145 to 0.425 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 3 and the highest during low flow at Site 1. Zinc concentrations recorded at all Sites were above DWAF (1996) and CCME (2012) guidelines. Zinc concentrations recorded at all Sites during high flow were above BCEPD (2006) and USEPA (2012) guidelines.

Zinc, a metallic element, is an essential micronutrient for all organisms as it forms the active site in various metalloenzymes (DWAF 1996). Zinc occurs in two oxidation states in aquatic ecosystems, namely as the metal, and as zinc (II) (DWAF 1996). It occurs in rocks and ores and is readily refined into a pure stable metal (DWAF 1996). It can enter aquatic ecosystems through both natural processes such as weathering and erosion, and through industrial activity (DWAF 1996). In aquatic ecosystems the zinc (II) ion is toxic to fish and aquatic organisms at relatively low concentrations (DWAF 1996). A variety of interactions affect the toxicity of zinc in

aquatic ecosystems. The toxicity of zinc is reduced in hard waters (DWAF 1996). Zinc is a trace metal which is also an essential micronutrient in all organisms (DWAF 1996). Severe imbalances can cause death, whereas marginal imbalances contribute to reduced fitness (DWAF 1996).

Sediment quality

In this section, the metals in the sediment are represented in mg/kg dry weight (dw) and compared to the guidelines (Table 3.8 and Table 3.9). Most of the metals have no guidelines but some have guidelines in the Canadian Council of Ministers of the Environment: Sediment Quality Guidelines (CCME 2012). The values of concentrations exceeding Sediment Quality Guidelines (SQG) are bolded.

Table 3.8: Metals concentrations in the sediments in the Steelpoort River during high flow.

Metals (mg/kg)	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd	Sediment quality guidelines
Aluminium	21905.15	28197	29704.3	79926.05	42540.95	40454.69	23303.69	No guidelines
Barium	61.4	54.75	72.05	69.55	46.9	60.93	10.41	No guidelines
Boron	10.7	11.3	16.4	40.2	10.8	17.88	12.7	No guidelines
Cadmium	<0.010	<0.010	4.7	2.4	0.8	2.63	1.96	0.6 mg/kg
Chromium	69.8	141.6	126.85	68.25	136.00	108.50	36.42	37.3 mg/kg dw
Cobalt	71.25	62.15	53.7	11.95	15.3	42.87	27.43	No guidelines
Copper	59.8	45.55	57.7	12.65	15.2	38.18	22.82	35.7 mg/kg dw
Iron	258839.35	215268.2	196820	26470.75	40710.2	147621.7	106622.59	No guidelines
Lead	<4.00	<4.00	6.5	4.4	<4.00	5.45	1.48	35.0 mg/kg dw
Manganese	1451.7	1282.55	1140.7	334.4	439.2	929.71	509.04	No guidelines
Nickel	1363.1	901.15	1872.7	432.8	479.55	1009.86	611.42	No guidelines
Selenium	<4.00	<4.00	5.9	4	<4.00	4.95	1.34	No guidelines
Strontium	50.35	63.85	71.8	177.45	95.65	91.82	50.62	No guidelines
Vanadium	1010.8	1481.35	823.9	91.55	126.9	706.9	596	No guidelines
Zinc	187.95	158.25	142.25	30.5	38.9	111.57	72.12	123.0 mg/kg dw

Table 3.9: Metals concentrations in the sediments in the Steelpoort River during low flow.

Metals (mg/kg)	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	±sd
Aluminium	21905.15	28197	29704.3	79926.05	42540.95	40454.69	23303.69
Barium	61.4	54.75	72.05	69.55	46.9	60.93	10.41
Boron	10.7	11.3	16.4	40.2	10.8	17.88	12.7
Cadmium	<0.010	<0.010	4.7	2.4	0.8	2.63	1.96
Chromium	69.8	141.6	126.85	68.25	136.00	108.50	36.42
Cobalt	71.25	62.15	53.7	11.95	15.3	42.87	27.43
Copper	59.8	45.55	57.7	12.65	15.2	38.18	22.82
Iron	258839.35	215268.2	196820	26470.75	40710.2	147621.7	106622.59
Lead	<4.00	<4.00	6.5	4.4	<4.00	5.45	1.48
Manganese	1451.7	1282.55	1140.7	334.4	439.2	929.71	509.04
Nickel	1363.1	901.15	1872.7	432.8	479.55	1009.86	611.42
Selenium	<4.00	<4.00	5.9	4	<4.00	4.95	1.34
Strontium	50.35	63.85	71.8	177.45	95.65	91.82	50.62
Vanadium	1010.8	1481.35	823.9	91.55	126.9	706.9	596
Zinc	187.95	158.25	142.25	30.5	38.9	111.57	72.12

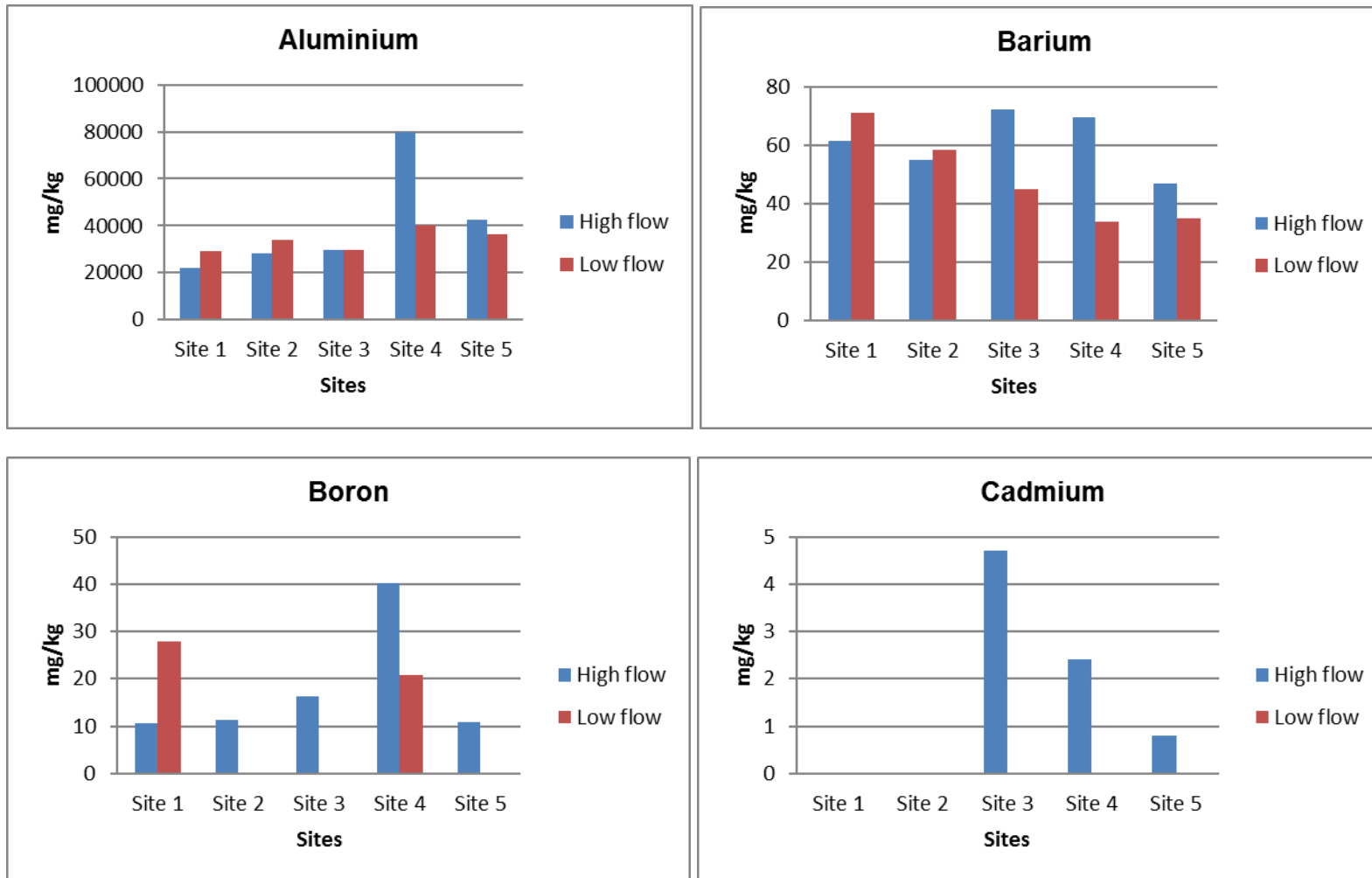


Figure 3.2: Graphic representation of selected metals (mg/kg) present in the sediment of the Steelpoort River.

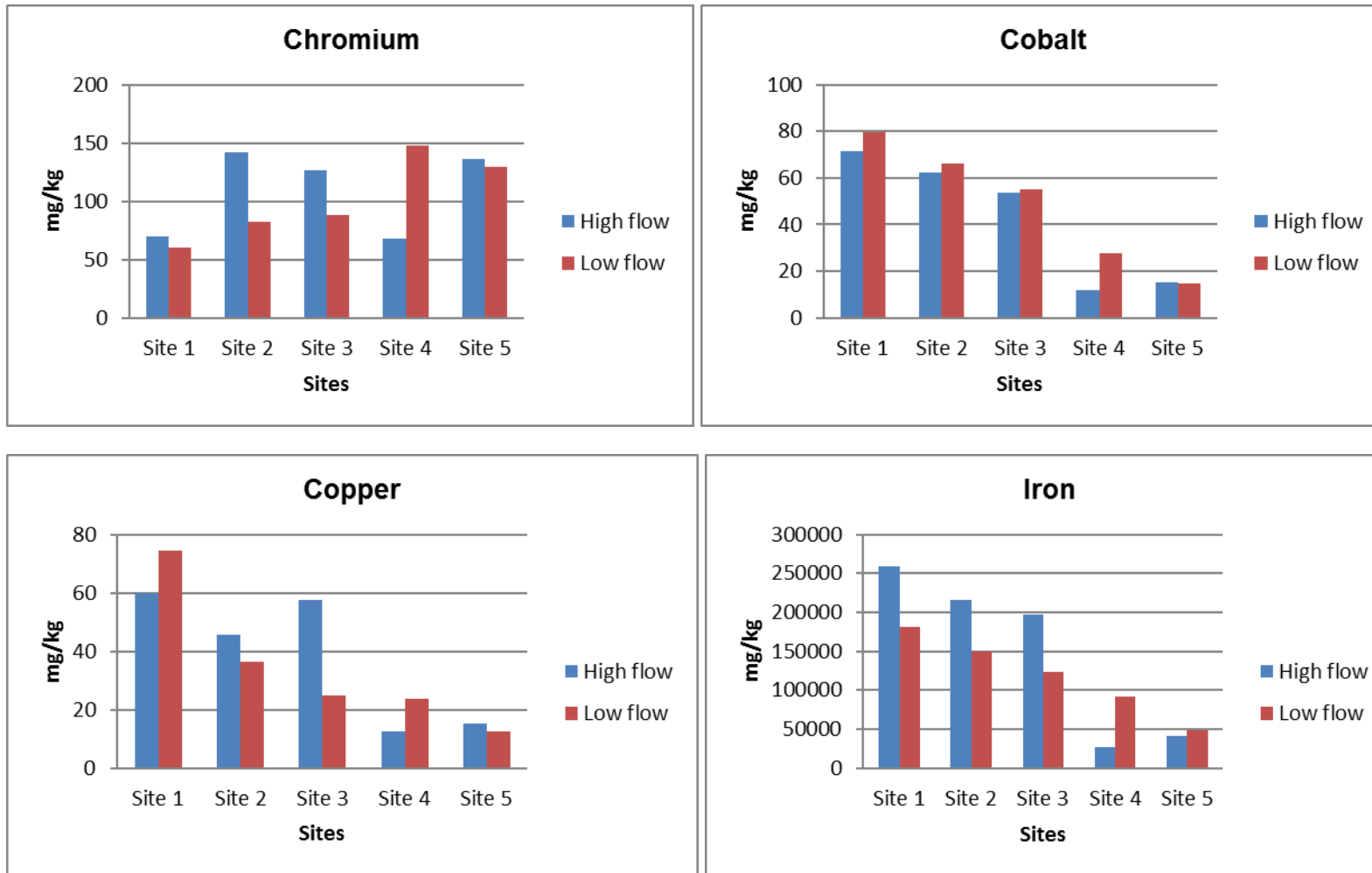


Figure 3.2 (cont.): Graphic representation of selected metals (mg/kg) present in the sediment of the Steelpoort River.

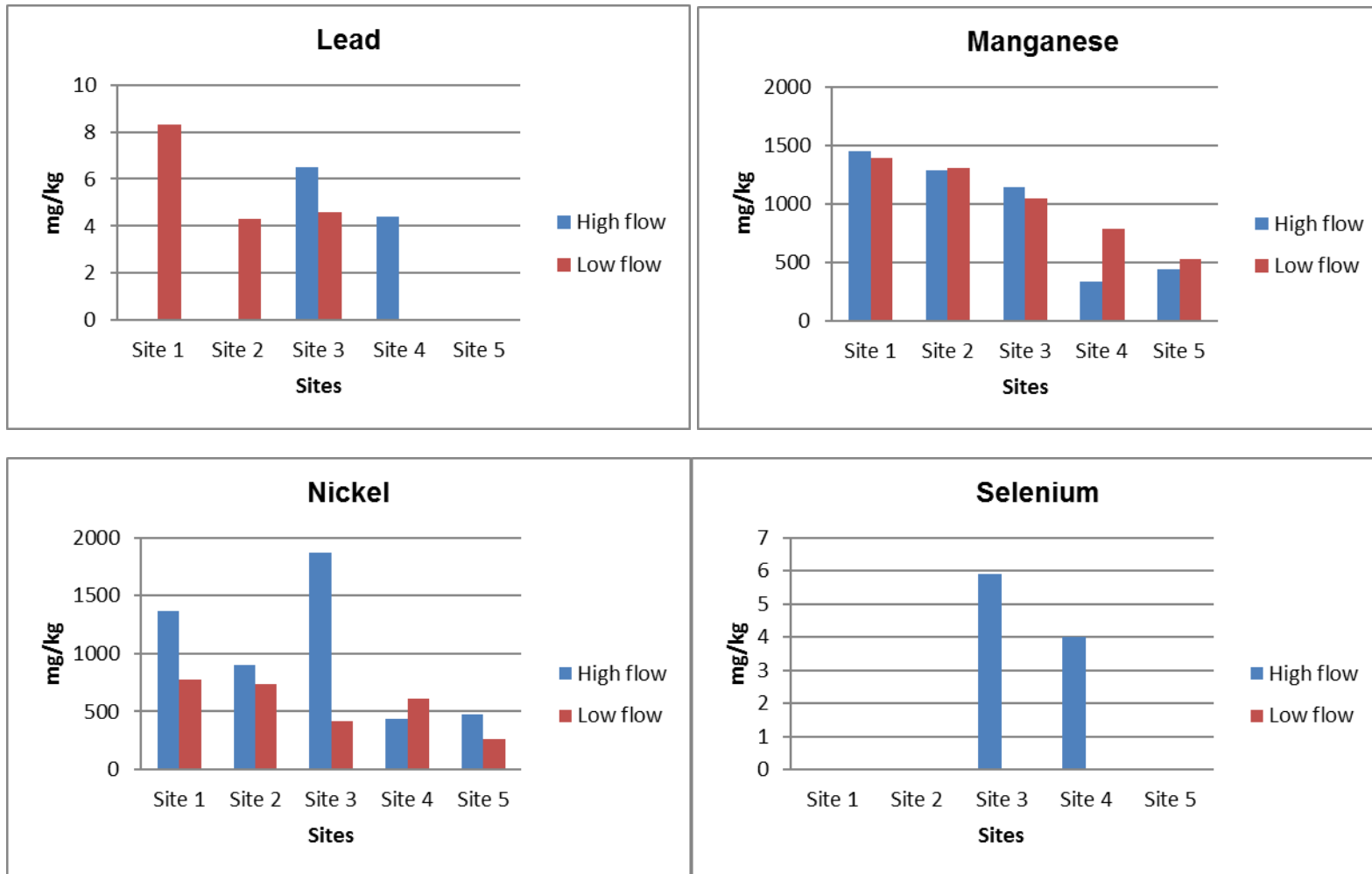


Figure 3.2 (cont.): Graphic representation of selected metals (mg/kg) present in the sediment of the Steelpoort River.

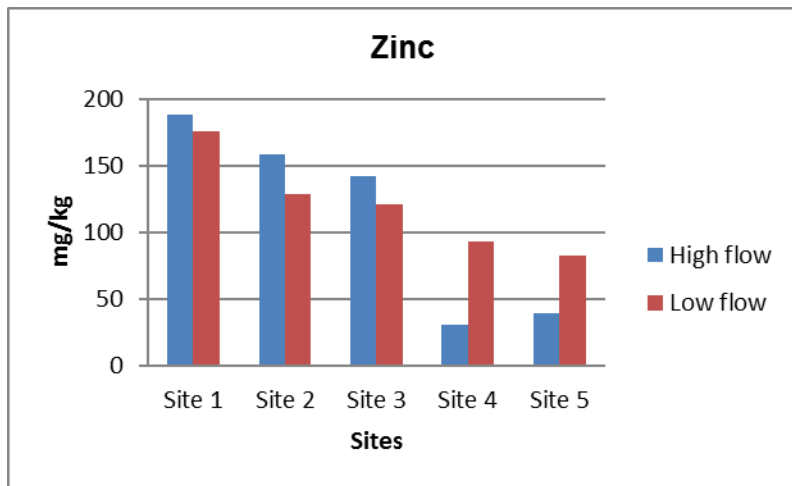
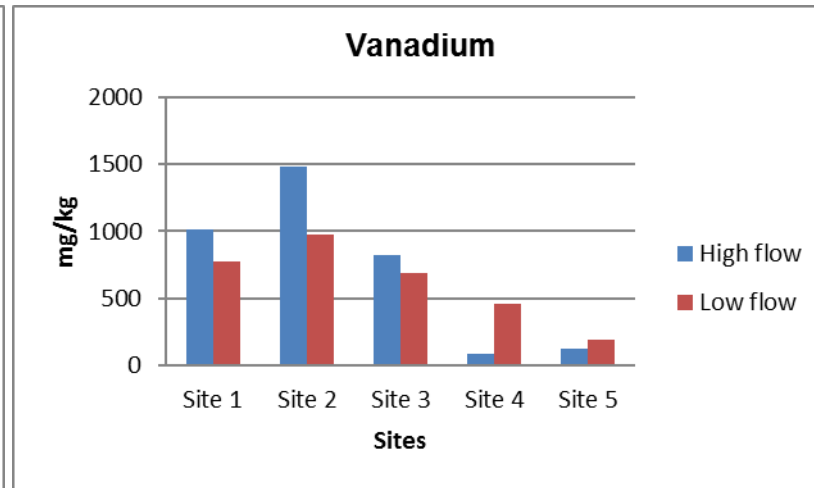
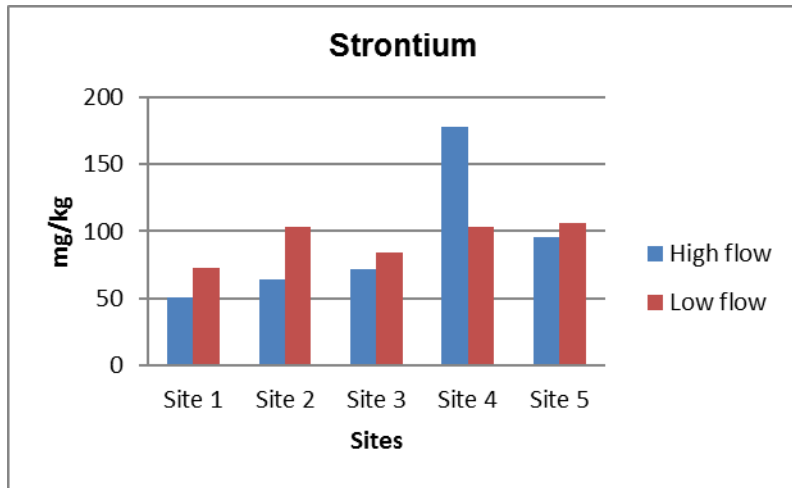


Figure 3.2 (cont.): Graphic representation of selected metals (mg/kg) present in the sediment of the Steelpoort River.

Aluminium (Al)

The Al concentrations ranged from 79926 to 28197 mg/kg. The highest concentration was at Site 4 lowest at Site 1 (Table 3.8). The highest concentrations were obtained during high flow (Table 3.8), hence, the lowest during low flow (Table 3.9). There was a statistically significant difference between sites ($p < 0.05$) during high flow but not during low flow ($p > 0.05$).

Barium (Ba)

The Ba concentrations ranged from 33.75 to 72.05 mg/kg. The highest concentration was at Site 3 (Table 3.8) lowest at Site 4 (Table 3.9). The highest concentrations were obtained during high flow (Table 3.8), hence, the lowest during low flow (Table 3.9). There was a non-statistically significant difference between sites ($p > 0.05$) during both high flow and low flow.

Boron (B)

The B concentrations ranged from below detection level to 16.4 mg/kg. The highest concentration was at Site 3 (Table 3.8) and those below detection were Sites 2, 3 and 5 (Table 3.9). The highest concentrations were obtained during high flow (Table 3.8) and the lowest during low flow (Table 3.9).

Boron is a widely occurring element in minerals found in the earth's crust and is the 51st most common element found in the earth's crust and is found at an average concentration of 8 mg/kg (ATSDR 2010). It is found in the environment primarily combined with oxygen in compounds called borates; common borate compounds include, boric acid, sodium tetraborates (also referred to as borax), and boron oxide (ATSDR 2010). Borate-containing minerals are mined and processed to produce borates for several industrial uses such as glass and ceramics (ATSDR 2010). Boron can be released into air, water, or soil after natural weathering of soils and rocks (ATSDR 2010). Smaller amounts of boron can be released from glass manufacturing plants, coal-burning power plants, copper smelters, agricultural fertilizer and pesticide usage (ATSDR 2010). It cannot be destroyed in the environment, it can only change its form or become attached or separated from particles in soil, sediment, and water (ATSDR 2010).

Cadmium (Cd)

The Cd concentrations ranged from below detection level to 4.7 mg/kg. The highest concentration was at Site 3 (Table 3.8) all the concentrations at all Sites during low flow were below detection level (Table 3.9). Concentrations at Sites 3, 4 and 5 were above CCME (2012) SQG during high flow.

Pure cadmium is a soft, silver-white metal. It is found in the earth's crust, associated with zinc, lead, and copper ores (ATSDR 2008). It is emitted to soil, water, and air by non-ferrous metal mining and refining, manufacture and application of phosphate fertilizers, fossil fuel combustion, and waste incineration and disposal (ATSDR 2008). It can accumulate in aquatic organisms and agricultural crops (ATSDR 2008). As an oxide, chloride, and sulphate, cadmium exists in air as particles or vapours (ATSDR 2008). It can be transported long distances in the atmosphere, where it will deposit onto soils and water surfaces (ATSDR 2008). In water it exists as the hydrated ion or as ionic complexes with other inorganic or organic substances. Soluble forms migrate in water (ATSDR 2008). Its insoluble forms are immobile and will deposit and absorb to sediments (ATSDR 2008). Cadmium and its compounds may travel through soil, but its mobility depends on several factors such as pH and amount of organic matter, which will vary depending on the local environment (ATSDR 2008).

Chromium (Cr)

The Cr concentrations ranged from 60.5 to 136 mg/kg. The highest concentration was at Site 5 (Table 3.8) lowest at Site 1 (Table 3.9). The highest concentrations were obtained during high flow (Table 3.8), hence, the lowest during low flow (Table 3.9). There was a non-statistically significant difference between sites ($p > 0.05$) during both high flow and low flow.

Chromium is a naturally-occurring element found in rocks, animals, plants, and soil. The three main forms of chromium are chromium (0), chromium (III), and chromium (VI). Small amounts of chromium (III) are considered to be a necessity for human health (DWAF 1996). Chromium can be found in air, soil, and water after release from the manufacture, use, and disposal of chromium-based products, and during the manufacturing process. In water and soil, it can change from one form to another depending on the conditions present (DWAF 1996). It exerts a toxic effect at different

concentrations in different groups of aquatic organisms, with fish being the most resistant and in some cases the toxicity of chromium(VI) is no greater than for chromium(III) (DWAF 1996).

Cobalt (Co)

The Co concentrations ranged from 11.95 to 79.55 mg/kg. The highest concentration was at Site 1 (Table 3.9) lowest at Site 4 (Table 3.8). The highest concentrations were obtained during low flow (Table 3.9), hence, the lowest during high flow (Table 3.8). There was a non-statistically significant difference between sites ($p>0.05$) during both low flow and high flow.

Cobalt is a naturally-occurring element that has properties similar to those of iron and nickel (ATSDR 2004). Small amounts of cobalt are naturally found in most rocks, soil, water, plants, and animals, typically in small amounts (ATSDR 2004). It is usually found in the environment combined with other elements such as oxygen, sulphur, and arsenic (ATSDR 2004). It's an essential element, required for good health in animals and humans (ATSDR 2004). Small amounts of these chemical compounds can be found in rocks, soil, plants, and animals (ATSDR 2004). In water it is found in a dissolved or ionic form, typically in small amounts (ATSDR 2004). It may enter the environment from both natural sources and human activities (ATSDR 2004). The cobalt that is released into water may stick to particles in the water column or to the sediment at the bottom of the body of water into which it was released, or remain in the water column in ionic form (ATSDR 2004). Plants can accumulate very small amounts of cobalt from the soil, especially in the parts of the plant that you eat most often, such as the fruit, grain, and seeds (ATSDR 2004). While animals that eat these plants will accumulate cobalt, cobalt is not known to bio-magnify up the food chain (ATSDR 2004).

Copper (Cu)

The Co concentrations ranged from 12.5 to 74.6 mg/kg. The highest concentration was at Site 1 (Table 3.9) lowest at Site 5 (Table 3.9). The highest concentrations were obtained during low flow (Table 3.9), hence, the lowest during high flow (Table 3.8). Concentrations of Sites 1 and 2 exceeded CCME (2012) SQG during both high flow and low flow, whilst Site 3 also exceeded CCME (2012) SQG during low flow.

There was a statistically significant difference between sites ($p < 0.05$) during high flow but not during low flow ($p > 0.05$).

Copper is a reddish metal that occurs naturally in rock, soil, water, sediment, and, at low levels, air; it is also naturally occurring in all plants and animals (ATSDR 2004). It is an essential element for all known living organisms including humans and other animals at low levels of intake. At much higher levels, toxic effects can occur (ATSDR 2004). The reddish colour of this element is most commonly seen in electrical wiring, and some water pipes (ATSDR 2004). Its various compounds are most commonly used in agriculture to treat plant diseases, like mildew, or for water treatment and as preservatives for wood, leather, and fabrics (ATSDR 2004). It can enter the environment through releases from the mining of copper and other metals, from factories that make or use copper metal or copper compounds and through waste dumps, domestic waste water, and combustion of fossil fuels and wastes (ATSDR 2004).

Iron (Fe)

The Fe concentrations ranged from 40710 to 258839.5 mg/kg. The highest concentration was at Site 1 (Table 3.8) lowest at Site 5 (Table 3.8). The highest and lowest concentrations were obtained during high flow (Table 3.8). There was a non-statistically significant difference between sites ($p > 0.05$) during both low flow and high flow.

Lead (Pb)

The Pb concentrations ranged from below detection level to 8.3 mg/kg. The highest concentration was at Site 1 (Table 3.9). The highest concentrations were obtained during low flow (Table 3.9). During high flow Concentrations at Sites 1, 2 and 5 were below detection level, whilst those below detection level for low flow were Sites 4 and 5. No concentrations recorded exceeded CCME (2012) SQG. There was a statistically significant difference between sites ($p < 0.05$) during low flow but not during high flow.

Lead is a naturally occurring bluish-grey metal found in small amounts in the earth's crust. It has no characteristic taste or smell (Wang & Rainbow 2008). Metallic lead does not dissolve well in water and does not burn (Wang & Rainbow 2008). Lead

behaves like many of the alkaline earth metals (Ca, Sr, and Ba) with respect to uptake, internal distribution, and secretion (Wang & Rainbow 2008). It is a bone seeking element so it is chemically similar to calcium (Wang & Rainbow 2008). In fish, lead deposits in active calcification areas such as scales, fin rays, vertebrae, and opercula (Wang & Rainbow 2008). It is listed by the Environmental Protection Agency as one of 129 priority pollutants; and is also listed among the 25 hazardous substances thought to pose the most significant potential threat to human health (Wang & Rainbow 2008).

Manganese (Mn)

The Mn concentrations ranged from 521 to 1451.7 mg/kg. The highest concentration was at Site 1 (Table 3.8) and the lowest at Site 5 (Table 3.9). The highest concentrations were recorded during high flow, hence, the lowest during low flow. There was a non-statistically significant difference between sites ($p>0.05$) during low flow but there was a statistically significant difference between sites ($p<0.05$) high flow

Nickel (Ni)

The Ni concentrations ranged from 266.4 to 1363.1 mg/kg. The highest concentration was recorded at Site 1 (Table 3.8) and the lowest at Site 5 (Table 3.9). The highest concentrations were recorded during high flow, hence, the lowest during low flow. There was a non-statistically significant difference between sites ($p>0.05$) during both low flow and high flow.

Nickel is the 24th most abundant element; in its pure form, it is a hard, silvery-white metal, which has properties that make it very desirable for combining with other metals to form mixtures called alloys (ATSDR 2005). In the environment, it is primarily found combined with oxygen or sulphur as oxides or sulphides. It is found in all soil, and is also emitted from volcanoes (ATSDR 2005). Some of the metals that nickel can be alloyed with are iron, copper, chromium, and zinc (ATSDR 2005). These alloys are used in making metal coins and jewellery and in industry for making items such as valves and heat exchangers (ATSDR 2005). Nickel is released into the atmosphere during nickel mining and by industries that make or use nickel, nickel alloys, or nickel compounds and also from industrial waste water (ATSDR 2005).

The nickel that comes out of the stacks of power plants attaches to small particles of dust that settle to the ground or are taken out of the air in rain or snow (ATSDR 2005). A lot of nickel released into the environment ends up in soil or sediment where it strongly attaches to particles containing iron or manganese (ATSDR 2005). Nickel does not appear to concentrate in fish. Studies show that some plants can take up and accumulate nickel (ATSDR 2005).

Strontium (Sr)

The Sr concentrations ranged from 50.3 to 177.45 mg/kg. The highest concentration was recorded at Site 1 (Table 3.8) and the lowest at Site 4 (Table 3.9). The highest and lowest concentrations were recorded during high flow. There was a non-statistically significant difference between sites ($p>0.05$) during low flow but there was a statistically significant difference between sites ($p<0.05$) high flow.

Vanadium (V)

The V concentrations ranged from 91.5 to 1481.35 mg/kg. The highest concentration was recorded at Site 2 (Table 3.8) and the lowest at Site 4 (Table 3.8). The highest and lowest concentrations were recorded during high flow. There was a non-statistically significant difference between sites ($p>0.05$) during both low flow and high flow.

Zinc (Zn)

The Zn concentrations ranged from 30.5 to 187.95 mg/kg. The highest concentration was at Site 1 (Table 3.8) lowest at Site 4 (Table 3.8). The highest and lowest concentrations were obtained during high flow (Table 3.8). Concentrations at Sites 1, 2 and 3 exceeded CCME (2012) SQG during both high flow and low flow, whilst only those at Site 1 exceeded CCME (2012) SQG during low flow. There was a non-statistically significant difference between sites ($p>0.05$) during low flow but not during high flow.

3.3 CONCLUSIONS

The physico-chemical parameters assessed in this study were found to be within TWQR at all the sites (DWAF 1996) (CCME 2012). The highest concentrations were recorded during high flow for water temperature, EC and turbidity. The highest concentrations were recorded during low flow for DO, pH, TDS, salinity and water hardness. Water temperature ranged from 13.15 (Table 3.3) to 23.45 °C (Table 3.2). The lowest temperatures were during low flow (Table 3.3) and the highest during high flow (Table 3.2). The highest values were recorded at Site 3 during high flow and the lowest at Site 3 as well during low flow. The highest DO concentration was 10.52 mg/L O₂ at Site 1 and the lowest 7.83 mg/L O₂ at Site 3 (Table 3.2); these concentrations were obtained during high flow. The pH ranged from 8.58-8.88 during high flow (Table 2.2) and from 8.84-9.3 during low flow (Table 3.3). The highest values were obtained during low flow (Table 3.3). The highest EC was 316.98 mS/m at Site 5 and the lowest 169.35 mS/m at Site 1 (Table 3.2). The highest and lowest values were obtained during high flow. Site 5 had the highest values, and this can be attributed to this site being the last section of the river before it flows into the Olifants River. The highest TDS concentration was 275 mg/L at Site 5 (Table 3.3) and the lowest 82.95 mg/L at Site 1 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). The highest salinity concentration was 0.27 at Site 5 (Table 3.3) and the lowest 0.08 ‰ at Site 1 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). The highest water hardness was 55 (mg/L CaCO₃) at Site 5 (Table 3.3) and the lowest 16.5 (mg/L CaCO₃) at Site 2 (Table 3.2). The highest value was obtained during low flow (Table 3.3) and the lowest during high flow (Table 3.2). The highest turbidity value was 23 NTU at Site 4 (Table 3.2) and the lowest 3 NTU at Site 2 (Table 3.2). The highest and lowest values were obtained during high flow (Table 3.2). Site 4 being the most turbid and Site 2 being the least turbid may be due to Site 2 is after De Hoop Dam so clearer water flows from it which explains why it's the least turbid.

Nutrients were available throughout the sites, with the highest concentrations of total nitrogen recorded at Site 5 and at Site 3 for phosphate values. The availability of these nutrients is not abundant given that none of the sites have algal blooms, which is pleasing. This river is mesotrophic according to both N and P.

The suite of available metals within the water was lower than those in the sediment. The Al concentrations ranged from 0.011 to 0.68 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. All the recorded concentrations were above TWQG for DWAF (1996) and CCME (2012). The Ba concentrations ranged from 0.06 to 0.108 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 1 and the highest during low flow at Site 2. All the recorded concentrations were below TWQG. The Fe concentrations ranged from 0.04 to 0.63 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. Sites 1, 2, 4 and 5 concentrations were above TWQG for CCME (2012) during high flow. The Mn concentrations ranged from 0.002 to 3.64 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 5 and the highest during low flow at Site 1. All the concentrations recorded during high flow were above DWAF (1996) TWQG whilst only Sites 1, 2 and 3 were above and CCME (2012) TWQG. The Sr concentrations ranged from 0.12 to 0.24 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 2 and the highest during low flow at Site 4. All the concentrations recorded were below TWQG. The V concentrations ranged from 0.002 to 2.506 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 3 and the highest during low flow at Site 1. There is no TWQR criterion for vanadium. The Zn concentrations ranged from 0.0145 to 0.425 mg/L (Figure 3.1). The lowest concentration was during low flow at Site 3 and the highest during low flow at Site 1. Zinc concentrations recorded at all Sites were above DWAF (1996) and CCME (2012) TWQG. Zinc concentrations recorded at all Sites during high flow were above BCEPD (2006) and USEPA (2012) TWQG.

The concentrations of metals in the sediment were higher than those in the water column. Some metals which were not detected in the water column (Cd, Ni, Se, Pb) were detected in the sediments. The following metals were above the SQG: Cd, Cu, Cr and Zn which can pose a threat to the health of fish through biomagnification and through the food chain (McGeer *et al.* 2004). The high concentrations of metals in the sediment indicate the chronic nature of metal pollution possibly due to the activities within the catchment. The hypothesis of this chapter was that, high levels of some metals and nutrients would be present within the water and sediment. This hypothesis was supported by the results.

CHAPTER 4: FISH METAL BIOACCUMULATION AND ACETYLCHOLINE ESTERASE INHIBITION

4.1 INTRODUCTION

Many South African rural communities have become dependent on freshwater fish as a protein supplement; protein sources have increased in cost (Heath *et al.* 2004). With the increase in rural populations that are poverty stricken, there is an increase in consumption of fish from contaminated river systems (Root 1973, Rahman *et al.* 2012). The potential health risks to humans consuming contaminated fish are, however, seldom addressed (Heath *et al.* 2004). In the previous chapter, metals were shown to have accumulated in both the water column and sediment. These available metals can be assimilated into fish tissues through direct uptake and the food chain, and if accumulated at unacceptable concentrations can affect the fish.

4.1.1 Metal bioaccumulation

Bioaccumulation is the ability of an organism to accumulate substances in their tissues through ingestion of food or the surrounding (Dallas & Day 2004). Heavy metals are non-biodegradable, they can be bioaccumulated by fish, either directly from the surrounding water or by ingestion of food (Dallas & Day 2004). In addition, Heath *et al.* (2004) indicated that when metals reach appropriately high concentrations in body cells they can alter the physiological functioning of the fish. As metal pollution increases world-wide, fishes are often the main choice when studying aquatic toxicology due to them serving as a food source and their economic importance (Brix *et al.* 2011). Fish are able to integrate the effects of toxicants within an aquatic system at low concentrations, and due to being long lived, capable of integrating the effects of toxicants over time (Brix *et al.* 2011).

4.1.2 Human health risk

Human communities that regularly consume contaminated fish are at risk of genotoxic, carcinogenic and non-carcinogenic health impairment from long-term exposure to toxic contaminants (du Preez *et al.* 2003). Thus, it has become increasingly important to assess the levels of metals in fish tissues as an indicator of metal pollution in aquatic systems and to determine whether the fish from impacted river systems are suitable for human consumption (Heath *et al.* 2004). To determine

the human health risk, a Target Hazard Quotient (THQ) is calculated. The THQ is a risk-based approach that assesses the possible health risks to humans when consuming fish from selected rivers in South Africa (Heath *et al.* 2004).

Table 4.1: Health effects on humans of selected metals

Metals	Effects	References
Al	Has been linked to neurological degenerative diseases such as Alzheimer's	ATSDR (2008)
Ba	Very large amounts can cause changes in heart rhythm or paralysis in humans, and death with no medical assistance. Smaller amounts bring about vomiting, abdominal cramps, diarrhoea, difficulties in breathing, increased or decreased blood pressure, numbness around the face, and muscle weakness.	ATSDR (2007)
B	Large amounts over short periods of time can affect the stomach, intestines, liver, kidney, and brain and can eventually lead to death.	ATSDR (2010)
Cd	High levels severely irritate the stomach, leading to vomiting and diarrhoea, and sometimes death. Lower levels over a long period of time can lead to a build-up in the kidneys that could lead to kidney damage and it can also cause bones to brittle.	ATSDR (2008)
Cr	Anaemia and irritation and ulcers to stomach and small intestine. Carcinogenic and also disrupts male reproductive system	ATSDR (2008)
Co	Nausea, vomiting, thyroid and heart irregularities on the heart.	ATSDR (2004)
Cu	Gut irregularities, kidney and heart damage and even death	(ATSDR 2004)
Fe	High levels in tissues result in liver cirrhosis	Dallas & Day (2004)
Pb	Primarily affects the nervous system. Causes weakness in fingers, wrists, or ankles. Causes small increases in blood pressure and anaemia. At high levels can severely damage the brain and kidneys and ultimately cause death. In pregnant women it may cause miscarriage and in men it can damage the organs responsible for sperm production. It is an Endocrine Disrupting Chemical. It is classified as a carcinogen to humans (Group 2A).	ATSDR (2007) IARC (2012)
Mn	Inhibits dopamine and thus causes a disturbance in the nervous system	ATSDR (2008)
Ni	Stomach aches and suffered adverse effects in their blood and kidneys. Serious reactions such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus.	ATSDR (2005)
Se	Brittle hair and deformed nails can develop. In extreme cases, people may lose feeling and control in arms and legs, this is called selenosis.	ATSDR (2003)
Sr	Bone defects	ATSDR (2004)
V	Nausea, mild diarrhoea, and stomach cramp when taking some vanadium	ATSDR (2009)
Zn	Reduced fitness, respiratory problems and death when in excess	ATSDR (2005)

4.1.3 Biomarker of response: AChE

Biomarkers are measurements in body fluids, cells or tissues indicating cellular or biochemical modifications, due to the presence and magnitude of toxicants, or of host response (Couture & Pyle 2011). Biomarkers offer promise as sensitive indicators demonstrating that toxicants have entered organisms, have been distributed between tissues, and are eliciting a toxic effect at critical targets (McCarthy & Shugart 1990). Pesticide contamination of freshwater biota is generally restricted to agricultural environments in South Africa (Beasley & Kneale 2002). The root causes of contamination of water resources are: crop spraying in agricultural environments, with pollution of water resources arising from irrigation wash off and spray drift (Beasley & Kneale 2002); lack of practical knowledge by farmers with regards to pesticide safety and disposal and the risks posed to human health and the environment; and over-use of pesticides as opposed to an integrated pest management system. The consequences of pesticide contamination are human and animal-health related (Heath *et al.* 2010). An example of the effects insecticides have is in a study by Khan & Law (2005), where organophosphate insecticide phenthoate were synergised by carbaryl in fish, resulting in the inhibition of AChE thus altering the morphology and behaviour of the fish. When choline esterases are inhibited, an accumulation of acetylcholine occurs, interfering with the normal nervous system function (Brungs *et al.* 1978). This AChE biomarker was chosen for this study because it is a biomarker of response (Brungs *et al.* 1978); it is also an uncomplicated and direct method, cheap and easily performed at the laboratories available at the University of Limpopo.

4.1.4 Objectives of the chapter

- To determine whether metals and metalloids accumulated in the fish muscle tissue have reached a toxic level.
- To assess the Human Health Risk upon consumption of metal contaminated fish.
- To determine whether pesticides in the river are inhibiting acetylcholine esterase in fish brain tissue.

4.2 METHODS AND MATERIALS

Fish species

In this study, two fish species were used at three sites for metal bioaccumulation and AChE analyses. Only three sites were chosen because of the availability of fish at those sites. The two fish species were selected because they are the most abundant species in the river and they represent different trophic levels (Rogers.S 2014) (pers comm).



Figure 4.1: The shortspine suckermouth or shortspine rock catlet *Chiloglanis pretoriae* (Image from www.google.co.za)

Chiloglanis pretoriae Van der Horst, 1931

This fish species (Figure 4.1) possesses a small dorsal fin is small, short dorsal spine and less than 50% fin length with both sexes possessing a caudal fin (Skelton 2001). The head is depressed with relatively small eyes and a large mouth possessing pre-maxillary tooth-pads, about twelve mandibular teeth in a straight band (Skelton 2001). This species has a dark brown colour with small lighter patches on the back, a distinctive series of vertical linear spots along the body, and the caudal fin has broad splayed black band over either lobe. The standard length (SL) on average is 65 mm (Skelton 2001).

This species occurs in shallow rocky reaches, riffles and rapids of permanent rivers. Has a wider altitudinal range than most species where it occurs (Skelton 2001). This species is a secondary level consumer, feeding on aquatic insects such as mayfly

nymphs, caddis fly and blackfly larvae (Skelton 2001). This species is of economic and biological importance because it is a potential aquarium species, a useful indicator species in studies on river conservation (Skelton 2001). It is widely distributed along the Incomati, Limpopo, middle and lower Zambezi, Pungubwe and Buzi systems (Skelton 2001).



Figure 4.2: The Lowveld largescale yellowfish *Labeobarbus marequensis* (Image from www.google.co.za)

Labeobarbus marequensis (A. Smith, 1841)

This fish species (Figure 4.2) has a dorsal fin positioned in front of origin of pelvis, a flexible primary ray (Skelton 2001). The mouth is sub-terminal, lips extremely variable, 2 pairs of barbels. Both sexes develop small tubercles on top, side of the head, and on the anal fin rays (Skelton 2001). Their colour varies with water clarity from pale olive to bright golden yellow but juveniles are silvery with dark blotches. It attains an average total length (TL) of 470 mm and a mass averaging 6.0 kg (Skelton 2001).

It's widely distributed from the middle and lower Zambezi south to the Phongola River System, also occurs with the bushveld smallscale yellowfish (*Labeobarbus polylepis*) in southern Limpopo tributaries, favouring flowing waters of perennial rivers (Skelton 2001). This species it is a primary to tertiary level consumer, feeding

primarily on algae and aquatic insect larvae, also small fishes, snails, freshwater mussels and drifting organisms such as beetles and ants (Skelton 2001).

4.2.1 Sampling

Collection of fish samples

In order to evaluate the fish for metal bioaccumulation and pesticides, electrofishing was used to capture the fish using an electro fisher (Smith-Root LR-24 backpack electro fisher). The fish species were identified as *Chiloglanis pretoriae* and *Labeobarbus marequensis* and a minimum of 5 specimens (relatively equal in size) of each species were required. The total length (TL in mm) of each fish was measured and the weight (g) determined on a Mettler Toledo (MS24KLIPE) balance. The fish were sacrificed for further analyses by severing the spinal cord behind the head. A skinned portion of the dorsal muscle tissue of the *L. marequensis* was harvested. In the case of *C. pretoriae* an un-skinned sample was collected due to the small size of the fish. The muscle tissue samples were wrapped in aluminium foil, labelled, frozen in a battery operated fridge/freezer (Engel 42L), transported to the laboratory and then stored in an ultra-deep freezer at -80°C at the Department of Biodiversity prior to metal analysis. The head of the fish was dissected and the brain case was opened and the brain tissue samples were collected and stored in 1.5 ml Eppendorf tubes containing 0.1 M phosphate saline buffer (pH 8.0), frozen in the field and stored at -80°C prior to enzyme analysis.

4.2.2 Analysis

Chemical analysis for metal bioaccumulation

The frozen muscle tissue samples were submitted to a South African National Accreditation System (SANAS) accredited laboratory for metal analysis. At the laboratory, the muscle tissue samples were dried, weighed, acid digested (refer to chapter 3), filtered according to the methods of Bervoets & Blust (2003). The samples were then analysed to determine a suite of 32 metals using sequential Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) analyses. The results were expressed in mg/kg dry weight.

The mean and standard deviation of the metal concentration in muscle tissue was calculated for each species for the three sites. Analysis of variance (ANOVA) was

performed for each species to determine whether there was a significant difference in metal content in the muscles tissue between the sites.

Human health risk assessment

A human health risk was conducted using a desktop methodology of the US Environmental Protection Agency (US-EPA 1997); this methods calculates the Target Hazard Quotient (THQ) in order to deduce the possible risks involved with the consumption of fish tissue with respect to the elemental concentrations present. The THQ was calculated using wet weight values of the fish tissue. The wet weight was calculated by dividing the dry weight with 0.75. The risk of chronic non-cancer health effects from oral exposure was calculated using the Average Daily Dose (ADD); expressed in mg/kg body weight per day.

$$ADD = \frac{(\text{average metal concentration in fish muscle}) \times (\text{mass of portion})}{(\text{adult body weight}) \times (\text{no. of days between fish meals})} \quad (1)$$

where the average fresh weight (fw) metal concentration in mg/ kg, mass of portion in kg, adult body weight in kg and number of days between fish meals in days (US-EPA 1997). In the calculation of the ADD some assumptions were made to characterize the population at risk: 150 g portion of fish muscle once a week; 70 kg adult; and 30 years exposure (not used in the calculation, but the basis of the risk assessment). The average metal concentration in muscle tissues for each site was used in the risk assessment. A THQ for each metal was calculated comparing the expected exposure of the populations to the Reference doses (RfD) for the respective metals; a threshold above which adverse health impacts could be expected:

$$HQ = \frac{ADD}{RfD} \quad (2)$$

where $HQ < 1$ suggests that adverse health effects are unlikely and $HQ > 1$ suggests a high probability of adverse health effects (US-EPA 1997). Reference Dose levels published by the US-EPA were used (US-EPA 1997). The body mass used was 70 kg according to Heath *et al.* (2004). The higher body mass used for the risk assessment may result in an underestimate of the THQ value for rural populations in South Africa.

To calculate THQ, the following method by US-EPA (2014) was used

$$THQ = \frac{(EF \times ED \times IRF \times C)}{(Rf \times Do \times BW \times AT)} \quad (3)$$

where:

THQ is the target hazard quotient

BW is the average body weight of individuals in the adult age class

AT is the average time over which the assessment was undertaken

EF is the exposure frequency (i.e. the number of days within a year fish is consumed by the study groups)

ED is the duration of the exposure (expressed in years)

IRF is the consumption rate of fish expressed as kilograms per day (kg/day).

Consumption rates were recalculated to determine how much fish muscle needs to be consumed for adverse health effects to occur. To accomplish this, THQ values for all elements were assumed to be 1 (THQ = 1), at this value and higher, health risks associated with consumption of fish are assumed to manifest (US-EPA 2015).

Bio-concentration factor

Calculation of bio-concentration factor (BCF) values serves not only as an indication of how many times greater a pollutant is in the biota compared to the environment (McGeer *et al.* 2003). The BCF were calculated according to the method of Chon *et al.* (2012); to determine the ratio in element concentrations between fish and environment (water and sediment), as well as to determine the partitioning of elements between different samples. The BCF was calculated as follows:

$$BCF = \frac{(fish\ muscle)}{(water)} \quad (4)$$

$$BCF = \frac{(fish\ muscle)}{(sediment)} \quad (5)$$

Where: the *(fish muscle)*, *(sediment)* (µg/g dry weight) and *(water)* (µg/L) are the fractions of each element in the fish tissues and environment (water and sediment). The BCF was calculated using the median concentration values of each of the elements present in the fish organs, water and sediment.

Analysis of AChE inhibition

In the laboratory at the University of Limpopo, the samples were thawed, then a weighed sample tissue homogenised in 0.1M phosphate buffer (pH 8.0) and centrifuged at 1200 rpm. An aliquot of the supernatant was extracted and analysed AChE activity measured in absorbance/min/mg protein (A/min/mgp) using the methods of Grabowski *et al.* (2001). The acetyl-choline esterase inhibition was calculated relative to the reference site, Site 1.

ANOVA was conducted in IBM SPSS V.23 (Statistical Package for the Social Sciences, SPSS Inc.) to determine whether the differences in AChE inhibition varied between the impacted sites, for the selected fish species.

4.3 RESULTS AND DISCUSSION

4.3.1 Fish metal bioaccumulation

The results presented in this section show that fish sampled at the three sites of the Steelport River accumulated some metals. The three sites sampled were Sites 1, 2 and 5, during high flow and low flow. These three sites were selected because they are a good reflection of the River and they're also accessible with machinery used to catch fish. The fish lengths for *C. pretoriae* ranged from 4.8 cm to 6.9 cm and weight from 1.09 g to 2.56. The length of *L. marequensis* ranged from 9.8 cm to 36.1 cm and weight ranged from 8.73 g to 519.24 g (Appendix 2). Site 1 presented with the largest fish and Site 5 with the smallest.

Table 4.2: The concentrations of metals in the muscle tissue of metals in the muscle tissue of *Chiloglanis pretoriae* for low flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.

Metals (mg/kg dw.)	Site 1		Site 2		Site 5		P value
	mean	±sd	mean	±sd	mean	±sd	
Al	286.56	267.70	150.02	45.62	187.47	1.26	0.012
B	15.03	6.34	6.93	1.77	7.52	0.11	0.581
Ba	15.16	9.18	8.66	2.89	8.97	0.20	0.497
Cd	0.13	0.10	0.05	0.05	0.08	0.00	0.234
Co	1.78	0.72	0.81	0.14	0.86	0.03	0.063
Cr	4.54	3.62	1.22	0.85	2.56	0.04	0.006
Cu	5.02	0.98	5.87	5.68	4.39	2.27	0
Fe	964.52	913.49	556.59	528.86	740.87	19.17	0.006
Mn	26.33	20.64	99.13	32.10	44.55	0.70	0.072
Ni	9.62	6.68	6.06	3.59	6.49	0.38	0.012
Pb	1.94	1.55	0.78	0.73	1.25	0.06	0.124
Se	0.81	0.20	0.61	0.16	0.45	0.03	0.443
Sr	19.52	1.10	15.41	2.91	9.73	1.46	0.004
V	4.00	1.92	3.40	3.22	3.13	0.18	0.022
Zn	114.37	27.26	58.84	21.52	55.50	5.44	0.619

Table 4.3: The concentrations of metals in the muscle tissue of metals in the muscle tissue of *Chiloglanis pretoriae* for high flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.

Metals (mg/kg dw.)	Site 1		Site 2		Site 5		p value
	mean	±sd	mean	±sd	mean	±sd	
Al	65.980	14.886	43.514	4.530	131.333	67.943	0.045
B	0.461	0.314	0.293	0.307	0.258	0.340	0.009
Ba	7.853	1.855	5.076	1.365	8.609	8.039	0
Cd	0.026	0.015	0.017	0.010	0.045	0.039	0.002
Co	0.357	0.039	0.230	0.059	0.926	0.495	0
Cr	0.797	0.702	0.098	0.024	0.526	0.182	0.018
Cu	1.966	0.335	1.297	0.188	2.491	0.366	0.289
Fe	63.229	20.911	52.024	16.622	133.391	53.637	0.092
Mn	7.031	3.279	12.425	2.649	7.050	2.071	0
Ni	1.038	0.593	0.748	0.274	1.460	0.585	0.024
Pb	0.177	0.243	0.327	0.201	0.027	0.053	0.037
Se	0.678	0.886	0.042	0.088	0.489	1.009	0
Sr	23.637	2.481	17.960	1.795	31.573	8.059	0
V	0.698	0.253	0.288	0.252	0.982	0.457	0.045
Zn	135.270	33.649	105.147	20.366	121.057	72.349	0

Table 4.4: The concentrations of metals in the muscle tissue of metals in the muscle tissue of *Labeobarbus marequensis* for low flow (mg/kg dry weight), at the three sampling sites at the Steelpoort River.

Metals (mg/kg dw.)	Site 1		Site 2		Site 5		p value
	mean	±sd	mean	±sd	mean	±sd	
Al	28.16	14.86	17.48	6.04	9.63	3.38	0.053
B	4.65	1.19	4.13	2.59	2.29	0.52	0.507
Ba	3.87	2.91	0.90	0.40	0.78	0.26	0.385
Cd	0.23	0.12	0.11	0.19	0.09	0.14	0.224
Co	0.68	0.26	0.30	0.32	0.32	0.32	0
Cr	1.15	0.44	0.93	0.77	0.78	0.64	0.815
Cu	3.37	1.76	2.42	0.67	3.25	0.83	0.771
Fe	62.06	30.73	37.15	6.84	24.31	5.31	0.642
Mn	4.93	2.52	2.24	0.39	1.81	0.50	0.426
Ni	1.24	0.37	0.80	0.17	0.99	0.56	0
Pb	1.40	1.67	0.48	0.52	0.25	0.28	0.836
Se	0.66	0.18	0.41	0.28	0.44	0.20	0.610
Sr	17.57	19.13	1.66	0.34	1.75	0.29	0.018
V	0.82	0.20	0.33	0.32	0.40	0.34	0.147
Zn	34.08	25.41	12.37	2.30	14.32	4.49	0.171

Table 4.5: The concentrations of metals in the muscle tissue of metals in the muscle tissue of *Labeobarbus marequensis* for high flow (mg/kg dry weight), at the Steelpoort River.

Metals (mg/kg dw.)	Site 1		Site 2		Site 5		p value
	mean	±sd	mean	±sd	mean	±sd	
Al	41.41	26.16	59.83	7.09	96.15	48.09	0.029
B	0.11	0.24	0.49	0.87	0.14	0.32	0.022
Ba	1.72	1.57	2.89	1.39	1.99	1.04	0.103
Cd	0.02	0.02	0.01	0.01	0.02	0.01	0.357
Co	0.13	0.10	0.17	0.05	0.18	0.09	0.659
Cr	0.20	0.10	0.11	0.04	0.59	0.22	0.414
Cu	2.25	0.41	2.04	0.65	2.06	0.34	0.021
Fe	57.57	69.96	87.42	39.74	83.44	45.69	0.013
Mn	3.68	3.77	15.95	3.93	4.24	1.69	0.260
Ni	0.75	0.72	0.87	0.41	0.95	0.44	0.212
Pb	0.08	0.18	0.21	0.22	0.31	0.36	0.192
Se	1.61	2.22	0.84	1.88	2.50	3.43	0.066
Sr	16.63	12.57	31.94	5.95	35.07	7.74	0.043
V	0.08	0.11	0.23	0.17	0.22	0.07	0.044
Zn	60.05	36.31	105.69	54.40	69.17	7.07	0.076

Chiloglanis pretoriae

The concentrations of Al, Fe and Zn were significantly high in the muscle tissue of the *C. pretoriae*. Other metals that had accumulated at high levels were Ba, B, Fe, Pb, Mn and Sr (Table 4.2 and 4.3). Site 2 had the highest metal accumulation concentrations of manganese and iron probably due to a small tributary from a mine within the catchment that is near this site. More metals accumulated in the muscle tissue of *C. pretoriae* than in *L. marequensis*.

Labeobarbus marequensis

The metals had accumulated in the muscle tissue of *L. marequensis* during both low flow (Table 4.4) and high flow (Table 4.5). The metals that were present in slightly higher concentrations in the fish tissue were Al, Fe, Sr, and Zn. The other metals (B, Ba, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se and V) were present in very low concentration (Tables 4.4 & 4.5). Site 2 had the highest metal accumulation concentrations followed by Sites 5, 4 and 1 respectively. The concentration levels of metals were higher during low flow than during high flow. The concentrations of Al and Sr within the *Labeobarbus* species muscle tissue in this study were higher than the studies done by Chadambuka (2015) at the Selati River Kekana (2013) in Flag Boshielo Dam Mahlatji (2014) at the Phalaborwa Barrage. In comparison to the studies mentioned, the values of Fe were lower in this study. Comparing the values of Zn with the same studies, the values in this study were higher compared to the studies done by Chadambuka (2015) and Kekana (2013) and lower compared to the study done by Mahlatji (2014). In this study, As and Sb were below detection level. The variations could be because even though all the studies were done within the Olifants River system, the catchments characteristics differ and they play a huge role on the impacts of the different tributaries of the Olifants River.

Effects of metals in fish

The metals present in the fish tissue have various effects on the fish and their functioning abilities (Luoma 1989). Aluminium interferes with basic cellular functions such as the phosphoinosi-tide and intracellular Ca²⁺ signalling pathways, which are involved in a myriad of cellular metabolic functions (Rengel 2004). It is also associated with the decrease in the accumulation of inositol phosphates causing changes in the membrane phospholipid composition (Shi *et al.* 1993). Aluminium can

also potentiate Fe-induced oxidative stress through increased production of reactive oxygen, resulting in lipid peroxidation and cytotoxicity of free-oxygen radicals (Strong *et al.* 1996).

The harmful toxicity of Cu is largely attributed to its cupric (Cu^{2+}) forms (Luoma 1989). Accumulation of copper can cause alterations in antioxidant enzyme activities and other biomarkers of oxidative stress (Luoma 1989). Copper can also cause osmoregulation, behaviour, growth, and metabolism problems (Heath *et al.* 2010).

Lead is a neurotoxin that causes behavioural deficits in fish within days of exposure at sub-lethal concentrations, and these effects can persist after removal from the contaminant (Heath *et al.* 2010). Lead also causes deficits or decreases in survival, growth rates, development, behaviour, learning, and metabolism, in addition to increased mucus formation (Heath *et al.* 2010). Lead is listed as a carcinogen, with sufficient evidence to be classed as an animal carcinogen (ATSDR 2007). It is an Endocrine Disrupting Chemical (EDC) (ATSDR 2007). The International Agency for Research on Cancer (IARC) has determined that inorganic lead and lead compounds are probably carcinogenic to humans (Group 2A) as there is insufficient information to determine whether organic lead compounds will cause cancer in humans, but sufficient evidence in animals. The lead metal is classified in Group 2B: limited evidence in humans and less than sufficient evidence in animals (IARC 2012).

Manganese at high doses reduces leukocytes, impairs growth, and increases mortality (Heath *et al.* 2010).

Although selenium, like Cu is an essential micronutrient, it can be toxic at high levels and the toxicity in some fish are associated with adverse effects (Schmidt *et al.* 2011).

The accumulation of Zn can cause respiratory problems and coupled with the accumulation of Cu, though essential in the diet can be harmful when large intake occurs (Luoma 1989).

In this study, there were also metals (Cd, Cr, Ni and V) present in low concentrations, but in excess could have had adverse effects on fish. Cadmium is a serious environmental contaminant and is an EDC that is also transported atmospherically (Heath *et al.* 2010). In fish, it can cause anaemia and vertebral fractures, osmoregulatory problems, decreased digestive deficiency, haematological

and biochemical processes, growth deficits, erratic swimming, and mortality (Heath *et al.* 2010). Chromium is an essential trace element that is abundant in the earth's crust. In fish, it can cause swimming deficits, feeding disruption, ulcerations, and death (Heath *et al.* 2010). Nickel and chromium produce a combined effect in altering the metabolic functions of the fresh water fish (McGeer *et al.* 2004). Vanadium accumulation shortens lifespan of fish (Chon *et al.* 2012).

4.3.2 Human Health Risk

In this section, the THQ results and their effects are represented on Table 4.1. Only lead was found to be greater than 1 in *C. pretoriae* during high flow, which means that it could pose a risk to the humans consuming them. Most of the metals were in low concentrations and didn't pose a health threat when ingested. The metals discussed are those that could pose a risk which are lead and cobalt. Even though cobalt values were below 1, they were high enough to indicate that in the near future they may be above 1 if the land-use characteristics within the catchment remain the same or escalate. The rest of the metals were not discussed because they did not indicate that they would pose a risk now or in the near future, even though this is not certain.

Table 4.6: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Chiloglanis pretoriae* during high flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg.

Site 1 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	71.6	3.8	3.8	0.0	0.4	1.1	1.3	241.1	6.6	2.4	0.5	0.2	4.9	1.0	28.6	
Average Daily Dose (µg/kg)	21.93	1.15	1.16	0.01	0.14	0.35	0.38	73.82	2.02	0.74	0.15	0.06	1.49	0.31	8.75	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.018	0.005	0.005	0.003	0.036	0.695	0.008	0.084	16.121	0.004	0.006	0.010	0.002	0.049	0.023	17.069

Site 2 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	37.5	1.7	2.2	0.0	0.2	0.3	1.5	139.1	24.8	1.5	0.2	0.2	3.9	0.8	14.7	
Average Daily Dose (µg/kg)	11.48	0.53	0.66	0.00	0.06	0.09	0.45	42.60	7.59	0.46	0.06	0.05	1.18	0.26	4.50	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.009	0.002	0.003	0.001	0.017	0.186	0.009	0.049	60.693	0.003	0.002	0.007	0.002	0.042	0.012	61.037

Table 4.6 (cont.)

Site 5 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	0.9	0.3	0.5	0.0	0.0	0.0	0.6	8.7	0.4	0.2	0.0	0.0	1.0	0.1	4.1	
Average Daily Dose (µg/kg)	0.27	0.08	0.15	0.00	0.01	0.01	0.17	2.66	0.11	0.06	0.01	0.01	0.31	0.03	1.25	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.000	0.000	0.001	0.000	0.002	0.022	0.003	0.003	0.918	0.000	0.000	0.001	0.000	0.004	0.003	0.957

Table 4.7: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Chiloglanis pretoriae* during low flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg.

Site 1 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	10.3	0.4	0.0	0.0	0.1	0.0	0.6	14.4	0.0	0.9	0.2	0.4	4.1	0.0	15.0	
Average Daily Dose (µg/kg)	3.17	0.13	0.01	0.00	0.02	0.01	0.17	4.41	0.01	0.28	0.06	0.12	1.27	0.01	4.59	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.004	0.002	0.000	0.001	0.016	0.055	0.003	0.006	0.108	0.003	0.003	0.008	0.002	0.009	0.028	0.248

Table 4.7 (cont.)

Site 2 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	15.0	0.7	0.1	0.0	0.0	0.0	0.5	21.9	0.1	4.0	0.2	0.2	8.0	0.1	26.5	
Average Daily Dose (µg/kg)	4.58	0.22	0.04	0.00	0.01	0.01	0.16	6.70	0.02	1.21	0.07	0.06	2.43	0.02	8.10	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.003	0.002	0.000	0.000	0.002	0.035	0.002	0.005	0.200	0.005	0.002	0.001	0.002	0.004	0.021	0.284

Site 5 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	24.0	0.5	0.0	0.0	0.1	0.0	0.5	20.8	0.1	1.1	0.2	0.6	8.8	0.1	17.3	
Average Daily Dose (µg/kg)	7.35	0.15	0.01	0.00	0.04	0.01	0.16	6.37	0.02	0.32	0.07	0.19	2.68	0.02	5.28	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.008	0.003	0.000	0.001	0.011	0.142	0.004	0.012	0.017	0.003	0.004	0.006	0.003	0.012	0.025	0.251

Table 4.8: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Labeobarbus marequensis* during high flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg.

Site 1 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	7.0	1.2	1.0	0.1	0.3	0.8	15.5	1.2	0.3	0.3	0.2	4.4	10.0	0.2	8.5	
Average Daily Dose (µg/kg)	2.16	0.36	0.30	0.02	0.09	0.26	4.75	0.38	0.09	0.11	0.05	1.34	3.07	0.06	2.61	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Target Hazard Quotient	0.002	0.001	0.001	0.005	0.023	0.516	0.095	0.000	0.758	0.001	0.002	0.215	0.004	0.010	0.007	1.164

Site 2 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	4.4	1.0	0.2	0.0	0.2	0.6	9.3	0.6	0.2	0.1	0.1	0.4	1.9	0.1	3.1	
Average Daily Dose (µg/kg)	1.34	0.32	0.07	0.01	0.07	0.19	2.84	0.17	0.06	0.04	0.03	0.13	0.57	0.02	0.95	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.001	0.001	0.000	0.002	0.019	0.370	0.057	0.000	0.490	0.000	0.001	0.020	0.001	0.004	0.003	0.969

Table 4.8 (cont.)

Site 5 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	2.4	0.6	0.2	0.0	0.2	0.8	6.1	0.5	0.2	0.1	0.1	0.4	1.7	0.1	3.6	
Average Daily Dose (µg/kg)	0.74	0.18	0.06	0.01	0.06	0.25	1.86	0.14	0.08	0.02	0.03	0.13	0.53	0.03	1.10	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.001	0.001	0.000	0.002	0.016	0.497	0.037	0.000	0.609	0.000	0.001	0.021	0.001	0.005	0.003	1.194

Table 4.9: The Target Hazard Quotient, calculated for metals found in the muscle tissue of *Labeobarbus marequensis* during low flow at the Steelpoort River. Based on one fish meal (150 g) eaten on a weekly basis by a human weighing 70 kg.

Site 1 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	10.3	0.4	0.0	0.0	0.1	0.0	0.6	14.4	0.0	0.9	0.2	0.4	4.1	0.0	15.0	
Average Daily Dose (µg/kg)	3.17	0.13	0.01	0.00	0.02	0.01	0.17	4.41	0.01	0.28	0.06	0.12	1.27	0.01	4.59	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.003	0.001	0.000	0.000	0.004	0.020	0.003	0.005	0.050	0.002	0.002	0.020	0.002	0.001	0.012	0.125

Table 4.9 (cont.)

Site 2 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	15.0	0.7	0.1	0.0	0.0	0.0	0.5	21.9	0.1	4.0	0.2	0.2	8.0	0.1	26.5	
Average Daily Dose (µg/kg)	4.58	0.22	0.04	0.00	0.01	0.01	0.16	6.70	0.02	1.21	0.07	0.06	2.43	0.02	8.10	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.004	0.001	0.000	0.000	0.002	0.027	0.003	0.008	0.126	0.007	0.003	0.010	0.003	0.003	0.022	0.219

Site 5 (n=5)	Al	Ba	B	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Se	Sr	V	Zn	Total
Metal concentration (mg/kg)	24.0	0.5	0.0	0.0	0.1	0.0	0.5	20.8	0.1	1.1	0.2	0.6	8.8	0.1	17.3	
Average Daily Dose (µg/kg)	7.35	0.15	0.01	0.00	0.04	0.01	0.16	6.37	0.02	0.32	0.07	0.19	2.68	0.02	5.28	
Reference Dose (µg/kg)	1000	200	200	3	3	0.4	40	700	0.06	140	20	5	600	5	300	
Hazard Quotient	0.006	0.001	0.000	0.000	0.012	0.028	0.003	0.007	0.189	0.002	0.003	0.031	0.004	0.003	0.014	0.303

Cobalt

At Sites 1 and 2, the cobalt THQ values for *C. pretoriae* during high flow (Table 4.7) were above 1 and Site 5 was almost 1. The values during low flow (Table 4.6) for Site 1 were above 0.5. Even if humans don't consume *C. pretoriae*, this is a good indicator of the condition of the river. This also means that the metals can bio-magnify through the food chain and the fish feeding on these fish species can acquire a large dose of metal concentration. Values for *L. marequensis* during high flow (Table 4.9) at all sites were below 1 but at Site 1 it was above 0.5 which is not cause for alarm but is an indication that if cobalt keeps accumulating then health risks will be posed to human beings consuming it. The THQ values of Co from this study in comparison to the other studies done within the Olifants River system on another *Labeobarbus* species vary. The THQ values of Co from this study were higher than studies done by Chadambuka (2015), Kekana (2013) and Mahlatji (2014); but they were lower compared to the study done by Wang (1987).

As a component of cyanocobalmin (Vitamin B12), cobalt is essential in the body. It has been identified in most tissues of the body, with the highest concentrations found in the liver (ATSDR 2004). Oral exposure in humans increases the numbers of erythrocyte; this effect has been observed in both normal subjects and in anaemic patients (ATSDR 2004). However, treatment of pregnant women with cobalt did not prevent the reduction in haematocrit and haemoglobin levels often found during pregnancy (ATSDR 2004). Exposure of humans to beer containing cobalt as a foam stabilizer resulted in severe effects on the cardiovascular system, including cardiomyopathy and death, as well as gastrointestinal effects and hepatic necrosis; however, the subjects were alcoholics, and it is not known what effect excessive alcohol consumption may have played in the development of the observed effects (ATSDR 2004).

Lead

The THQ values for lead were >1 for *C. pretoriae* at Sites 1 (16.121) and 2 (60.693) and almost 1 at Site 5 (0.918) during high flow and the rest of the values for low flow were <1 (Tables 4.6 and 4.7). The high values for *C. pretoriae* can be due to the unskinned sample that was used instead of a skinned sample. The fish skin also accumulates metals. During high flow *L. marequensis* THQ values were <1 for all

sites (Table 4.9). The THQ values for *C. pretoriae* were high during high flow, which is a cause for concern given the potential effects that lead has on humans. This could be due to large concentrations of metals flowing into the river from the catchment. Site 2 had the highest THQ and this could be due to a tributary connecting to the Steelpoort River that flows close to a mine and then connects at this site. Humans consuming 150g per week of *L. marequensis* given its high THQ values may pose a serious threat to their health. The THQ values of Pb from this study in comparison to the other studies done within the Olifants River system on another *Labeo* species vary. The THQ values of Pb from this study were <1 and the THQ values in the studies done by Chadambuka (2015), Wang (1987) were >1. The THQ values in this study were however higher than those in the study done by Mahlatji (2014).

Lead is a common and toxic trace metal which readily accumulates in living tissue (ATSDR 2007). Metabolically, it interacts with iron and therefore interferes with haemoglobin synthesis (ATSDR 2007). It also affects membrane permeability by displacing calcium at functional sites, and inhibits some of the enzymes involved in energy metabolism (ATSDR 2007). Lead that has been absorbed by vertebrate organisms is largely deposited in the bony skeleton, where it does not usually exhibit toxic effects (ATSDR 2007). If stress results in decalcification or deossification, lead deposits may result in toxic effects (ATSDR 2007). It has been shown that rainbow trout develop spinal deformities after exposure to lead in soft water, while no deformities were evident in hard water (ATSDR 2007). Low concentrations of lead affect fish by causing the formation of a film of coagulated mucous over the gills and subsequently over the entire body; This has been attributed to a reaction between lead and an organic constituent of the mucous (ATSDR 2007). Death of fish is due to suffocation brought about by the mucous layer since insoluble lead is apparently not toxic to fish (ATSDR 2007). Lead is bio-accumulated by benthic bacteria, freshwater plants, invertebrates and fish, though it does not appear to bio-magnify through the aquatic food web (ATSDR 2007). Lead is a carcinogen and can cause cancer (ATSDR 2007) as mentioned previously within the bioaccumulation section .

4.3.3 Bio-concentration Factor (BCF)

This section represents the results for the BCF in relation to water and sediment quality.

Table 4.10: The bio-concentration factor values calculated between mean metal concentrations in water compared to muscle tissue levels of the two fish species from the Steelpoort River. (LBM- *Labeobarbus marequensis*, CPRE- *Chiloglanis pretoriae*) (b/d- below detection).

Water (mg/L)	Site 1		Site 2		Site 5	
Metals	LBM	CPRE	LBM	CPRE	LBM	CPRE
Al	135.00	684.38	108.97	6.28	119.35	152.67
Ba	39.05	140.30	54.22	2.59	9.64	21.70
B	590.90	2318.87	115.79	20.03	10.18	24.60
Cd	b/d	b/d	b/d	b/d	b/d	b/d
Cr	4.89	9.33	2.82	3.31	2.54	1.20
Co	7.26	10.16	6.82	1.49	11.40	3.55
Cu	407.00	44.22	350.00	0.13	b/d	b/d
Fe	124.25	2042.73	170.86	11.96	167.41	331.19
Pb	b/d	b/d	b/d	b/d	b/d	b/d
Mn	2.07	6.81	7.37	0.92	5.10	8.88
Ni	230.02	488.00	410.85	1.19	326.84	367.29
Se	14317.85	1104.40	b/d	b/d	1370.02	195.39
Sr	209.96	159.50	127.51	1.25	138.12	117.02
V	0.36	1.87	0.22	8.38	1.66	3.59
Zn	306.70	813.57	515.92	1.58	277.69	458.24

Table 4.11: The bio-concentration factor values calculated between mean metal concentrations in sediment compared to muscle tissue levels of *Labeobarbus marequensis* and *C. pretoriae* at the Steelpoort River (b/d- below detection).

Sediment (mg/kg dw.)	Site 1		Site 2		Site 5	
	LBM	CPRE	LBM	CPRE	LBM	CPRE
Al	0.01	0.03	0.00	0.00	0.00	0.00
Ba	0.59	2.12	0.06	0.11	0.05	0.12
B	b/d	b/d	b/d	b/d	b/d	b/d
Cd	b/d	b/d	b/d	b/d	b/d	b/d
Cr	0.08	0.14	0.00	0.00	0.01	0.00
Co	0.10	0.13	0.02	0.01	0.11	0.04
Cu	6.29	0.68	0.48	0.09	0.92	0.17
Fe	0.00	0.01	0.00	0.00	0.00	0.00
Pb	0.00	0.00	0.12	11.56	b/d	b/d
Mn	0.00	0.00	0.01	0.01	0.00	0.01
Ni	0.22	0.46	0.00	0.00	0.00	0.00
Se	b/d	b/d	b/d	b/d	b/d	b/d
Sr	3.40	2.58	0.24	0.20	0.21	0.18
V	0.00	0.00	0.00	0.00	0.00	0.00
Zn	1.69	4.47	0.41	0.57	0.69	1.14

An abundant number of metals were present in the sediment even when they were either not present or very low in the water column. The bio-concentration values of *C. pretoriae* were in most cases higher than those of *L. marequensis* (Tables 4.10 and 4.11). Some of the metals were below detection level in the water and sediment so BCF could not be calculated. The metal concentrations in the sediments were higher than that of the water and this can be attributed to the fact that sediment functions as natural 'sinks' within aquatic ecosystems (Courtney & Clements 2002). The differences in BCF between the water and sediment can additionally result from the different dietary habits of the fish species (Addo-Bediako *et al.* 2014).

4.3.4 Acetylcholine esterase inhibition

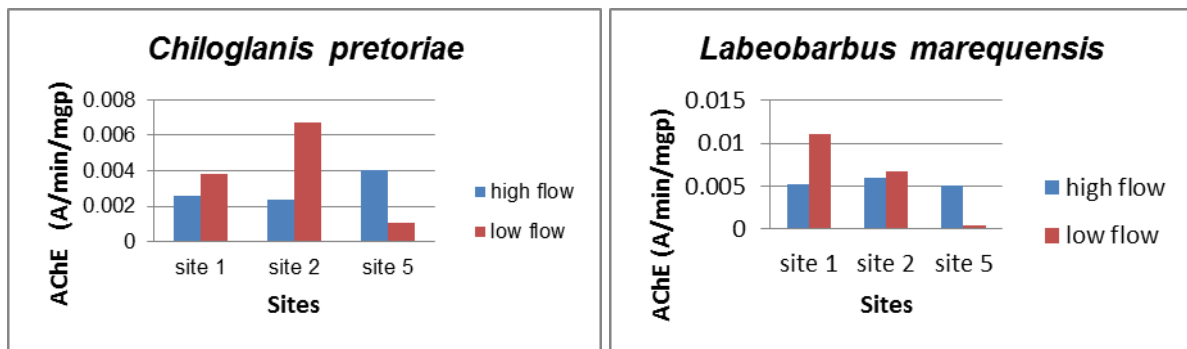


Figure 4.3: Results for AChE activity within the brain of fish in the Steelpoort River

The results for AChE activity in *C. pretoriae* brain tissue (Figure 4.3) were higher during low flow than high flow at Sites 1 and 2; at Site 5, the brain activity was lower for low flow than for high flow and this was so in the case of *L. marequensis* (Figure 4.3). According to Brönmark & Hansson (2002) the inhibition of AChE is higher during spring (low flow) than all the other seasons, because spring is pesticides spraying season and pesticides inhibit AChE activity.

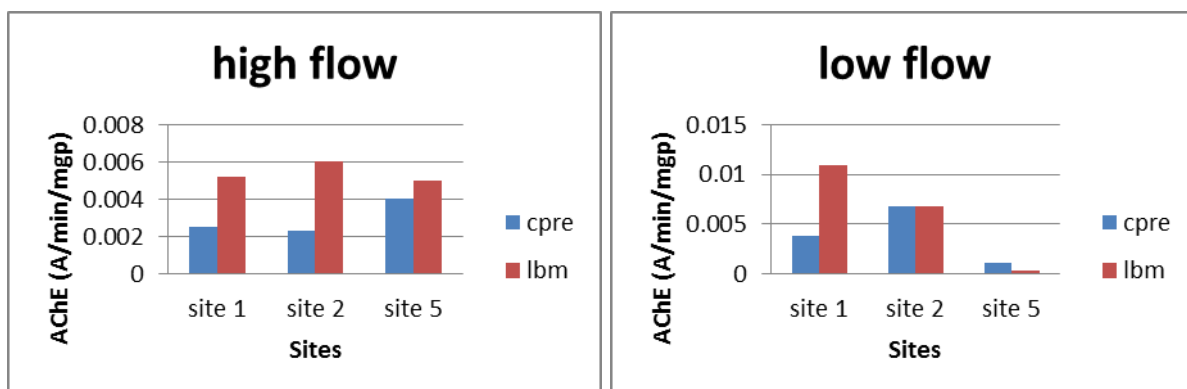


Figure 4.4: Results for AChE activity within the brain of fish in the Steelpoort River

AChE activity in brain tissue of *C. pretoriae* and *L. marequensis* showed differences during high and low flow (Figure 4.4). The brain activity of *L. marequensis* were higher than that of *C. pretoriae* during high flow for all the three sites. Which means there is less AChE inhibition in the brain of *L. marequensis* during high flow. The calculated values during low flow, showed that in *L. marequensis* AChE brain activity were higher at Site 1 and lower at Site 5 and equivalent at Site 2 in comparison to *C. pretoriae*. There was no statistically significant difference between sites during the

high flow trip for *C. pretoriae* ($p > 0.05$) and also for *L. marequensis* ($p > 0.05$). During the low flow trips, there was a statistically significant difference between sites for *C. pretoriae* ($p < 0.05$) and *L. marequensis* ($p < 0.05$). Compared to the results of Chadambuka (2015) from the Selati River which is within the Olifants River system, the values obtained for both high and low flows were higher in this study.. The highest and lowest values for AChE activity for high flow on the study done by Chadambuka (2015) were (0.0015) and (0.0010) respectively and were (0.006) and (0.0021) in this study. The highest and lowest values for AChE activity for low flow on the study done by Chadambuka (2015) were (0.0035) and (0.0013) respectively and were (0.012) and (0.001) in this study.

4.4 CONCLUSIONS

Both *C. pretoriae* and *L. marequensis* reflected that metals were bioaccumulated from the river system. The highest concentrations of metals were within the muscle tissue of *C. pretoriae* and this can be because unskinned samples were used in the case of *C. pretoriae*. A variety of metals were present in both fish species but Al, Fe, Sr and Zn were present in the high concentrations. The sites varied vastly in concentrations of accumulated metals, Site 1 had the highest concentrations of Zn, Ni and Cr; Site 2 had the highest concentration of Mn and Site 5 had the highest concentrations of Al, Ba, Cu, Fe and Sr.

The risk assessment revealed that Pb could cause health related impacts to the humans who consume a single 150 g fish meal once a week in *C. pretoriae*; and predicted that Co can cause potential risks in the near future. Even though the THQ lead values of *C. pretoriae* were alarming, it is highly unlikely that the community at the Steelpoort valley feed on this fish because of its relatively small size. Larger fishes feeding on this fish species can acquire excessive concentrations of lead and humans may consume those fish.

The BCF values revealed that the metals had bioconcentrated more in *C. pretoriae* than in *L. marequensis* and this can be due to the same reason mentioned for metal bioaccumulation and also due to their diet.

The AChE activity was higher for *L. marequensis* during high flow at all sites and higher at site 1 during low flow but lower at Site 5 and equivalent at Site 2 to the AChE activity for *C. pretoriae*. This could be due to the ability of *L. marequensis* to

expel pesticides from their system before the pesticides affect brain activity by decreasing the function of AChE. Both fish species had more AChE activity during low flow for Sites 1 and 2 than during high flow. The high flow AChE activity was higher for Site 5 in both fish species. Compared to the results of Chadambuka (2015) from the Selati River which is within the Olifants River system, the high and low flow highest and lowest values were higher in this study. The highest and lowest values for AChE activity for high flow on the study done by Chadambuka (2015) were (0.0015) and (0.0010) respectively and were (0.006) and (0.0021) in this study. The highest and lowest values for AChE activity for low flow on the study done by Chadambuka (2015) were (0.0035) and (0.0013) respectively and were (0.012) and (0.001) in this study. Pesticides were found to be inhibiting AChE activity in fish brains.

The hypotheses were that metals will be present in fish tissue and will have an effect on human health; and that the biomarker AChE will show signs of inhibition within brain tissue. The hypotheses of this chapter were proven to be correct.

The objectives of this chapter were to determine whether toxic metals and metalloids are present in the fish tissue and if they would have any health risks for humans who ingest them; and also to determine whether pesticides in the river are inhibiting acetylcholine esterase in fish brain tissue. Both of these objectives were met, metals were present in fish tissue and there is a human health risk or potential health risk for some metals.

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

The aims of this study were, to determine the effects of water and sediment quality on fish of the Steelpoort River system and the effects of land-use on the water and sediment quality of the Steelpoort River system. These aims were accomplished by; determining the water and sediment quality of the Steelpoort River at five sites, the level of metal bioaccumulation of two fish species at three sites, the effects of pesticides on two fish species of the Steelpoort River by evaluating the acetylcholine esterase inhibition in brain tissue, and evaluating the land-use change and its impacts of land-use on water quality in the Steelpoort River.

5.1 WATER AND SEDIMENT QUALITY

The physico-chemical parameters assessed in this study were found to be within acceptable limits at all the sites. The highest concentrations were recorded during high flow for water temperature, EC and turbidity. The highest concentrations were recorded during low flow for DO, pH, TDS, salinity and water hardness. Site 5 had the highest values for most parameters and this can be attributed to this site being the last section of the river before it flows into the Olifants River. Site 1 had the lowest values and this could be due to this site being situated where there are fewer land-use characteristics, hence it is the reference Site.

Nutrients were available throughout the sites, with the highest nitrogen concentration recorded at Site 5 and the highest phosphate concentrations at Site 3. The availability of these nutrients is not abundant given that none of the sites have algal blooms, thus the river is mesotrophic according to both N and P.

The suite of available metals within the water was lower than those in the sediment. The concentrations of metals in the sediment were higher than those within the water column. Metals available in the water were within DWAF standards except in some cases where Al, Ba, Mn and Zn were above those standards. Some metals which were not detected in the water column (Cd, Ni, Se and Pb) were detected in the sediments. In the sediment these metals were above the SQG: Cd, Cu, Cr and Zn which can pose a threat to the health of fish through biomagnification through the food chain (McGeer *et al.* 2004). The high concentrations of metals in the sediment

indicate the chronic nature of metal pollution possibly due to the activities within the catchment.

5.2 FISH METAL BIOACCUMULATION AND ACETYLCHOLINE ESTERASE INHIBITION

Both *C. pretoriae* and *L. marequensis* had bioaccumulated metals from the river system. The highest concentrations of metals were within the muscle tissue of *C. pretoriae* and this can be because unskinned samples were used in the case of *C. pretoriae*. A variety of metals were present in both fish species but Al, Fe, Sr and Zn were present in higher concentrations. The sites varied vastly in concentrations of accumulated metals, Site 1 had the highest concentrations Zn, Ni and Cr; Site 2 had the highest concentration of Mn and Site 5 had the highest concentrations of Al, Ba, Cu, Fe and Sr.

The risk assessment revealed that Pb could cause health related impacts to the humans who would ever consume a single 150 g fish meal once a week in *C. pretoriae*; and that Co can cause potential risks in the near future. Even though the THQ lead values of *C. pretoriae* were alarming, it is highly unlikely that the community at the Steelpoort valley feed on this fish because of its relatively small size. Larger fishes feeding on this fish species can acquire excessive concentrations of lead and humans may consume those fish.

The BCF values revealed that the metals had bioconcentrated more in *C. pretoriae* than in *L. marequensis* and this can be due to the same reason mentioned for metal bioaccumulation and also due to their diet.

The AChE activity was higher for *L. marequensis* during high flow at all sites and higher at site 1 during low flow but lower at site 5 and equivalent at site 2 to the AChE activity for *C. pretoriae*. Both fish species had more AChE activity during low flow for sites 1 and 2 than during high flow. The high flow AChE activity was higher for site 5 in both fish species.

Toxic metals and metalloids were present in the fish tissue and could pose a threat to people ingesting 150g per week; and pesticides in the river were proven be inhibiting AChE in fish brain tissue.

5.3 EFFECTS OF LAND-USE CHANGE

The agricultural activity has decreased over time within the catchment, from 2000 to 2014 and this can be due to a decrease of fertile land. There has also been a decrease in the mining activity from 2000 to 2009 and then an increase during 2014 where the decrease could be due to the closure of mines at the end of their life span; and the increase in 2014 could be due to the opening of new mines. Residential areas have increased from 2000 through to 2014. The water from 2000 to 2009 decreased then increased drastically in 2014. The drastic increase is due to the multiple dams that have been built over time in order to provide water resources to the increased population, and also for irrigation purposes.

The water quality showed an increase in the NO_3 , SO_4 and PO_4 ; meanwhile the EC has decreased over time. The decrease in EC means that there are fewer ions in the river which gives off less conductivity. The increase in phosphates and nitrates could mean there is a lot of fertilizers and pesticides within the agriculture .

The land-use characteristics have an effect on the water quality, therefore, the sediment and the biota within the River system.

5.4 RECOMMENDATIONS

The study indicated that the land-use characteristics within the catchment do in fact affect the water and sediment quality of the Steelpoort river system, hence the biota (fish). The bioaccumulation of carcinogens such as Pb could pose a chronic risk to the surrounding beings ingesting *C. pretoriae* and *L. marequensis* fish on a weekly basis. The pesticides in use within the catchment, in the residential areas and agriculture, pose a risk to the bio-marker AChE which is vital in fish brain. The land-use characteristics within the catchment have changed over time and it is advised that, mitigation factors for the effects these characteristics inflict within the Steelpoort river system be put in place. More studies need to be conducted in this river system for monitoring purposes.

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APPENDIX 1: Water and Sediment quality results

Table 1: Water quality parameters in the Steelpoort River

Water Quality Parameters	Site 1				Site 2				Site 3			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Water temperature	23.6	15	17.1	23.8	26.4	19.9	17.2	14.3	26.9	20	15.4	15.3
Dissolved oxygen (mg/L O ₂)	10.8	10.24	12.53	9.04	7.97	8.75	12.66	12.04	7.57	8.08	11.1	7.5
Dissolved oxygen (%)	116.3	114.2	146.1	118.2	95.4	96.4	145.5	126.3	89.4	97.6	121.6	100.7
pH	8.53	8.63	8.37	9.3	8.96	7.84	8.7	9.2	8.83	8.92	8.7	9.5
Conductivity (EC) mS/m	149.35	189.35	236.85	289.3	204	177.7	183.25	201.9	214.2	260.85	326	363
TDS mg/L	69.1	96.8	113.7	136.7	91.6	86	89.9	95.7	94.5	127.9	162.5	164.4
Salinity (‰)	0.07	0.09	0.11	0.13	0.09	0.08	0.09	0.09	0.09	0.12	0.16	0.16
Water Hardness (CaCO ₃)*	18	21	33	41	16	17	27	22	21	22	45	37
Turbidity NTU	24	1	9	14	4	2	4	3	16	3	4	4
Nitrate (mg/L NO ₃ -N)	0.97	1	1.12	<0.5	0.54	1.71	0.5	0.6	0.8	0.92	1.1	<0.5
Nitrite (mg/L NO ₂ -N)	0.097	0.013	0.019	0.02	0.052	0.012	0.02	<0.010	0.046	0.015	0.03	0.02
Ammonium (mg/L NH ₄ -N)	0.277	0.072	0.012	0.032	0.102	0.077	0.006	0.058	0.106	0.036	0.01	0.043
Total Nitrogen	1.344	1.202	1.151	0.047	0.694	1.799	0.501	0.658	0.952	0.971	1.138	0.061
Ortho-Phosphate (mg/L PO ₄)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.05	<0.05	<0.05	<0.05	0.05	<0.05
Phosphorus (mg/L P)	0.3	2.982	0	<0.010	< 0.5	2.607	0	0	< 0.5	2.959	0	0
Calcium (mg/L)	20	33	25.9	26.4	29	59	20.8	19.8	38	57	26.8	24.5
Magnesium (mg/L)	11	18	13	12.6	9	10	9.7	8.3	12	14	14.1	12.4
Potassium (mg/L)	<1.0	1.8	1.7	1.9	1.7	2.3	2.4	2.3	1.5	2.852	1.9	2
Sodium (mg/L)	9	8	15	12	8	17	12.3	8.2	17	11	42.9	22.6

Table 1 (cont.)

Water Quality Parameters	Site 4				Site 5			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Water temperature	26.2	16.5	15.8	10.5	27.3	17.2	19.8	16.2
Dissolved oxygen (mg/L O ₂)	6.9	8.96	10.12	10.91	7.85	9.54	10.21	8.06
Dissolved oxygen (%)	91.46	94.38	113.5	113.2	90	106.7	120.9	101.7
pH	8.68	8.74	9	9	8.73	8.84	9.3	9.3
Conductivity (EC) mS/m	217.7	356.8	482	505	226.3	407.65	572	580
TDS mg/L	98.4	178.8	241	243	101.4	208	279	271
Salinity (‰)	0.1	0.17	0.23	0.23	0.13	0.2	0.27	0.26
Water Hardness (CaCO ₃)*	26	30	53	46	34	39	55	55
Turbidity NTU	38	8	7	24	37	5	8	15
Nitrate (mg/L NO ₃ -N)	0.87	1.72	2.1	1.1	0.78	3.58	3.6	1.4
Nitrite (mg/L NO ₂ -N)	0.067	0.048	0.03	0.04	0.028	0.037	0.03	0.03
Ammonium (mg/L NH ₄ -N)	0.1	0.223	0.203	0.24	0.097	0.043	<0.010	0.052
Total Nitrogen	1.037	1.991	2.372	1.376	0.905	3.66	3.63	1.484
Ortho-Phosphate (mg/L PO ₄)	<0.05	<0.05	0.07	<0.05	<0.05	<0.05	<0.05	<0.05
Phosphorus (mg/L P)	0.4	1.209	0.1	0.2	0.4	0.916	0	0
Calcium (mg/L)	35	102	33.1	30.5	31	67	32.4	28.3
Magnesium (mg/L)	19	12	24.3	21.2	27	14	36.5	29.8
Potassium (mg/L)	1.5	2.127	1.7	2.1	1.6	3	1.8	1.9
Sodium (mg/L)	24	16	65.8	35.8	25	11	69.4	35.2

Table 2: Metals present in the water of the Steelpoort River.

Metals mg/L	Site 1				Site 2				Site 3			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Aluminium	<0.100	0.49		0.025	0.143	0.91		0.011	0.106	0.73	0.015	<0.010
Barium	0.031	0.175	0.04	0.08	0.029	0.186	0.026	0.018	0.026	0.18	0.021	0.026
Boron	<0.025	<0.025	0.007	0	<0.025	<0.025	0.011	0.001	<0.025	<0.025	0.003	0.0175
Cadmium	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.005	<0.005	0	<0.010
Chromium	<0.025	0.138	<0.025	<0.025	<0.025	0.184	<0.025	<0.025	<0.025	0.251	0	<0.010
Cobalt	<0.025	0.241	<0.025	<0.025	<0.025	0.19	<0.025	<0.025	<0.025	0.199	0	<0.010
Copper	<0.025	0.079	<0.025	<0.025	<0.025	0.056	<0.025	<0.025	<0.025	0.048	0.001	<0.010
Iron	0.169	0.477	0.182	0.178	0.601	0.351	0.054	0.045	0.24	0.391	0.065	0.098
Lead	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	0	<0.010
Manganese	<0.025	3.637	0.033	0.006	0.122	3.178	<0.025	0.015	0.054	3.486	0.009	0.026
Nickel	<0.025	<0.025	<0.025	0.003	<0.025	<0.025	<0.025	0.002	<0.025	0.072	0.002	<0.010
Selenium	<0.020	<0.020	<0.020	0.001	<0.020	<0.020	<0.020	0	<0.020	<0.020	0.001	<0.010
Strontium	0.094	0.145	0.15	0.152	0.085	0.299	0.108	0.124	0.104	0.228	0.171	0.137
Vanadium	<0.025	2.506	<0.010	0.003	<0.025	2.501	<0.010	0.002	<0.025	2.407	0.002	<0.010
Zinc	<0.020	0.425	0.03	0.005	<0.020	0.309	0.03	0.005	<0.020	0.355	0.002	0.027

Table 2 (cont.)

Metals	Site 4				Site 5			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Aluminium	0.315	0.136	0.018	<0.010	0.531	0.78		0.017
Barium	0.031	0.152	0.022	0.033	0.031	0.138	0.035	0.023
Boron	<0.025	<0.025	0.014	0.0304	<0.025	<0.025	0.028	0.017
Cadmium	<0.005	<0.005	0	<0.010	<0.010	<0.010	<0.010	<0.010
Chromium	<0.025	0.292	0.001	<0.010	<0.025	0.268	<0.025	<0.025
Cobalt	<0.025	0.053	0	<0.010	<0.025	0.033	<0.025	<0.025
Copper	<0.025	<0.025	0.001	<0.010	<0.025	<0.025	<0.025	<0.025
Iron	0.381	0.125	0.049	0.226	0.674	0.101	0.196	0.045
Lead	<0.020	<0.020	0	<0.010	<0.020	<0.020	<0.020	<0.020
Manganese	0.062	1.477	0.002	0.027	0.033	1.285	<0.025	0.002
Nickel	<0.025	0.094	0.002	<0.010	<0.025	<0.025	<0.025	0.002
Selenium	<0.020	<0.020	0.002	<0.010	<0.020	<0.020	<0.020	0.002
Strontium	0.129	0.353	0.219	0.18	0.141	0.236	0.164	0.216
Vanadium	<0.025	0.799	0.037	0.05	<0.025	0.464	0.049	0.033
Zinc	<0.020	0.172	0.004	0.027	<0.020	0.324	0.026	0.004

Table 3: Metals present in the sediment of the Steelpoort River (mg/kg dry weight)

Metals mg/kg	Site 1				Site 2				Site 3			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Aluminium	22210.3	21600	19600	38400	31194	25200	36400	31600	19808.6	39600	29200	30000
Barium	62.2	60.6	70	72.1	65.3	44.2	74.4	42.4	48.3	95.8	72	18
Boron	10.7	<4.00	<4.00	28	11.3	<4.00	<4.00	<4.00	16.4	<4.00	<4.00	<4.00
Cadmium	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	4.7	<0.010	<0.010	<0.010
Chromium	81.5	58.1	55.2	65.8	66.3	216.9	73.6	91.6	137.9	115.8	100.4	75.1
Cobalt	69.7	72.8	96	63.1	37.8	86.5	76	56.3	79.5	27.9	79.6	30.7
Copper	76.7	42.9	<0.010	74.6	52.8	38.3	22.4	50.7	58.5	56.9	19.2	30.5
Iron	297279	220400	190800	170400	139736	290800	140400	157600	313640	80000	156400	89600
Lead	<4.00	<4.00	<4.00	8.3	<4.00	<4.00	<4.00	4.3	6.5	<4.00	<4.00	4.6
Manganese	1225	1678.4	1454.8	1318	906.7	1658.4	1271.2	1333.2	1231	1050.4	1394.4	700
Nickel	2065	661.2	13.6	1531.9	998.7	803.6	28.8	1448	2202.2	1543.2	28.8	805.7
Selenium	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	4.5	7.3	<4.00	<4.00
Strontium	45.7	55	58	88.3	76.6	51.1	119.6	86.8	47.8	95.8	91.2	77.3
Vanadium	1137.4	884.2	1002.4	552.8	665.8	2296.9	1000.4	945.8	1423.1	224.7	962.8	410.1
Zinc	194.1	181.8	170	181.9	90.1	226.4	123.6	132.9	220.8	63.7	142	99.2

Table 3 (cont.)

Metals mg/kg	Site 4				Site 5			
	Feb	May	Aug	Oct	Feb	May	Aug	Oct
Aluminium	84252.1	75600	54400	26400	51481.9	33600	31200	41200
Barium	87.1	52	60.8	6.7	48.6	45.2	55.2	14.9
Boron	40.2	<4.00	<4.00	20.9	10.8	<4.00	<4.00	<4.00
Cadmium	2.4	<0.010	<0.010	<0.010	0.8	<0.010	<0.010	<0.010
Chromium	77.3	59.2	116.8	178.2	136	136	107.2	152.5
Cobalt	13.6	10.3	21.2	34.5	14.4	16.2	13.2	16.4
Copper	17.8	7.5	<0.010	23.7	16.1	14.3	<0.010	12.5
Iron	25741.5	27200	50000	133200	40220.4	41200	40400	55200
Lead	4.4	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
Manganese	294	374.8	590.8	986	336	542.4	514	528
Nickel	210.4	655.2	37.6	1189.2	314.3	644.8	31.2	501.6
Selenium	4	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
Strontium	183.9	171	141.2	65.7	112.5	78.8	94.4	118.1
Vanadium	76.8	106.3	319.6	602.2	110.3	143.5	185.6	209.1
Zinc	36.9	24.1	68.8	117.5	44	33.8	129.6	34.8

APPENDIX 2: Metal bioaccumulation results

Table 1: metal bioaccumulation for low flow for *Chiloglanis pretoriae* (CPRE) and *Labeo-barbus marequensis* (LMB).

CPRE	Site 1					Site 2					Site 5				
	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
Al	85	70	74	52	49	41	41	48	38	49	128	249	84	89	108
Sb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.639
As	0.169	0	0	0	0	0	0	0	0	0	0.123	0	0.115	0.025	2.79
Ba	5.53	8.65	9.83	8.99	6.3	3.61	5.83	3.63	5.77	6.54	3.86	23	4.68	6.07	5.52
B	0.428	0.356	0.675	0.014	0.829	0	0	0.255	0.677	0.532	0	0.798	0.385	0.109	0
Cd	0.026	0.031	0.046	0.021	0.005	0.015	0.015	0.006	0.034	0.015	0.002	0.035	0.034	0.045	0.109
Cr	0.874	0.441	0.312	0.368	2	0.086	0.071	0.09	0.11	0.133	0.504	0.846	0.427	0.432	0.42
Co	0.361	0.408	0.299	0.364	0.35	0.278	0.219	0.194	0.301	0.158	0.612	1.09	0.625	1.73	0.574
Cu	1.83	2.2	2.42	1.8	1.6	1.08	1.29	1.14	1.44	1.53	2.91	2.4	2.19	2.84	2.12
Fe	98	56	65	53	44	79	35	41	52	53	114	229	102	118	104
Pb	0.234	0.075	0.576	0	0	0.67	0.239	0.149	0.313	0.264	0	0.014	0	0	0.121
Mn	12	8.5	6.54	4.03	4.2	16	11	10	11	14	5.94	9.55	8.5	4.3	6.95
Ni	1.29	1.91	0.463	0.549	1	1.14	0.542	0.934	0.588	0.539	1.27	2.41	1.01	1.01	1.59
Se	0	0.103	0	1.74	1.5	0	0.012	0	0	0.199	0	0	2.29	0	0.153
Ag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.98
Sr	25	25	25	24	19	18	20	19	16	16	30	32	25	25	45
Sn	0.008	0	0	0	0	0	0	0	0	0	0.015	0	0	0	0.139
V	0.42	1	0.498	0.95	0.652	0.045	0.104	0.179	0.607	0.506	0.738	1.08	0.597	1.74	0.757
Zn	114	106	155	185	116	110	103	130	74	109	83	121	87	246	69

LBM	Site 1					Site 2					Site 5				
	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
Al	13	66.9	13	54	60	71	59	60	56	53	56	74	97	178	75
Sb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ba	0	2.93	0	2.97	2.7	1.72	1.4	2.84	4.67	3.84	1.13	1.6	2.08	3.75	1.41
B	0	0	0	0.534	0	0	0	2.01	0.427	0	0	0.718	0	0	0
Cd	0.009	0.003	0	0.028	0.037	0.008	0.002	0.002	0.002	0.014	0.02	0.025	0.026	0.018	0.002
Cr	0.155	0.253	0.251	0.293	0.048	0.177	0.081	0.098	0.084	0.084	0.491	0.666	0.703	0.819	0.244
Co	0.047	0.274	0.05	0.1	0.17	0.202	0.231	0.178	0.107	0.148	0.098	0.1	0.231	0.309	0.181
Cu	2.28	2.08	1.64	2.63	2.6	1.51	1.27	2.54	2.14	2.77	1.52	2.21	2.42	2.15	2.01
Fe	4.2	170	3.7	30	80	68	130	130	46	64	38	64	117	145	52
Pb	0	0	0	0	0.406	0.387	0.475	0.17	0	0	0.79	0.063	0.609	0.063	0.019
Mn	0	5.3	0	4.23	8.9	18	21	11	17	12	3.6	4.5	5.4	6	1.7
Ni	0	1.57	0	1.16	1	0.901	1.334	1.2	0.42	0.482	0.46	1.13	1.31	1.36	0.492
Se	4.88	0	0.189	2.96	0	0	4.201	0	0	0	6.19	0	0	0	6.34
Ag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sr	3.44	23	2.54	27	27	40	33	23	32	31	27	32	35	48	33
Sn	0.014	0.008	0.011	0	0	0	0	0	0	0	0	0	0	0	0
V	0	0.252	0.013	0.122	0.032	0.474	0.351	0.122	0.139	0.081	0.151	0.29	0.207	0.279	0.155
Zn	26	66	22	78	108	51	71	84	182	141	60	73	63	72	77

Table 2: Metal bioaccumulation for high flow for *Chiloglanis pretoriae* (CPRE) and *Labeo-barbus marequensis* (LMB).

CPRE	Site 1					Site 2					Site 5				
	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
Al	84.515	70.286	73.553	52.254	49.29	41.128	41.331	48.062	38.477	48.571	127.623	248.864	83.544	88.989	107.646
Sb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.639
As	0.169	0	0	0	0	0	0	0	0	0	0.123	0	0.115	0.025	2.791
Ba	5.534	8.654	9.829	8.989	6.257	3.607	5.827	3.63	5.775	6.542	3.86	22.91	4.681	6.074	5.517
B	0.428	0.356	0.675	0.014	0.829	0	0	0.255	0.677	0.532	0	0.798	0.385	0.109	0
Cd	0.026	0.031	0.046	0.021	0.005	0.015	0.015	0.006	0.034	0.015	0.002	0.035	0.034	0.045	0.109
Cr	0.874	0.441	0.312	0.368	1.989	0.086	0.071	0.09	0.11	0.133	0.504	0.846	0.427	0.432	0.42
Co	0.361	0.408	0.299	0.364	0.35	0.278	0.219	0.194	0.301	0.158	0.612	1.094	0.625	1.726	0.574
Cu	1.825	2.204	2.417	1.795	1.591	1.083	1.293	1.145	1.439	1.527	2.914	2.4	2.19	2.836	2.118
Fe	98.202	55.589	65.028	53.135	44.191	78.634	35.282	41.267	51.869	53.068	114.139	228.592	101.78	117.988	104.456
Pb	0.234	0.075	0.576	0	0	0.67	0.239	0.149	0.313	0.264	0	0.014	0	0	0.121
Mn	11.888	8.498	6.542	4.029	4.199	16.399	10.691	10.275	10.855	13.904	5.943	9.555	8.498	4.304	6.95
Ni	1.294	1.913	0.463	0.549	0.971	1.14	0.542	0.934	0.588	0.539	1.268	2.414	1.012	1.011	1.593
Se	0	0.103	0	1.744	1.545	0	0.012	0	0	0.199	0	0	2.29	0	0.153
Ag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.982
Sr	25.314	25.12	24.697	23.716	19.337	18.26	20.292	18.914	16.248	16.086	30.188	32.259	25.264	25.252	44.901
Sn	0.008	0	0	0	0	0	0	0	0	0	0.015	0	0	0	0.139
V	0.42	0.965	0.498	0.954	0.652	0.045	0.104	0.179	0.607	0.506	0.738	1.082	0.597	1.735	0.757
Zn	114.37	105.93	154.825	185.095	116.129	109.937	102.79	130.48	73.894	108.632	83.418	120.856	86.526	245.87	68.616

LBM	Site 1					Site 2					Site 5				
	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5	Fish 1	Fish 2	Fish 3	Fish 4	Fish 5
Al	12.895	66.86	13.489	54.046	59.746	71.471	58.8	60.036	56.073	52.744	55.944	74.27	97.442	178.058	75.04
Sb	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
As	0	0	0	0	0	0	0	0.575	0.309	0.237	0.21	0.277	0.107	0.182	0.006
Ba	0	2.929	0	2.971	2.704	1.716	1.397	2.838	4.671	3.836	1.129	1.596	2.082	3.747	1.409
B	0	0	0	0.534	0	0	0	2.015	0.427	0	0	0.718	0	0	0
Cd	0.009	0.003	0	0.028	0.037	0.008	0.002	0.002	0.002	0.014	0.02	0.025	0.026	0.018	0.002
Cr	0.155	0.253	0.251	0.293	0.048	0.177	0.081	0.098	0.084	0.084	0.491	0.666	0.703	0.819	0.244
Co	0.047	0.274	0.05	0.1	0.17	0.202	0.231	0.178	0.107	0.148	0.098	0.1	0.231	0.309	0.181
Cu	2.281	2.083	1.643	2.625	2.621	1.508	1.266	2.536	2.14	2.767	1.518	2.21	2.415	2.148	2.014
Fe	4.198	169.798	3.697	30.37	79.802	67.673	130.2	129.748	45.543	63.923	37.962	64.474	116.83	145.484	52.468
Pb	0	0	0	0	0.406	0.387	0.475	0.17	0	0	0.79	0.063	0.609	0.063	0.019
Mn	0	5.297	0	4.231	8.873	17.759	20.94	11.495	17.131	12.422	3.596	4.498	5.397	5.995	1.699
Ni	0	1.571	0	1.163	1.025	0.901	1.334	1.203	0.42	0.482	0.46	1.129	1.306	1.363	0.492
Se	4.878	0	0.189	2.962	0	0	4.201	0	0	0	6.185	0	0	0	6.336
Ag	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sr	3.444	22.925	2.543	27.118	27.141	39.677	33.335	23.025	32.274	31.402	27.353	32.362	34.567	48.048	33.016
Sn	0.014	0.008	0.011	0	0	0	0	0	0	0	0	0	0	0	0
V	0	0.252	0.013	0.122	0.032	0.474	0.351	0.122	0.139	0.081	0.151	0.29	0.207	0.279	0.155
Zn	25.767	66.254	22.13	78.126	107.96	50.588	70.503	84.031	182.024	141.293	60.465	73.089	62.96	72.475	76.872

