Assessment of the effects of environmental contaminants on feral fish populations in the Olifants River system

by

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DECLARATION

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

Surname, Initials (Title) **Date**

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ABSTRACT

Freshwater ecosystems are the most threatened systems globally, suffering from channel modification, over extraction of water and, of particular concern, pollution. In South Africa, Olifants River is categorised as the third most polluted river system. Acid mine drainage seeping from derelict and abandoned mines has been described as the primary stressor in the upper Olifants catchment. The increase of metal concentration in the water, sediment and fish tissues has been evident over the past few decades. As a result, there has been an increasing concern regarding the effects of increased metal concentrations on the health of fish and the safety of communities consuming fish from the polluted Olifants River system.

This study used enzymatic and histopathologic biomarkers to assess the physiological response of *Oreochromis mossambicus* and *Labeo rosae* to environmental contaminants. The study further investigated the metal accumulation trend of across different fish tissues and assessed the edibility of *O. mossambicus* and *L. rosae* from Loskop and Flag Boshielo dams. Water, sediment and fish samplings were carried out concurrently during low flow and high flow seasons in 2014. Water and sediment sampling were done at the inflow, middle and dam wall.

A minimum of 10 fish specimens for each species were collected from Loskop and Flag Boshielo dams during each sampling. For bioaccumulation analysis, liver, gill and muscle tissues were dissected out, wrapped with aluminium foil and frozen. Frozen samples were sent to SANAS accredited laboratory for metal analysis. For Histopathology, tissues were fixed in 10% neutral buffered formalin prior processing. Tissue processing was done at the Pathology laboratory of the University of Pretoria, Onderstepoort campus. For biomarker analysis, liver and brain tissues were fixed in liquid nitrogen in the field and transferred to the -80°C biofreezer at the University of Limpopo, Biotechnology Unit laboratory. Metal concentrations in the muscle tissue were used to calculate hazard quotient for human health risk assessment which was based on the assumptions that an adult weighting 70 kg consume 150 g portion once per week.

Alkaline pH was observed in the water throughout the study. Most water constituents were within the guidelines at both dams. The water at Flag Boshielo Dam was oligotrophic with Loskop Dam showing mesotrophic conditions. Concentrations were below detection level for most metals; however, significant concentrations were recorded in the bottom sediment. Although Loskop Dam is being described as a repository for pollutants from the upper Olifants catchment, no significant differences ($p>0.05$) were observed for metal concentrations in sediment between the two dams.

Coinciding with sediment metal concentrations, liver, gills and muscle have shown notable concentrations for both species at Loskop and Flag Boshielo dams. The common trend of liver accumulating higher metal concentration followed by gill and muscle (liver>gills>muscle) was observed for most metals on *O. mossambicus* and *L. rosae* at both dams. In contrast, lead, strontium and manganese showed higher concentrations in the gills. Muscle exhibited lowest concentrations for most metals.

Remarkable trends on the activities of biomarkers, lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), glutathione-S-transferase (GST) and acetylcholinesterase (AChE) were detected for both species at Loskop and Flag Boshielo dams. *Labeo rosae* population have shown a significantly high ($p<0.05$) LDH activities at Loskop Dam and no significant difference ($p>0.05$) was observed for *Oreochromis mossambicus*. Glucose-6-phosphate dehydrogenase activities exhibited no significant difference ($p>0.05$) for both species between the two dams. *Labeo rosae* have shown a significantly high ($p<0.05$) GST activities at Loskop Dam whereas *Oreochromis mossambicus* exhibited no significant difference ($p>0.05$) between the two dams. Acetylcholinesterase (AChE) has shown no significant difference ($p>0.05$) for both species between the Loskop and Flag Boshielo dams. LDH, G6PDH and GST activities have shown relationship with metal concentrations, which makes them good biomarkers of metal exposure.

The condition factor indicated that overall conditions of *O. mossambicus* and *L. rosae* from Loskop and Flag Boshielo dams were good. Hepatosomatic index results were not conclusive. Most histopathological alterations were recorded on both species at both dams, but with different magnitude of severity. Regressive changes

were more prominent in the liver and gills of both species at Loskop and Flag Boshielo dams followed by progressive change. Gills of *Oreochromis mossambicus* exhibited moderate modifications (score >20) at Loskop Dam and slight modification (score <20) at Flag Boshielo Dam. *Labeo rosae* populations have shown slight modifications (score <20) in the gills at both dams. Both species have shown significant difference ($p<0.05$) on the gill index between the two dams. Liver index has also exhibited significant difference ($p<0.05$) for each species between Loskop and Flag Boshielo dams. Slight modifications (score <20) were observed in the liver for both species at Loskop and Flag Boshielo dams.

Both species have shown to accumulate metals within their tissues with liver accumulating higher concentration for most metals, followed by gills and muscle, respectively. Although muscle showed to accumulate lesser metal concentrations, it still raise a serious concern as it is the tissue consumed by human. Lead, chromium, cobalt and antimony concentrations have been the only metals of concern in this river system over the past few years; nevertheless, the present study has shown that other metals *viz.* arsenic, silver and selenium have exceeded international levels for safe consumption. Given the metal concentration trend reported over the past two decade in fish tissues, there is a need for urgent intervention to address the acid mine drainage problem to ensure sustainable development of the Olifants River and safety of communities depending on it for their livelihood.

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CHAPTER 1

INTRODUCTION

1.1 Background

Water is the most fundamental and indispensable of all natural resources (Ashton 2002). It covers approximately 71% of the planet earth, however, less than 1% is available as surface water in freshwater ecosystems i.e. rivers, lakes and artificial impoundments (Davies & Day 1998). Freshwater ecosystems are the most threatened habitats globally. Biodiversity decline is far greater in freshwater ecosystems than in most affected terrestrial ecosystems. Anthropogenic activities driving the decline of biodiversity in freshwater ecosystems include engineering schemes, mining, industrialisation, urbanisation and agriculture, and coal-fired power stations (Dudgeon *et al.* 2006; Vorosmarty *et al.* 2010). Effects of the aforementioned anthropogenic activities in freshwater ecosystems include physical alteration which result in change in flow regime, habitat loss and, or particular concern, pollution (Revenga *et al.* 2005; Vorosmarty *et al.* 2010).

Pollution as a potential stressor for freshwater ecosystems has received an increasing attention over the past few decades (Heath 1995; Austin 1998). Water pollution may result from point or non-point sources. Point source comprises those which can be traced and controlled e.g. sewage treatment works, industry and intensive animal enterprises. Nonpoint sources include those which cannot be traced or controlled e.g. agricultural runoff, urban runoff and atmospheric deposition (Dallas & Day 2004). In semi-arid country like South Africa where larger part receives less than 450 mm of rainfall per annum (DWAF 2004a), pollution compromises the well-being of freshwater ecosystems and human communities. Biomonitoring programmes were therefore, developed to assess the effect of pollution in freshwater ecosystems.

However, biomonitoring programmes were not developed to replace physico-chemical monitoring. Dallas (2000) suggested that biological monitoring should be

viewed as complementary to physico-chemical monitoring. Biomonitoring is less detailed but provides a bigger picture of both the past and the present conditions of a river whereas physico-chemical analysis can give very accurate measure of the amount of individual substances in the water of a river, but it only consider the water passing at the moment of collection; they are thus instant “snapshots” of the environment (Davies & Day 1998). Fish is considered to be a good indicator due to its mobility, relatively long life span and its position at the top of the food chain (Palmer *et al.* 2004; Khan *et al.* 2012). Several indices using fish such as fish assemblage integrity index (FAII) (Kadye 2008), fish response assessment index (FRAI) (Avenant 2010; Adamu *et al.* 2015) and fish health assessment index (HAI) (Heath *et al.* 2004) have been developed to be used in biomonitoring.

Fish as indicator species for biomonitoring have a high public profile and consequently are used extensively for conservation status and bioaccumulation studies. Fish are found near or at the top of the food chain and are capable of accumulating metals from the surrounding water environment (Heath & Claassen 1999). Davies & Day (1998) reported that the concentration of a particular chemical substance in the body or tissue of an organism is seldom directly proportional to the concentration of it in the surrounding water. However, Coetzee *et al.* (2002) reported that the measurement of metal concentrations in these organisms provides the basis for the use of bioaccumulative indicators of the degree of metal pollution in various aquatic ecosystems.

In South Africa, the Olifants River is one of the main river systems threatened by pollution (Heath *et al.* 2010). The Olifants River system is characterised by elevated levels of metals, nutrients and sulphate (De Villiers & Mkwelo 2009; Oberholster *et al.* 2010; Dabrowski *et al.* 2014). Reports of unexplained fish, terrapin and crocodile mortalities (Fig. 1.1) have abounded over the past few decades. These mortalities have resulted in the establishment of the ‘Consortium for the Restoration of the Olifants Catchment’ initiative which was led by South African National Parks (SANParks) (De Villiers & Mkwelo 2009). The land use dominating the upper catchment of the Olifants River system include mining, heavy industries, coal-fired

power station, urbanisation, water treatment works and agriculture (Oberholster 2009). Acid mine drainage (AMD) emanating from abandoned mines in the upper catchment were found contributing heavily to the pollution of the Olifants River system (Dabrowski *et al.* 2013). Acid mine drainage may increase the concentrations of sulphates, dissolved salts, metal ions and, in some instances, radionuclides to the already stressed river and impoundments (De Villiers & Mkwelo 2009; CSIR 2010). Reduced pH as a result of AMD may increases the solubility of trace metals locked up in sediments and releases them into the overlying water (CSIR 2010).

Figure 1.1 a. Dead fish at the shoreline of Loskop Dam; b. Dead fish in the Lower Olifants River near Phalaborwa Gate of the Kruger National Park; c. Dead crocodile found at the shore of Loskop Dam in 2007.

Loskop and Flag Boshielo dams have been showing alkaline pH and high metal concentration in sediment over the past few years (Oberholster *et al.* 2010; Lebepe

et al. 2016). Given the on-going coal mining activities in the upper Olifants River catchment (Hodgson & Krantz 1998), the AMD from derelict and abandoned mines may result in pH drop, which may consequently remobilise metals back into the water column. Therefore, there is a need for regular monitoring of physico-chemical properties of water and the health of aquatic biota so that any drastic increase of metal concentrations in the water can be detected in time. Moreover, there are human communities consuming fish from this river system for protein supplements. Given that elevated metal concentrations have been reported in fish from the Olifants River system, it is thus, imperative to monitor metal concentrations in target fish species and assess the human health implications which may be associated with the consumption these fish to ensure the safety of human communities.

1.2 Rationale of the study

Episodic mortalities of aquatic biota (Fig. 1.1) have been prominent in the Olifants River system since early 1980s (Botha *et al.* 2011). In 2006, the Olifants River at Loskop Dam has experienced its largest fish kill to date, with thousands indigenous fish being found dead along the shoreline of the dam, including a large number of *Oreochromis mossambicus* as well as *Labeo rosae* (Legrange 2007). Mortalities of aquatic biota have also been reported at the Olifants River Gorge in the Kruger National Park (KNP). Veterinarians from South African National Parks (SANParks) established the cause of death as pansteatitis (Fig. 1.2), a nutritional disease which is caused by consumption of large amount of unsaturated fats, rancid fish and/or diet deficiency of vitamin E (Huchzermeyer 2012). It remains a mystery that recent mortalities of aquatic biota have only occurred at Loskop Dam in the Upper Olifants and the Olifants River gorge in KNP which is in the lower catchment (Oberhoster *et al.* 2012; Woodborne *et al.* 2012). No recent mortalities were recorded for indigenous fish species at Flag Boshielo Dam which is situated in the middle Olifants River (Dabrowski *et al.* 2014).

Figure 1.2 Normal (top) and panteatitis affect (bottom) *Oreochromis mossambicus* observed at Loskop Dam during 2014 surveys (Bowden *et al.* 2016).

Although these mortalities in the Olifants River system were linked to diet related disease, water quality deterioration has been a cause for concern over the past few decades. Austin (1998) reported that the exposure of fish to high concentration of pollutants such as metals, agricultural pesticides, hydrocarbons, nutrients etc. may lead to large scale mortalities. Studies conducted in the Olifants River system have shown elevated concentrations of sulphate, nutrients and metals in the water and sediment. These concentrations were reported to be driven by acid mine drainage emanating from derelict and operating coal mines in the upper catchment (De Villiers & Mkwelo 2009; Oberholster *et al.* 2010; Jooste *et al.* 2015).

Moreover, Flag Boshielo Dam in the middle catchment has been on the spotlight due to the pesticides, organophosphates and carbamates which are regularly applied (aerial application) to the crops in Groblersdal and Marble Hall areas (Bollmohr *et al.* 2008; Kekana 2013). A complaint was written by a general practitioner, which suggested the occurrence of pesticide-related symptoms in humans in the Groblersdal area whereby patients have shown to be suffering from headaches, dizziness, asthma, nausea and red eyes which seemed to be the side effects from pesticide exposure. Blood samples from patients were analysed and showed chronic levels of organophosphate and carbamate exposure (Bollmohr *et al.* 2008).

Concern was raised over the effect of these contaminants (metals, nutrients, ions and pesticides) in aquatic biotas in the Olifants River system. Studies were conducted in this river system to investigate the effect of environmental contaminants on the health of fish; however, a macroscopic assessment was employed (Madanire-Moyo *et al.* 2012; Watson *et al.* 2012). Although the macroscopic assessment proved to be relatively rapid and inexpensive method to detect change in fish populations, it was not informative enough. Van der Oost *et al.* (2003) reported that changes at higher levels of biological organisations are always preceded by changes at the lower levels. Moreover, USEPA (1990) emphasised that the response at lower levels of biological organisations are more rapid and can be more clearly associated with toxic exposure (Van der Oost *et al.* 2003). Therefore, the present study evaluates the enzymatic and histopathologic response to contaminants exposure on *Oreochromis mossambicus* and *Labeo rosae* at Loskop and Flag Boshielo dams.

Moreover, Olifants River catchment is home to about 10% of South Africa's population dominated by rural communities with low income (Coetzee *et al.* 2002; Van Vuuren 2010). Due to the serious need for alternative dietary protein supplements, these communities are opting for freshwater fish which are cheap and readily available from local rivers and dams. Metals in aquatic ecosystems do not degrade but accumulate and incorporate into food webs, and biomagnify up the food chain until organism at the top accumulating lethal dose (Davies & Day 1998;

Coetzee *et al.* 2002). Metal accumulation may pose a toxicity risk to organisms higher in the food chain (Chapman & Wang 2000). Fish are found near or at the top of the food chain and have been reported to accumulate metals from their surrounding water environment. Fish consumption is one of the routes of human exposure to metals (Barbour *et al.* 1999; Llobet *et al.* 2003).

Elevated concentrations of metals have been reported in different fish species in the Olifants River system over the past few decades, however, only few studies (Addo-Bediako *et al.* 2014b, a; Jooste *et al.* 2015; Lebepe *et al.* 2016) focused on assessing human health risks which may be associated with the consumption of this contaminated fish. Given that Loskop and Flag Boshielo dams are being considered for the development of inland fisheries (Lebepe *et al.* 2016), it is therefore, necessary to monitor metal concentration in all target fish species and evaluate if the concentration is safe for human consumption.

1.3 Aim

The aim of the study was to assess the effects of environmental contaminants on feral fish populations in the Olifants River system.

1.4 Objectives

Objectives of the study were to:

- Analyse water and sediment quality at Loskop and Flag Boshielo dams by measuring selected constituents.
- Measure the concentration of selected metals in the liver, gills and muscle tissues.
- Assess physiological response of fish to contaminants exposure by measuring activities of glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH) and glutathione S-transferase (GST).
- Measure acetylcholinesterase (AChE) inhibition in the brain of *O. mossambicus* and *L. rosae* at Loskop and Flag Boshielo dams.

- Assess the overall condition of the *O. mossambicus* and *L. rosae* using length-weight relationship, condition factor and hepatosomatic indices.
- Conduct histopathological assessment in the liver and gills of *O. mossambicus* and *L. rosae* at both dams.
- Assess the human health risks associated with the consumption of *O. mossambicus* and *L. rosae* from Loskop and Flag Boshielo dams by performing a desktop study.

1.5 Thesis outline

This thesis comprises eight chapters with the inclusion of concluding chapter. Each results chapter has got its own materials and methods. **Chapter 1:** Present the background information on the status of freshwaters, biomonitoring and Olifants River system, rationale, as well as aims and objectives on the study. **Chapter 2:** Literature reviews including study area and sentinel fish species description. **Chapter 3:** Physico-chemical properties of water and bottom sediment from Loskop and Flag Boshielo dams. **Chapter 4:** Metal concentration assessment in the liver, gills and muscle tissues of *O. mossambicus* and *L. rosae* from Loskop and Fag Boshielo dams. **Chapter 5:** Enzymatic biomarker responses to water pollution. **Chapter 6**: Overall fish conditions and histopathologic responses observed for *O. mossambicus* and *L. rosae* at both dams. **Chapter 7:** Human health risk assessment. **Chapter 8**: Concluding remarks and recommendations for future studies.

Given the manner in which this thesis has been structured, repetition of information such as introductory background and methodologies was inevitable. Results on water quality and metal concentrations in fish tissues were incorporated from chapter 4 – 7. Metal concentration results in muscle tissues were repeated in human health risk assessment chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Olifants River catchment

The Olifants River catchment comprises portions of two Southern African Development Community (SADC) countries, South Africa and Mozambique (Ashton *et al.* 2001). About 85% of the catchment is located in the north-eastern part of South Africa with a large portion in Limpopo and Mpumalanga provinces and a smaller portion in Gauteng (Fig. 2.1) (Ashton *et al.* 2001; Heath *et al.* 2010). The South African portion of the Olifants River catchment covers about 74 500 km^2 and is home to about 10% of human population (Van Vuuren 2010). The Olifants River catchment is subdivided into upper, middle, the Steelpoort and lower catchment (Claassen *et al.* 2005; Ashton 2010).

The upper Olifants River catchment comprises the drainage areas of the Steenkoolspruit, Klein Olifants River and Wilge River with all tributaries down to the Loskop Dam. The main impoundments in the upper catchment include Witbank, Middelburg, Bronkhorstspruit and Premiere Mine dams (Heath *et al.* 2010). The middle catchment comprises the drainage areas of the Olifants River downstream of Loskop Dam down to the Flag Boshielo Dam. The major tributaries of the middle Olifants River catchment include Selons, Moses, Bloed, Makhutswi and Elands rivers (Ashton 2010; Heath *et al.* 2010).

The Steelpoort catchment covers part of the Sekhukhuneland and since it is dominated by mountains, the catchment is also referred to as mountain region. Conservation areas, Sterkspruit and Lydenburg Nature Reserves are found in this catchment (De Lange *et al.* 2005). The lower Olifants River catchment covers the area between the Steelpoort confluence and the Mozambique border. The major tributaries include the Steelpoort, Blyde and Ga-Selati rivers (Fig. 2.1) (Heath *et al.* 2010; DWA 2011a).

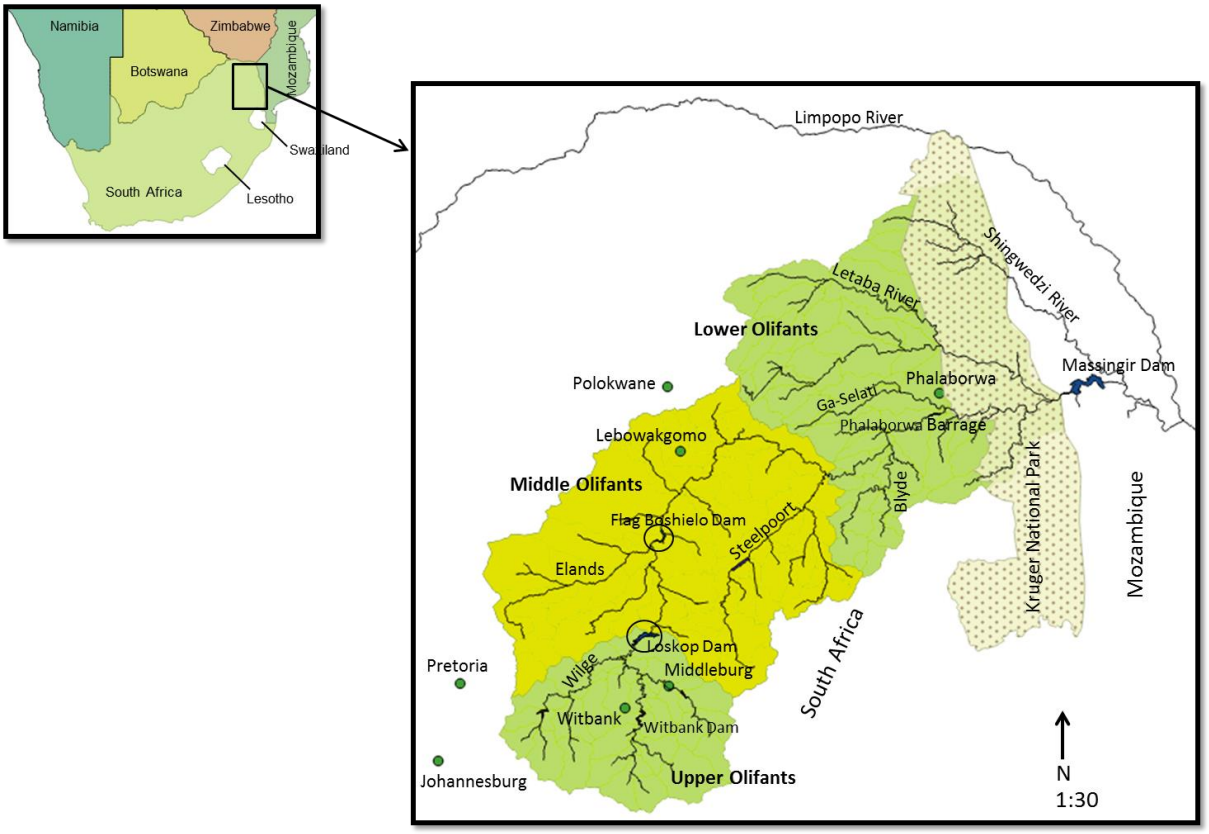

Figure 2.1 The Olifants River catchment map with Loskop and Flag Boshielo dams encircled.

2.1.1 Geology

The catchment is dominated by exposed rocks from the early Precambrian Era that comprises three common types, sedimentary, igneous and metamorphic. The oldest exposed rock formation in the catchment includes Archaean Granite and Gneiss Basement Complex which forms the basement rock complex for other rock systems. These Granite and Gneis formations are found in the extreme east Lowveld part of the Olifants River catchment, and are mined for use as polished stones. The southern part of the catchment is dominated by carbon-rich sedimentary rocks of the Karoo System containing enormous economic reserves of coal. The intensive coal mining activities occur in the Karoo System (Ashton *et al.* 2001; DWA 2010).

2.1.2 Land use

The land use in the Olifants River catchment consists primarily of irrigated and dryland cultivation, improved and unimproved grazing, mining, industries, coal-fired power generation plants, water treatment works, forestry, and urban and rural settlements (Fig. 2.2 & 2.3) (IWMI 2008; Oberholster *et al.* 2010; Ashton & Dabrowski 2011; Dabrowski *et al.* 2013).

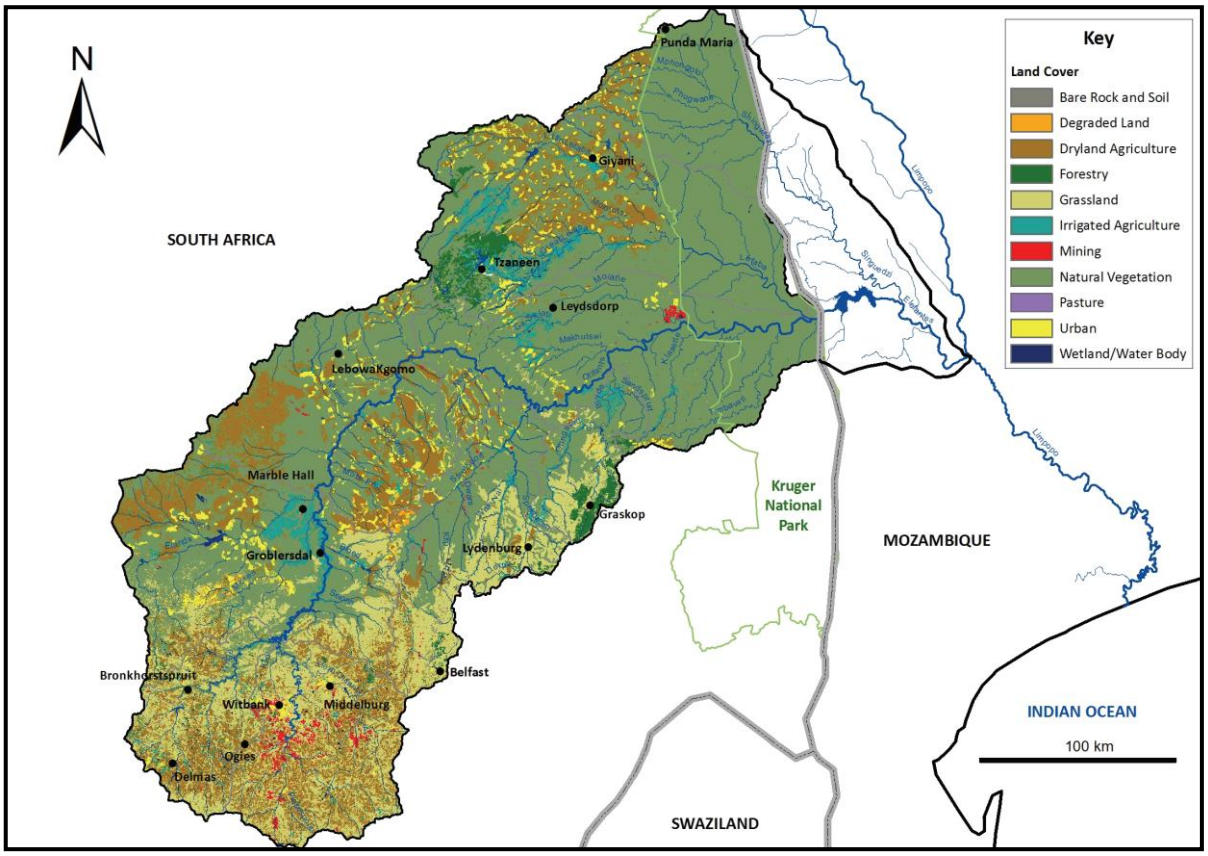

Figure 2.2 Land cover and different land uses in the Olifants River catchment (Ashton & Dabrowski 2011).

Figure 2.3 Land uses observed in the upper Olifants River catchment. **a**. Coal-fired power station; **b**. Agricultural activities; **c**. Colliery (coal mine).

A total of 348 mines and quarries producing a wide spectrum of mineral commodities are located in the Olifants River catchment (Fig. 2.4) (Ashton & Dabrowski 2011). The upper Olifants River catchment is characterised by an extensive mining of coal, with some of them remaining abandoned (Netshitungulwana & Yibas 2012; DMR 2014). Over 50% of the South Africa's coal is produced by Witbank, Ermelo and Highveld coal fields (Mccarthy & Pretorius 2009). The upper Olifants River catchment further exhibit large scale agricultural activities, irrigation schemes and human settlements (Fig. 2.2) (Ashton & Dabrowski 2011).

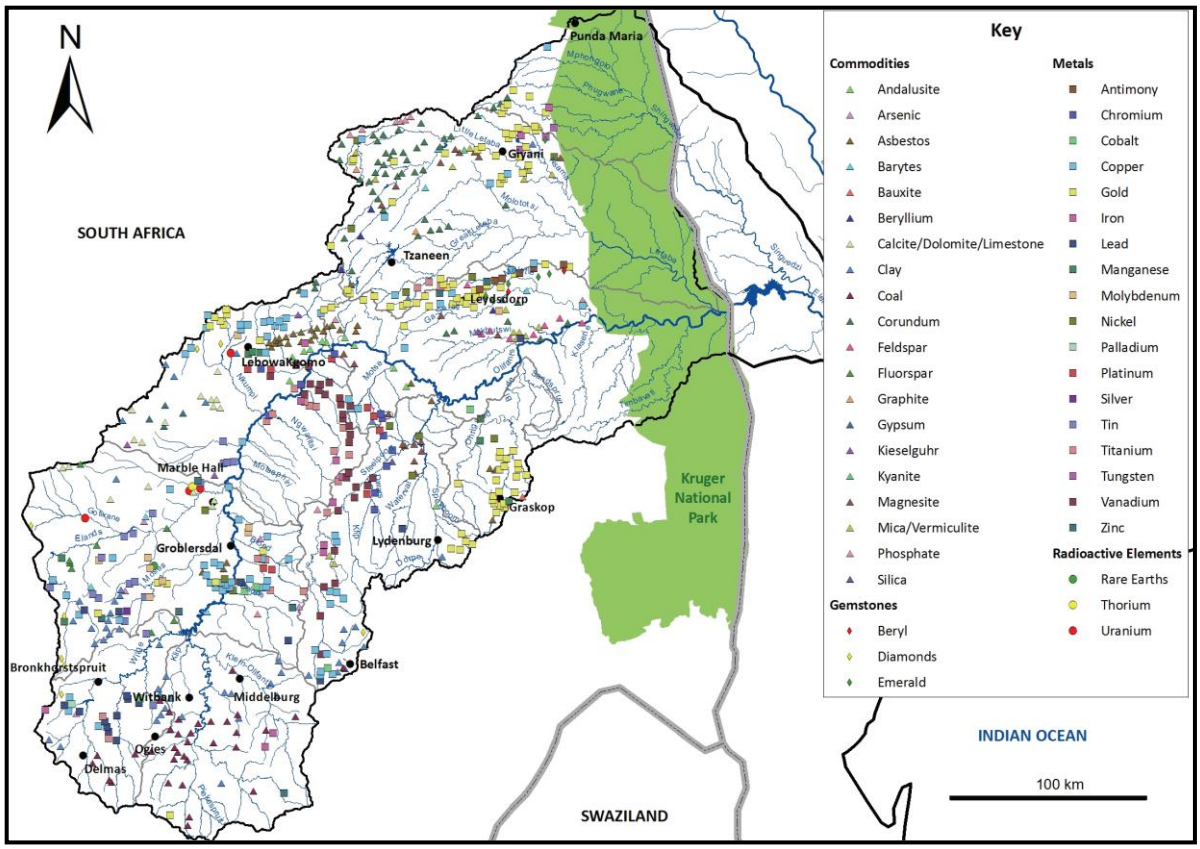

Figure 2.4 Mines and quarries across the Olifants River catchment (Ashton & Dabrowski 2011)

The middle catchment is characterised by agricultural activities, both dry-land and irrigated; small towns i.e. Groblersdal, Marble Hall, Settlers, Siyabuswa and Dennilton; and mining activities in the catchments of Klipspruit, Moses River and Loopspruit as well as the area east of Marble Hall (Heath *et al.* 2010; Ashton & Dabrowski 2011; Dabrowski *et al.* 2014). Minerals mined in the middle Olifants River catchment over the past few decades, gold, diamond-kimberlite, andalusite, manganese, chrome, platinum, clay minerals, limestone, zinc, tin, feldspar, pegmatite-muscovite, asbestos, vanadium, pegmatite-tantalum and fluorspar (Fig. 2.4) (Ashton *et al.* 2001; Ashton & Dabrowski 2011).

The Steelpoort catchment is characterised by rural settlements with small-scale agricultural activities, few towns i.e. Lydenburg, Burgerfort and Vermont, and mines (Claassen *et al.* 2005; DWA 2011b). Minerals mined in the Steelpoort catchment

include, iron, vanadium, chrome, platinum, andalusite, vermiculite, dimension stone and copper (Ashton *et al.* 2001).

The lower Olifants River catchment consists entirely of small-scale subsistence agriculture and some irrigation, conservation areas i.e. Kruger National Park (KNP) as well as few mines in the Phalaborwa area (Ashton *et al.* 2001; Heath *et al.* 2010). Minerals mined in this catchment include gold in the Blyde sub-catchment, antimony, gold, clay minerals, copper, emerald, titanium, vermiculite, phosphate, silver, zinc and zirconium in the Ga-Selati sub-catchment (Ashton *et al.* 2001).

2.1.3 Hydrology

The Olifants River system is classified as perennial, however, some of its tributaries experience low to seasonal flows particularly in the lower catchment where it flows through the KNP (Claassen *et al.* 2005). The catchment receives summer rainfall and the mean annual rainfall varies greatly across different regions. The Sekhukhune area and the northern parts of the eastern Lowveld receives rainfall varies between 325 and 550 mm/annum. In the Highveld region and the southern part of the eastern Lowveld, the rainfall varies between 550 and 750 mm/annum. The escarpment receives a rainfall between 750 and 1000 mm/annum with Wolkberg area receiving an annual rainfall exceeding 1000mm/annum (DWA 2011b).

There are more than 200 dams in the Olifants River catchment, 30 of which are major dams having a capacity of over 2 million m^3. The major dams include, Phalaborwa Barrage, Witbank Dam, Loskop Dam, Flag Boshielo Dam, Middleburg Dam etc. (Heath *et al.* 2010; DWA 2011b). The total mean annual runoff in the Olifants River catchment is estimated at 2400 million m^3 per year (Ballance *et al.* 2001; De Villiers & Mkwelo 2009).

2.2 Loskop Dam

Loskop Dam (Fig. 2.5) (25° 26' 57.05" S, 29° 19' 44.36" E) is located in the upper reach of the Olifants River within the Loskop Dam Nature Reserve. The dam is situated approximately 32 km south (upstream) of the town of Groblersdal in

Mpumalanga province, South Africa. The dam was constructed in 1938 by the Department of Water Affairs and in 1979 the wall was raised to its current height of 54 m above the foundation (Botha *et al.* 2011). The impoundment has a surface area of 24.27 km^2 and a volume of 374 X 106 m^3 at full supply capacity, and was designed primarily to supply water for agricultural irrigation downstream of the dam wall (DWAF 2004b). Due to its location, Loskop Dam has been described as a repository for pollutants from the upper catchment (Oberholster *et al.* 2010).

Figure 2.5 a. Loskop Dam wall; **b.** Loskop Dam inflow with reeds on the edge.

The water quality of Loskop Dam has been deteriorating due to anthropogenic activities dominating the upper catchment. Of particular concern is the acid mine drainage emanating from abandoned coal mines in the upper catchment which has resulted in increasing sulphate concentration in Loskop Dam (Oberholster *et al.* 2010; Dabrowski *et al.* 2013; Jooste *et al.* 2015). Coal mining in the upper Olifants (Witbank and Middelburg area) commenced in 1894 to supply coal to the growing diamond and gold mining industries. Some mines in the region lie abandoned, some have collapsed and most are decanting acidic water. The water enters tributaries of the Olifants River where it is slowly neutralised by dilution and various chemical and biological reactions. However, the water remains highly saline and sulphate concentrations are particularly elevated (McCarthy 2011).

The development of algal blooms (mainly *Microcystis* sp. and *Euglena* sp.) is suggesting eutrophic to hypertrophic condition in Loskop Dam (Oberholster *et al.* 2010; Van Campenhout *et al.* 2010). However, a recent study has classified the dam as meso-eutrophic (Dabrowski *et al.* 2013). The dam has experienced mortalities of aquatic biota over the past two decades which was linked to pansteatitis. Although the cause of disease in Loskop Dam still remains elusive, it has been proposed that bioaccumulation of aluminium and iron via algae within body fat was the cause of yellow discolouration of fat of *Oreochromis mossambicus* in Loskop Dam (Oberholster & Botha 2011; Huchzermeyer 2012).

2.3 Flag Boshielo Dam

Flag Boshielo Dam (Fig. 2.6) (24° 46' 51.46"S 29° 25' 32.57"E) is situated in the lower middle catchment, about 80 km downstream of Loskop Dam. The dam was constructed in 1987 and raised by 5 meters in 2005 (Ashton 2010). Flag Boshielo Dam was built to provide water for irrigation, for domestic and industrial supply and also for recreational purposes (Mccartney *et al.* 2004). Despite mining and industrial activities occurring in the upper catchment and agricultural activities dominating the middle catchment, recent studies has shown that Flag Boshielo Dam is still oligotrophic (Madanire-Moyo *et al.* 2012; Dabrowski *et al.* 2014). Most of the contaminants from the upper Olifants River catchment are trapped by Loskop Dam. Moreover, no pansteatitis induced mortalities were reported in crocodiles (*Crocodylus niloticus*) and *O. mossambicus* in Flag Boshielo Dam (Woodborne *et al.* 2012; Dabrowski *et al.* 2014).

Figure 2.6 a. Flag Boshielo Dam with an island in the middle (arrow); **b.** Crocodile (solid arrow) and a bird (broken arrow) on the island at Flag Boshielo Dam.

2.4 Biomonitoring

Biomonitoring is the scientific procedure which uses aquatic biota to monitor the ecological state of an ecosystem. The aquatic biota used during biomonitoring is termed a bioindicator. Furthermore, biomonitoring is used to provide resource information by repetitive measurement, continued observation and evaluation of environmental data (Dallas 2000; Taylor *et al.* 2007). In South Africa, several indices i.e. Riparian Vegetation Index (RVI) (Kemper 2001), South African Scoring System (SASS 5) (Dickens & Graham 2002) and Biological Diatom Index (BDI) (De la Rey *et al.* 2008), Health Assessment Index (HAI) (Avenant-Oldewage *et al.* 1995), Fish Assemblage Integrity Index (FAII) (Kleynhans 1999) have been developed and used to monitor the health of aquatic ecosystems. All these indices and organisms proved to be reliable with regard to providing a rapid and inexpensive indication of the occurrence of change in a freshwater ecosystem.

2.5 Bioindicator

A bioindicator may be a species or group of species that readily reflects the abiotic or biotic state of an environment and represents the impact of environmental change on a habitat, community or ecosystem or is indicative of the diversity of a subset of taxa or the whole diversity within an area (Gerhardt 2002; Hamza-Chaffai 2014). Bioindicators can be divided into effect and accumulative bioindicators (Sures 2001;

Gerhardt 2002). Effect bioindicators may further be divided into compliance, diagnostic and early warning indicators. Compliance indicator uses a population, community or the ecosystem as a whole whereas diagnostic and early warning indicators (also known as biomarker) are measured at biochemical, cellular and/or tissue levels. Diagnostic and early warning indicators are sensitive and respond rapidly to environmental change (Gerhardt 2002). Accumulative indicators are organisms accumulating and concentrating contaminants from their surrounding environment and/or through feeding so that the analysis of their tissues provide a long record of the available concentration of these contaminants (Gerhardt 2002; Osman & Kloas 2010).

2.5.1 Fish as bioindicator

Fish has become a reliable bioindicator of aquatic pollution due to its sensitivity to change in physico-chemical properties of the water (Hobbs *et al.* 2008), long life span, high diversity, and being easily identifiable (Dabrowski & de Klerk 2013). Moreover, fish occur in wide variety of habitats and there is enough published information on their habits and occurrence (US-EPA 2008). Different indices employing fish have been developed to determine the ecological integrity of an aquatic ecosystem (Kadye 2008; Avenant 2010). The use of lower levels of biological organisations (biomarkers) has emerged over the past few decades (Richardson *et al.* 2010; Abdel-Moneim *et al.* 2012; Javed & Usmani 2016) and has even attracted an increasing attention due to its relevance in detecting early warning signs of environmental contaminants exposure.

Various fish species have been studied in order to determine their suitability and reliability on assessing the health of aquatic ecosystem. Although almost all fish species have proven to be reliable, they respond differently to different kinds of pollutants (US-EPA 2008). For ecotoxicological assessment study, native fish species are recommended since most non-native are invasive and highly tolerant to environmental stressors such as pollution and habitat modification (Fedorenkova *et al.* 2013).

2.6 Biomarkers

Biomarkers are defined as modification at molecular, biochemical or cellular level which can be related to exposure to or toxic effects of environmental contaminants. The response to environmental contaminants in an organism occurs in a sequential order (Fig. 2.7). Therefore, the effects at higher hierarchical levels of biological organisation are always preceded by modifications at the lower level. This scenario has triggered research to establish early-warning markers which may reflect exposure or response to contaminants toxicity (Van der Oost *et al.* 2003). Biomarkers may thus, give early warning signals of exposure to toxic chemicals and the magnitude of organism's response to contaminants (McCarthy & Shugart 1990). Hence, biomarkers are subdivided into three classes (Van der Oost *et al.* 2003; Hamza-Chaffai 2014) as follows:

- Biomarker of exposure is described as those which can be used to assess or to confirm the exposure of individuals or populations to particular contaminants, providing a link between external exposure and internal dosimetry.
- Biomarker of effect may be used to document either preclinical alterations or adverse health effects induced by external exposure and absorption of a chemical
- Biomarker of susceptibility help to elucidate variations in the degree of responses to toxicant exposure observed between different individuals.

In ecotoxicology studies, biomarkers may be used in a predictive way, allowing the initiation of bioremediation strategies before irreversible environmental damage of ecological consequence occurs (Melancon 1995; Hamza-Chaffai 2014). Enzymatic and histopathologic biomarkers have recently received an increasing attention as a new and potentially powerful tool for detecting exposure to and the effect of environmental contaminants in an aquatic ecosystem (Adedeji *et al.* 2012; Hamza-Chaffai 2014).

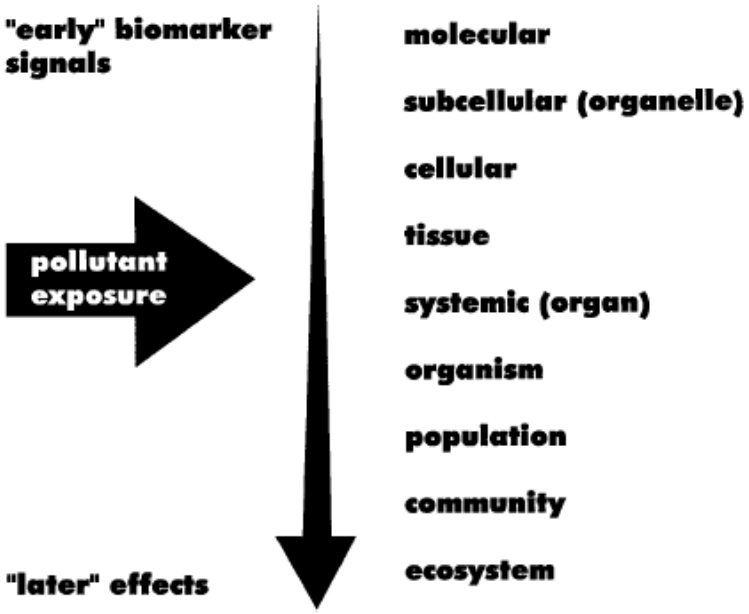

Figure 2.7 A schematic representation of a sequential order of responses to environmental contaminants stress in a biological system (Van der Oost *et al.* 2003).

2.6.1 Histopathologic biomarker

Histopathology is the study of the structure of abnormal, diseased tissues. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies (Camargo & Martinez 2007). Fish respond to changes in their surrounding water environment, which in turn reflect prevailing health status of an aquatic ecosystem (Qadir & Malik 2011). Early responses may be evident at biochemical levels, followed by cellular and tissues (histopathology) before significant changes can be identified in fish behaviour or external appearance. Severe or prolonged physiological and biochemical alterations may lead to structural alterations which become more pronounced over time resulting in increased magnitude of severity (Van Dyk *et al.* 2009b; McHugh *et al.* 2011).

The advantages of using histopathology as a biomarker as suggested by Hinton & Lauren (1990) and Van der Oost *et al.* (2003) include the following:

- Different organs can be assessed.
- Histological responses can indicate potential problems before the effects appear at higher organization levels.
- No geographical or ecosystem limitations.
- Histology sections retain *in situ* relationships of different cell types and tissues in organs.
- Many alterations persist even after exposure to a toxicant has ceased so that host response to prior toxicity can also be used to determine effects.
- Acute changes are seen when contact levels are sufficiently high, while chronic duration is required to determine sub lethal aspects of change.

The disadvantage is that the ability to detect alterations depends on the investigators expertise i.e. experience in recognition and interpretation of different alterations in different tissues and lack of specificity of certain detected lesions in reference to causality (Hinton & Lauren 1990).

2.6.1.1 Gill as a target organ

A fish gill is a respiratory organ comprised of a long and narrow lamellae bearing filament. Lamellae dramatically increase the surface area of the gill filament epithelium and result in a small diffusion distance between the blood that perfuses each lamella and the respiratory water. Gills constitute up to 90% of the total body surface area (Evans *et al.* 2005). In a fish, gills perform a variety of critical physiological functions such as ion regulation, maintenance of acid-base balance and excretion of nitrogenous wastes (Georgieva *et al.* 2014).

Gills are among the most delicate organs of fish and their vulnerability is thus considerable due their external location and necessarily intimate contact with the external water environment which means they are susceptible to damage by any irritant material, whether dissolved or suspended (Marchand 2008; Pereira *et al.* 2012). Moreover, gills are described as the primary uptake site of toxicologically effective concentration of waterborne metals and a crucial organ when it comes to the induction of compensatory responses, whether adaptive or pathological of the organism (Taylor *et al.* 2002; Ackermann 2008).

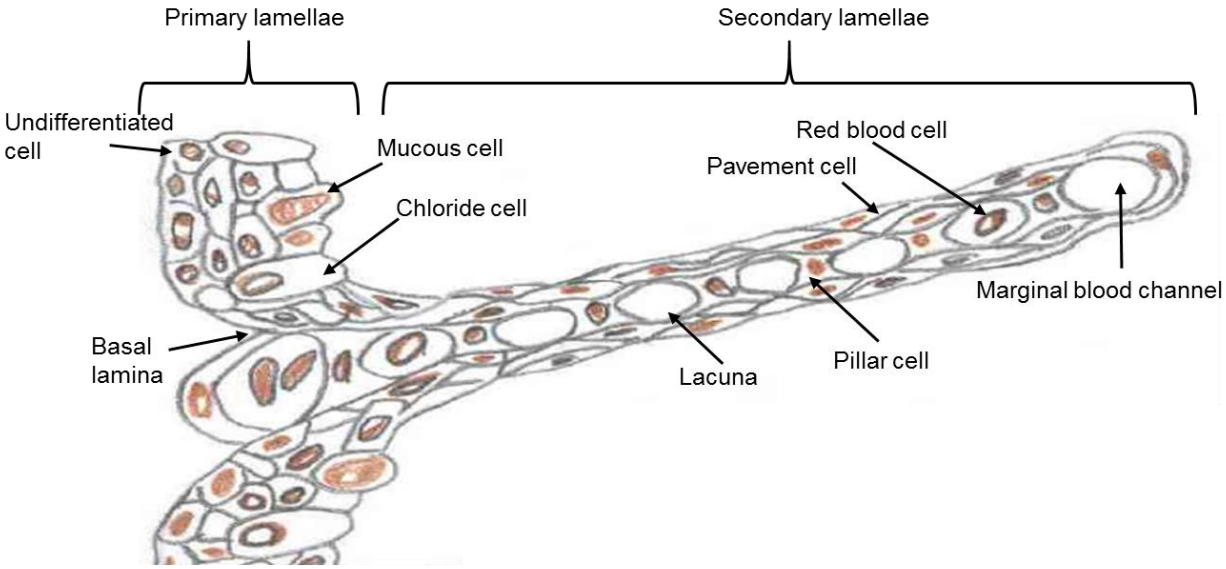

Figure 2.8 Normal gill lamella (Adapted from Mallatt (1985)).

2.6.1.2 Liver as a target organ

Fish liver is the largest mass of glandular tissues dominated by parenchymal cells (hepatocytes) arranged as tubules of cells. Hepatocytes range in shape from oval to irregular polygons endowed with secretory and biosynthetic structures such as Golgi bodies and endoplasmic reticulum to complement liver functions (Heath 1995; Roberts 2012). Liver plays an important role in vital functions in basic metabolism. It is a major organ for accumulation, biotransformation and excretion of contaminants in fish (Authman *et al.* 2013). However, some metabolites produced may be highly

reactive and ultimately more toxic, affecting the structural integrity of deoxyribonucleic acid (DNA) which results in carcinogenesis process and perturbations in fecundity, longevity and growth in fish populations (Ameur *et al.* 2012).

Detoxification mechanisms in the liver have been mainly centred on phase I and Phase II enzyme system. The phase I enzyme activities include oxidation, reduction, and hydrolysis reactions. The phase I cytochrome P450 superfamily of enzymes (CYP450) is generally the first defence employed by the body to biotransform xenobiotics. When phase I metabolises toxins, it either transform it to a less toxic state, makes it water soluble or converts it to a more chemically active form to be passed on to Phase II (Jeffery 2005; Wassmur 2012; Hodges & Minich 2015). The products of phase I metabolism are frequently substrates for phase II enzymes. Typically, Phase II enzymes add bulky water-soluble molecules to xenobiotics, often creating inactive products termed conjugates, that are excreted in bile and urine (Jeffery 2005; Wassmur 2012).

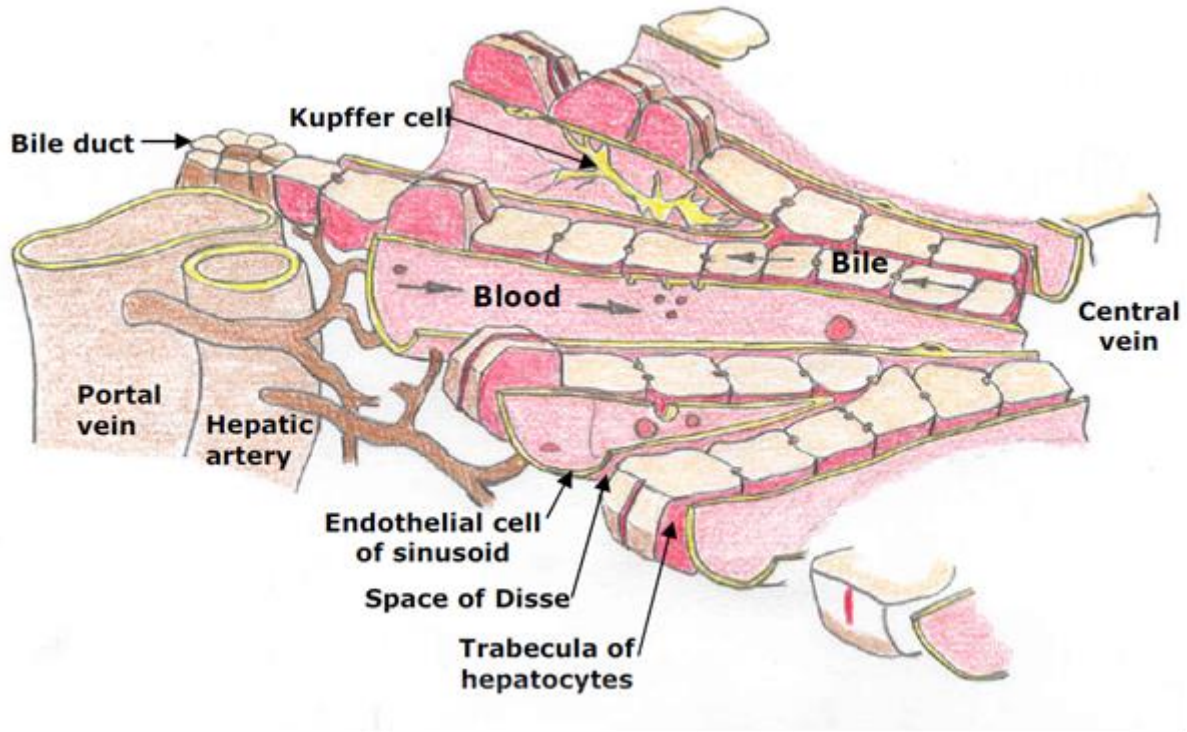

Figure 2.9 Classical vertebrate liver. Arrangement of hepatocytes and sinusoids in the classical liver lobe (Ackermann 2008).

2.6.2 Enzymatic biomarkers

2.6.2.1 Glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase (G6PDH) is the key enzyme of pentose phosphate pathway which supplies riboses for DNA and RNA synthesis, particularly in proliferating cells (Winzer *et al.* 2002; Gul *et al.* 2004). The enzyme is also involved in energy metabolism and production nicotinamide adenine dinucleotide phosphate (NADPH) (Carney 2008). Studies have shown that G6PDH is a regulatory enzyme in NADPH-dependent xenobiotic biotransformation and defences against oxidative stress (Winzer *et al.* 2002). Glucose-6-phosphate dehydrogenase is an antioxidant enzyme which has long been used as a biomarker of pollution-induced carcinogenesis in fish (Osman *et al.* 2010).

2.6.2.2 Lactate dehydrogenase

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme which is involved in carbohydrate metabolism and has also been used as an indicative criterion of exposure to chemical stress (Osten *et al.* 2005). This enzyme is widely used in toxicology and clinical chemistry to diagnose tissues and organ damage (Kumari *et al.* 2011). Lactate dehydrogenase commonly reflects the metabolic capacity of a cell or tissue (Osman *et al.* 2010). Deviation from normal activity pattern of LDH has been observed in fish exposed to xenobiotics such as metals and pesticides (Osman *et al.* 2010; Kumari *et al.* 2011).

2.6.2.3 Glutathione S-Transferase

Glutathione S-transferase (GST) is an important group of intracellular enzymes of the second stage of xenobiotic metabolism with the main function in detoxification processes by catalysing the conjugation of tripeptide glutathione (GSH) with some endogenous toxic metabolites and many environmental contaminants (Slatinska *et al.* 2008; Rudneva *et al.* 2010). Together with antioxidant enzymes GSTs protect the organisms from peroxidative damage and have also been used in detoxification of toxicants including pesticides, metals, cyclic hydrocarbons, oil and other xenobiotics because they help to eliminate the oxidative by-products (Rudneva *et al.* 2010). Glutathione S-transferase has been used as biomarker of liver toxicity in environmental monitoring studies (Lushchak *et al.* 2001; Saliu & Bawa-Allah 2012; Carvalho-Neta & Abreu-Silva 2013).

2.6.2.4 Acetylcholinesterase

Acetylcholinesterase (AChE) is the key enzyme in the nervous system of animals. It controls a large proportion of physiological and behavioural responses in the animal; thus any changes to these regulatory abilities could be potentially detrimental to fish (Richardson *et al.* 2010). Acetylcholinesterase plays an important role in the transmission of nervous influx and is the specific target for most nerve agents and pesticides (Jebali *et al.* 2013). The inhibition of AChE may impair the neurotransmission (Kumari *et al.* 2011). This enzyme has been used in

environmental monitoring as biomarker of organophosphorus and carbamates exposure (Oliveira *et al.* 2007). It is regarded as an effective biomarker of neurotoxic chemical exposure because it can be inhibited by low concentration of pesticides (Van der Oost *et al.* 2003).

2.7 Condition factor

The condition factor is an index based on weight-length relationship. The index has been widely used in fish biology with several purposes i.e. to estimate the mean fish weight based on the known length, morphometric interspecific and intra-population comparison and to assess the well-being of the fish population (da Costa & Araújo 2003; Gomiero & Braga 2005). The relative condition factor of a fish assumes that heavier fish are in better condition than lighter fish of the same length (Jenkins 2004; Gomiero & Braga 2005). The condition factor may be strongly influenced by biotic and abiotic environmental conditions and can be used to assess the status of the aquatic ecosystem in which fish live (Anene 2005).

The fish condition factor may vary among species, however, an index value of one is regarded as ideal (Jooste *et al.* 2005). Barnham & Baxter (2003) reported that a condition factor of 1.60 indicate an excellent condition (trophy class fish) while 0.80 indicate extremely poor fish resembling a barracouta, big head and narrow, thin body. Several studies have used condition factor to assess the health of fish from contaminated impoundments and it has shown to be useful in distinguishing fish from pristine and nutrient-rich impoundments (Madanire-Moyo *et al.* 2012; Watson *et al.* 2012).

2.8 Hepatosomatic index

The hepatosomatic index measures the liver mass relative to the body mass and is expressed in percentage (Chellappa *et al.* 1995; Di Giulio & Hinton 2008). The liver has the ability to degrade toxic compounds, but it may be overwhelmed by elevated levels of these compounds resulting in structural alterations (Ross & Wojciech 2011). The hepatosomatic index may increase due to pathological changes such as hyperplasia or hypertrophy which serve as an adaptive response to increase the

detoxification capacity (Salamat & Zarie 2012). However, the hepatosomatic index may decline in response to starvation because it is associated with liver energy reserves and metabolic activities (Pyle *et al.* 2005; Di Giulio & Hinton 2008; Liebel *et al.* 2013).

Together with condition factor, the hepatosomatic index has proven to be useful in field and laboratory fish studies, and is often employed to support the findings of qualitative and quantitative assessment of fish health (Van Dyk *et al.* 2007; Di Giulio & Hinton 2008; Marchand *et al.* 2008). The normal hepatosomatic index values for Osteichthyes range from 1 – 2% (Munshi & Dutta 1996). The hepatosomatic index value below 1% indicates possible atrophy of hepatocytes whereas a value above 2% indicates possible hypertrophy of hepatocytes (Busacker *et al.* 1990).

2.9 Bioaccumulation of metals

Bioaccumulation is the process by which organisms accumulate contaminants such as metals within their tissues. The process occurs across the food chain with organisms at the top accumulating elevated concentration (Davies & Day 1998). In an aquatic ecosystem, fish are near or at the top of the food chain and may concentrate large amounts of some metals from the surrounding water environment (Rauf *et al.* 2009). Fish accumulate toxicants directly from the water and through their diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Osman & Kloas 2010; Qadir & Malik 2011).

Metal distribution between different tissues depends on the mode of exposure. Gills are in a direct contact with the water and thus, accumulate metals from the water. Therefore, metal concentrations in the gills may reflect the concentration in water the fish inhabit (Rauf *et al.* 2009). Metals entering fish through diet may accumulate in the liver since it is the centre for biotransformation and detoxification. Metals, such as Fe, Cr, Cu, Zn and Mn are essential metals since they play important roles in biological systems, however, they become toxic at higher concentrations, whereas

Pb and Cd have no documented role in living organism and can be toxic even in trace amounts.

Several studies on bioaccumulation have reported elevated metal concentrations in the liver, kidneys, gills with muscle showing lesser concentrations (Abdel-Baki *et al.* 2013; El-Moselhy *et al.* 2014). Substantially high metal concentrations in fish tissues may result in structural alteration and/or even mortalities (Austin 1998). Although metals accumulate relatively low metal concentrations, it is the tissue consumed by human. Therefore, human consuming contaminated fish are exposing themselves to metals toxicity (Llobet *et al.* 2003).

2.10 Sentinel species

2.10.1 Oreochromis mossambicus

Oreochromis mossambicus (Peters, 1852) (Family: Cichlidae) (Fig. 2.8a) is widely distributed in Malawi, Mozambique, Swaziland, Zambia, Zimbabwe and South Africa. It is found along the eastern coast of Africa, in the lower Zambezi and its tributaries, eastward flowing rivers and coastal lagoons southward to the Bushmen's River, South Africa. It is a euryhaline species with high tolerance for excessive salinity concentrations (Skelton 2001). *Oreochromis mossambicus* feeds on algae, especially diatoms and detritus. However, large individuals may consume large aquatic invertebrates as well as small fry. The species is a mouth brooder (Skelton 2001). This fish species contributes about 4% of the total Tilapia aquaculture production in southern Africa and is valued more when used for hybridisation (Gupta & Acosta 2004). *Oreochromis mossambicus* are abundant in the Olifants River system and were among the species died in recent fish mortalities in the system (Huchzermeyer 2012).

2.10.2 Labeo rosae

Labeo rosae (Steindachner, 1894) (Family: Cyprinidae) is a small headed fish with a compressed body and is susceptible to the impacts of weir and net fishing as well as sedimentation (Bills *et al.* 2007). The base colour is golden green with silver pink

scales, reddish eye and snout with red tubercles (Fig. 2.8b). This species is distributed in the lower reaches of Limpopo, Inkomati and Phongolo River systems (Skelton 2001). *Labeo rosae* prefers sandy stretches of larger perennial and intermittent rivers. The species is prevalent in Flag Boshielo and Loskop dams. It feeds on detritus, algae and small invertebrates. It is an active fish, leaping at barriers when migrating upstream in swollen rivers to breed in summer (Reid 1985; Skelton 2001). It attains sexual maturity at about 150 mm total length (Skelton 2001).

Figure 2.10 Fish species used in this study: **a.** Mozambique tilapia, *Oreochromis mossambicus*; **b.** Rednose labeo, *Labeo rosae*.

CHAPTER 3

PHYSICO-CHEMICAL PROPERTIES OF LOSKOP AND FLAG BOSHIELO DAMS

3.1 Introduction

Water quality differs from continent to continent and even from region to region due to differences in climate, geomorphology, geology and soils, and biotic composition. Natural compounds in natural waters are derived from weathering of rocks over which they flow or from which they drain, soil leaching, volcanic actions and other natural processes (Chapman 1996; Dallas & Day 2004). However, anthropogenic activities such as mining and agriculture disturb the earth's surface; thus, enhance these processes resulting in the increase on the input of natural compounds into the aquatic ecosystem. Moreover, effluents from industries and municipal waterworks have been reported to contribute significantly to the increase of chemical compounds in aquatic ecosystems (Chapman 1996; Wang *et al.* 2010). Water pollution has recently become a cause for concern due to the deleterious effects it poses on aquatic biota, particularly in countries where freshwater is limited (Ashton 2002).

South Africa is a semi-arid country with limited freshwater resources, and a seasonal and variable rainfall pattern. A large part of South Africa receives less than 450 mm of rainfall per annum, well below the world average rainfall of 860 mm per annum. As a result, South Africa's water resources are, in global terms, regarded as scarce and extremely limited. The combined flow of all South African rivers amounts to approximately 49 000 million cubic metres per year (m^3/a), less than half of that of the Zambezi River, the closest large river to South Africa (DWAF 2004a; CSIR 2010). Most of South African's main rivers are receiving effluents from mining and industrial activities, and partially-treated and/or untreated sewage from wastewater treatment works (DWAF 2004a). Therefore, freshwater pollution is becoming an increasing problem in South Africa.

The Olifants River system is one of the most polluted freshwater sources in South Africa. The Olifants catchment is characterised by mining, agriculture, coal-fired

power stations, and manufacturing and metallurgic industries (De Villiers & Mkwelo 2009; DWA 2011b). The Olifants River system serves as a repository for acid mine drainage emanating from a number of abandoned and operating coal mines. Moreover, the discharge of untreated and partially treated industrial and municipal wastewaters into the Olifants River have been prominent over the past few decades (De Villiers & Mkwelo 2009; Oberholster *et al.* 2011). This has been supported by signs of water quality deterioration which have been evident since 1980s (Botha *et al.* 2011).

The water quality deterioration in the Olifants River system started received an increasing attention after unexplained mortalities of aquatic fauna at Loskop Dam in the upper catchment and at the Olifants River gorge in Kruger National Park (KNP) which is in the lower catchment (De Villiers & Mkwelo 2009; Heath *et al.* 2010). The mortality remained a mystery since none was reported at Flag Boshielo Dam which is located in the middle catchment, about 100km downstream of Loskop Dam. Loskop Dam is the first main water body which collects water from all upper catchment Olifants River tributaries, and thus, serve as a repository for contaminants emanating from the entire upper catchment (Fig. 2.1) (Oberholster *et al.* 2010). Given mysterious incidents reported in this river system and anthropogenic activities which may affect the water quality, this section evaluated physico-chemical properties of the water and sediment at Loskop and Flag Boshielo dams. Furthermore, the trend of metal concentrations in the water and sediment over the past recent years was explored.

3.2 Materials and methods

3.2.1 Water sampling and analysis

To represent different sites in the dams, water samples were collected from the inflow, middle and dam wall during winter (May/Jun) and summer (Nov/Dec) 2014 surveys. Water temperature, dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC), salinity and pH were measured *in situ* at all sites using a handheld multi parameter instrument (YSI 556 Multi Probe System) (Fig. 3.1). Water samples were collected at a 50 cm depth using acid pre-treated sampling bottles, frozen and sent to a South African National Accreditation System (SANAS) accredited laboratory (ISO/IEC 17025:2005) for chemical analyses.

In the laboratory, major ions, nutrients, and metals were analysed. Certified standard from De Bruyn spectroscopic solutions: 500MUL20-50 STD2 was used to determine analytical accuracy with recoveries being within 10% of the certified values (Jooste *et al.* 2015). Metal levels were measured by means of an inductively coupled plasma-mass spectrophotometer (ICP-MS). Selected metals include aluminium (Al), arsenic (As), antimony (Sb), barium (Ba), boron (B), cadmium (Cd), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), lithium (Li), manganese (Mn), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), lead (Pb), vanadium (V) and zinc (Zn). These metals were selected based on their highest importance to fisheries in practice and because they have been recommended by the United State Environmental Protection Agency (USEPA) for fish chemical contaminants monitoring (Svobodova *et al.* 1993; Heath *et al.* 2004). The water quality parameters were evaluated using DWAF (1996) target water quality range (TWQR) and CCME (2012) water quality guidelines as the main set of criterion for the evaluation process.

3.2.2 Sediment sampling and analysis

Concurrently with water sampling, sediment samples were collected using a Friedlinger mudgrab at the inflow, middle and dam wall (3 grab composite per site). Sediment samples were kept in acid treated sampling bottles and frozen (-20°). Frozen samples were later sent to a SANAS accredited laboratory (ISO/IEC

17025:2005) in Pretoria for metal analysis. In the lab, sediment samples were dried and digested in nitric and hydrochloric acid before metal analyses. Metal concentrations were analysed using inductively coupled plasma-mass spectrophotometer (ICP-MS) and measured in mg/kg dry weight. Certified standard from De Bruyn spectroscopic solutions: 500MUL20-50 STD2 was used to determine analytical accuracy with recoveries being within 10% of the certified values (Jooste *et al.* 2015). Metal concentrations in sediment were evaluated using threshold effect level (TEL) and probable effect concentrations (PEC) as stipulated by MacDonald *et al.* (2000). Threshold effect level is the concentration below which adverse effects are not expected to occur while PEC is the concentration above which adverse effects are expected to occur more often than not (MacDonald *et al.* 2000).

Figure 3.1 YSI meter used to measure system variables at Loskop and Flag Boshielo dams during 2014 surveys.

3.2.3 Data analysis

Shapiro-Wilk test was carried out to determine the normality of the data, whereas Levene's test was used to determine the homogeneity of variance. Depending on whether assumptions were satisfied, an independent t-test or Wilcoxon-Mann-Whitney U-test was used to evaluate the difference on sediment and water quality constituents between the two dams. Statistical analyses were performed using R-

3.1.1 (R Development Core Team 2014). The level of significance was set at 5% ($p<0.05$). To determine the site and season associations of water quality constituents and correlations, principal component analysis (PCA) was performed and plotted using R-3.1.1. Water quality constituents which were below detection limit where not included in PCA.

To evaluate differences in the multivariate metal concentrations in sediment between the two dams, the procedures described by Jooste *et al.* (2014) were followed. Non-metric multi-dimensional scaling (NMDS) plot was prepared to visualise the metal concentrations sediment from both Loskop and Flag Boshielo dams. A distance-based test of homogeneity of multivariate dispersion and a multiple analysis of variance (Anderson 2001a, b) were performed to determine whether metal concentrations in sediment exhibited significant difference between the two dams using the betadisper and adonis functions in VEGAN, hereafter DISPER and Multivariate Analysis of Variance (MANOVA).

3.3 Results

Concentrations or levels of water and sediment variables recorded during summer and winter were pooled, and presented in box and whisker plots in Figures 3.2 & 3.4. Means of the pooled water and sediment data were calculated and presented in Table 3.1 and Table 3.2, respectively.

3.3.1 System variables

The mean concentrations for non-toxic variables together with guidelines set for aquatic ecosystems are presented in Table 3.1. Loskop Dam has shown water temperature of 17.4°C during winter with 26.8°C being recorded during summer. The water temperature of 17.1°C and 25.8°C was recorded at Flag Boshielo Dam during winter and summer, respectively. No significant difference ($p>0.05$) was observed for water temperature between the two dams (Table 3.1). Associated with the water temperature is the dissolved oxygen which showed no significant difference ($p>0.05$) between Loskop and Flag Boshielo dams (Table 3.1). Both dams exhibited an alkaline pH throughout the study (Table 3.1), and no significant difference ($p>0.05$) was observed for the pH between the two dams. Total dissolved solids, electrical conductivity and water hardness have shown significantly higher levels at Flag Boshielo Dam as compared to Loskop Dam (Table 3.1) (Fig. 3.2). Loskop Dam exhibited slightly hard water with Flag Boshielo Dam showing moderately hard water. Moreover, a strong positive correlation was observed for total dissolved solids, electrical conductivity and water hardness (Fig. 3.3). No significant difference ($p>0.05$) was observed for salinity between the two dams (Table 3.1).

Table 3.1 Mean levels ± standard deviations of water constituents recorded at Loskop and Flag Boshielo dams during 2014 surveys. Units in mg/ℓ unless specified otherwise. TWQR = Target Water Quality Range for aquatic ecosystems according to DWAF (1996)* or CCME (2012)**.

Variables	Loskop Dam	Flag Boshielo Dam	Guidelines	P value
pH	8.17 - 10.06	9.39 - 9.89	6.5 - 9**	0.581
Temperature (°C)	20.10 ± 5.79	21.58 ± 4.21	-	0.564
DO	13.33 ± 1.81	12.89 ± 1.03	-	0.650
DO (%)	162.3 ± 31.18	164.38 ± 22.72	80% - 120%**	0.907
TDS	243.2 ± 24.93	330.2 ± 51.48	-	0.002
Conductivity (mS/m)	307.05 ± 73.33	505.03 ± 101.68	-	0.002
Salinity ‰	0.26 ± 0.07	0.36 ± 0.03	-	0.137
Water hardness	126.63 ± 11.80	165.34 ± 13.92	-	0.001
Ca	26.42 ± 2.38	32.10 ± 3.31	-	0.012
Mg	14.75 ± 1.41	20.68 ± 2.50	-	0.002
Na	23.01 ± 5.84	47.90 ± 9.24	-	0.001
K	5.62 ± 1.04	5.86 ± 0.24	-	0.640
Bicarbonate as $CaCo_3$	54.67 ± 6.90	84.67 ± 29.95	-	0.290
Carbonate as $CaCo_3$	6.67 ± 5.23	30.67 ± 12.47	-	0.097
Chloride	16.30 ± 3.13	41.6 ± 8.49	120**	0.039
SO_4	104.99 ± 17.68	101.33 ± 9.29	-	0.693
NH_4	0.28 ± 0.04	0.27 ± 0.05	-	0.928
NH_3	0.25 ± 0.01	0.25 ± 0.04	0.007*	0.968
NO_3	0.54 ± 0.19	0.14 ± 0.10	-	0.002
NO_2	0.01 ± 0.00	nd	-	-
Total nitrogen	1.14 ± 0.95	0.49 ± 0.29	-	0.116
Ba	0.05 ± 0.01	0.06 ± 0.01	-	0.006
Fe	0.02 ± 0.01	0.02 ± 0.01	0.3**	0.106
Li	0.01 ± 0.00	0.01 ± 0.00	-	0.076
Mn	0.02 ± 0.00	0.02 ± 0.00	0.18*	0.261
Sr	0.14 ± 0.003	0.18 ± 0.02	-	0.006
Zn	0,01 ± 0.00	nd	0.03**	-

nd=not detected

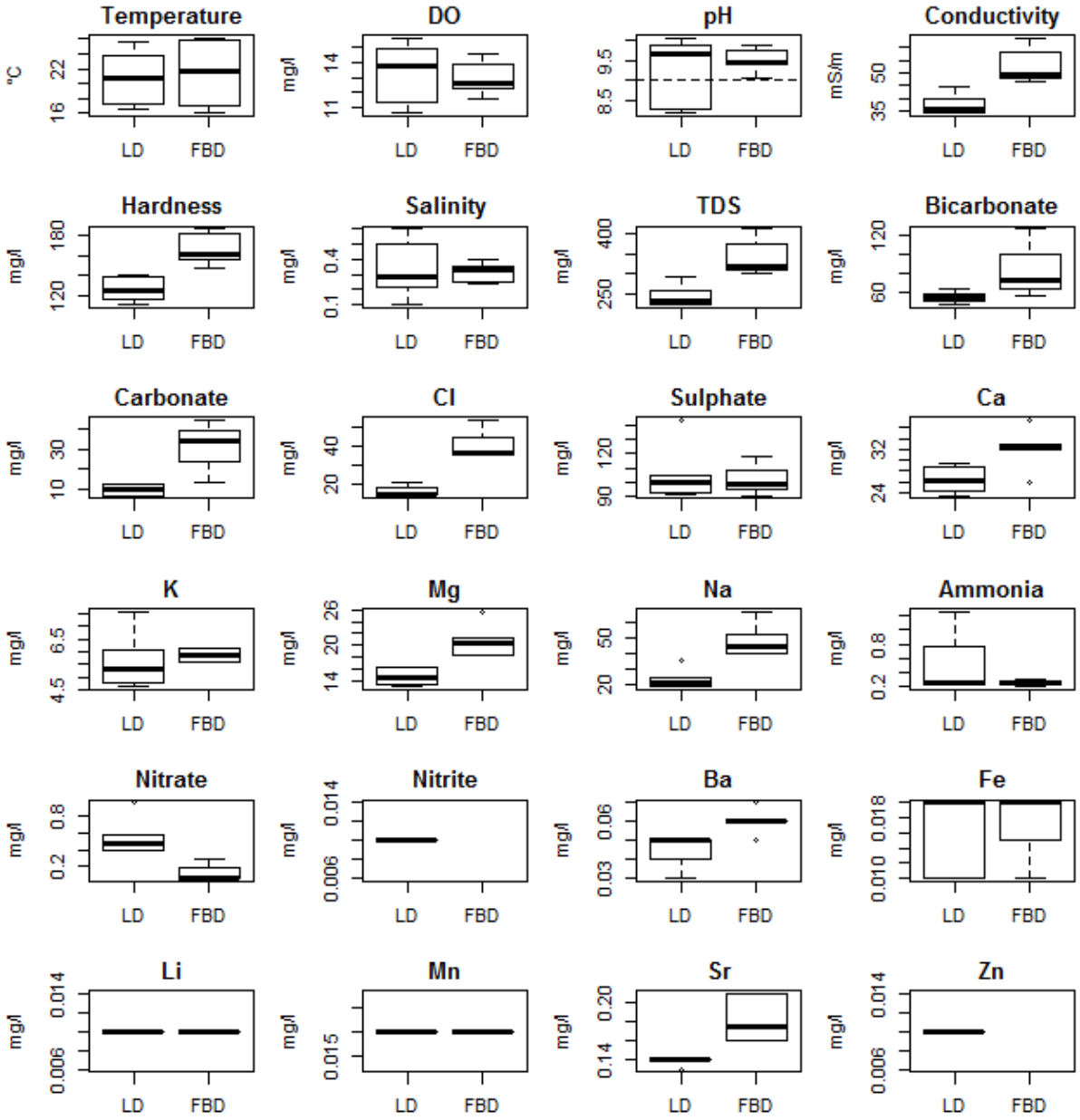

Figure 3.2 Levels of water constituents detected at Loskop Dam (LD) and Flag Boshielo Dam (FBD) during summer and winter in 2014. Ablines: guidelines where applicable.

3.3.2 Major ions

Four cations i.e. calcium, potassium, magnesium and sodium were measured in this study and the results are presented in Table 3.1. Calcium, magnesium and sodium have shown a significant difference ($p<0.05$) between Loskop and Flag Boshielo dams (Table 3.1) with strong positive correlations being observed for magnesium and sodium (Fig. 3.3). No significant difference was observed for potassium; however, the mean concentrations of 5.62 mg/ℓ and 5.86 mg/ℓ were recorded at Loskop and Flag Boshielo dams, respectively (Table 3.1).

Measured anions include bicarbonate, carbonate, chloride and sulphate. No significant difference ($p>0.05$) was observed for bicarbonate, carbonate and sulphate between the two dams, however, positive correlation was observed among them (Fig. 3.3). There was a significant difference ($p<0.05$) for chloride between Loskop and Flag Boshielo dams. The chloride mean concentration of 16.30 mg/ℓ was observed at Loskop Dam with 41.6 mg/ℓ being recorded at Flag Boshielo Dam (Table 3.1).

3.3.3 Nutrients

Ammonia and ammonium ion have shown no significance difference ($p<0.05$) between the two dams (Table 3.1). The ammonia mean concentration of 0.25 mg/ℓ was recorded at Loskop and Flag Boshielo dams (Table 3.1). Although nitrite concentration was below detection level at Flag Boshielo Dam, the concentration of 0.1 mg/ℓ was recorded at Loskop Dam (Table 3.1). Nitrate concentration has shown a significant difference ($p<0.05$) between Flag Boshielo and Loskop Dam, with latter showing higher concentration (Table 3.1). The overall total nitrogen was significantly higher at Loskop Dam than Flag Boshielo Dam. Phosphorus concentration was below detection level (<0.05) at both dams.

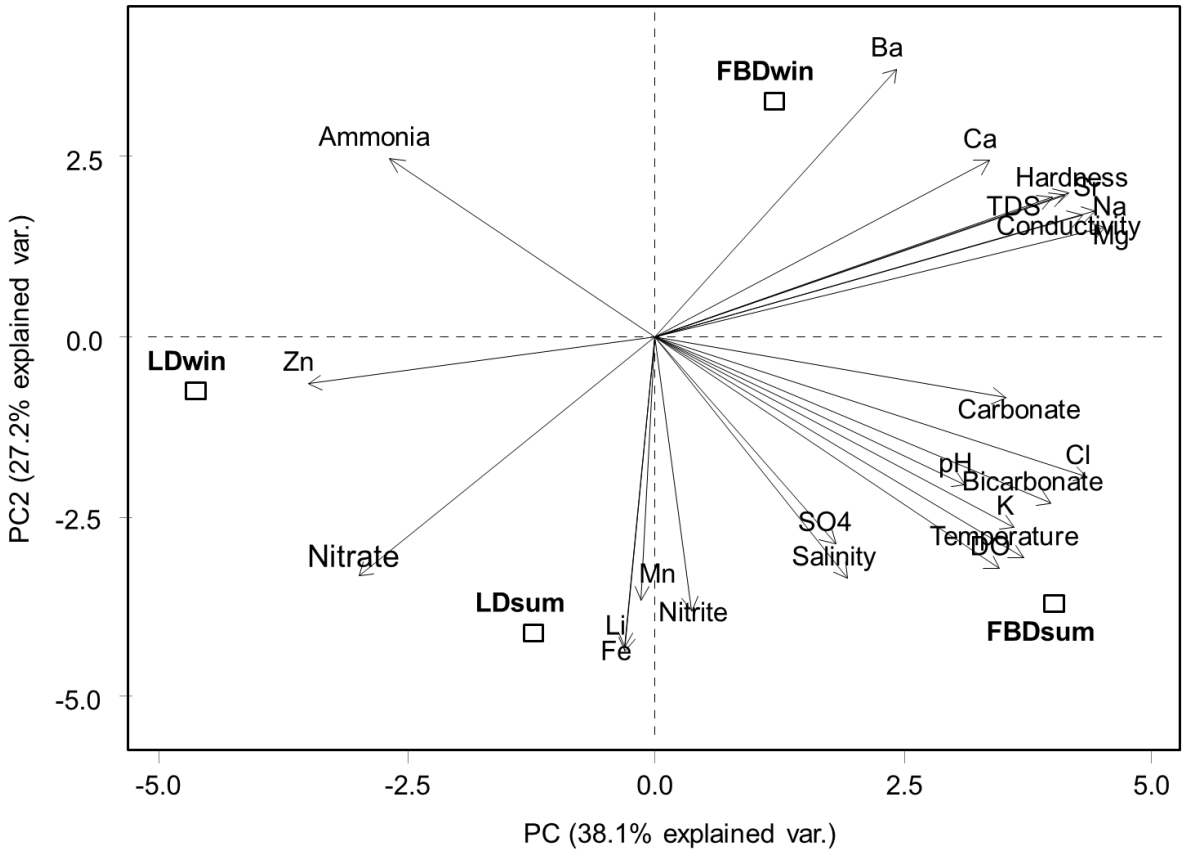

Figure 3.3 Principal components analysis summarising water quality constituents correlation, and seasons and sites association.

3.3.4 Metals

Although most selected metals were detected in sediment at both dams, only barium, iron, lithium, manganese and strontium were detected in the water column at Loskop and Flag Boshielo dams (Table 3.1 & Fig. 3.2). Zinc was below detection level at Flag Boshielo Dam (Table 3.1 & Fig. 3.2). A significant difference ($p<0.05$) was observed for barium and strontium concentration in the water between the two dams. The concentration of iron, lithium and manganese in the water have shown no significant difference ($p>0.05$) between Loskop and Flag Boshielo dams (Table 3.1).

Results for metal concentration ranges recorded in sediment at Loskop Dam and Flag Boshielo dams are presented in Figure 3.4, and mean concentrations together with guidelines are presented in Table 3.2. Although significant differences ($p<0.05$)

were observed for barium and strontium in the water column, none ($p>0.05$) was reported in sediment for all metals between the two dams (Table 3.2). Antimony and cadmium concentrations were below detection level in the water column and sediment at both dams.

Table 3.2 Mean metal concentrations ± standard deviation detected in sediment at Loskop and Flag Boshielo dams. Units in mg/kg dry weight. TEL = threshold effect level; PEL = probable effect level for aquatic ecosystems stipulated by MacDonald *et al.* (2000).

Metals	Loskop Dam	Flag Boshielo Dam	TEL	PEC	P values
Ag	nd	nd	-	-	-
Al	35266.67 ± 29418.74	26933.33 ± 19775.63	-	-	0.612
As	6.90 ± 1.43	10.43 ± 0.63	5.9	17	0.060
B	7.35 ± 1.75	8.35 ± 1.95	-	-	0.515
Ba	182.59 ± 108.24	102.42 ± 65.03	-	-	0.193
Cd	nd	nd	0.596	3.53	-
Co	30.30 ± 18.66	11.69 ± 4.76	-	-	0.156
Cr	84.35 ± 49.73	46.02 ± 34.16	37.3	90	0.189
Cu	27.20 ± 17.11	17.32 ± 12.92	35.7	197	0.343
Fe	32466.67 ± 24313.96	37533.33 ± 17887.67	-	-	0.716
Li	28.90 ± 14.98	14.28 ± 10.67	-	-	0.204
Mn	832.27 ± 805.41	697 ± 382.31	-	-	0.744
Ni	328.09 ± 238.12	346.11 ± 167.39	18	36	0.893
Pb	18.12 ± 12.44	16.33 ± 9.91	35	91.3	0.806
Sb	nd	nd	-	-	-
Se	13.15 ± 18.73	19.87 ± 0.66	-	-	0.179
Sn	nd	nd	-	-	-
Sr	27.79 ± 11.13	17.78 ± 9.41	-	-	0.247
V	61.47 ± 44.81	42.52 ± 23.75	-	-	0.429
Zn	129.6 ± 104.84	77.37 ± 38.07	123	315	0.390

nd = not detected

The NMDS plot exhibited no separation for metal concentrations in sediment between Loskop and Flag Boshielo dams (Fig. 3.5). The PERMIDISP analysis revealed significant differences ($p<0.05$) in the variability of metal concentrations between the two dams with greater variability being observed at Loskop Dam (centroid distance = 5.68) as compared to Flag Boshielo Dam (centroid distance =

3.46). MANOVA results (p=0.43) have shown no significant difference which confirm the no clear separation trend observed in the NMDS plot.

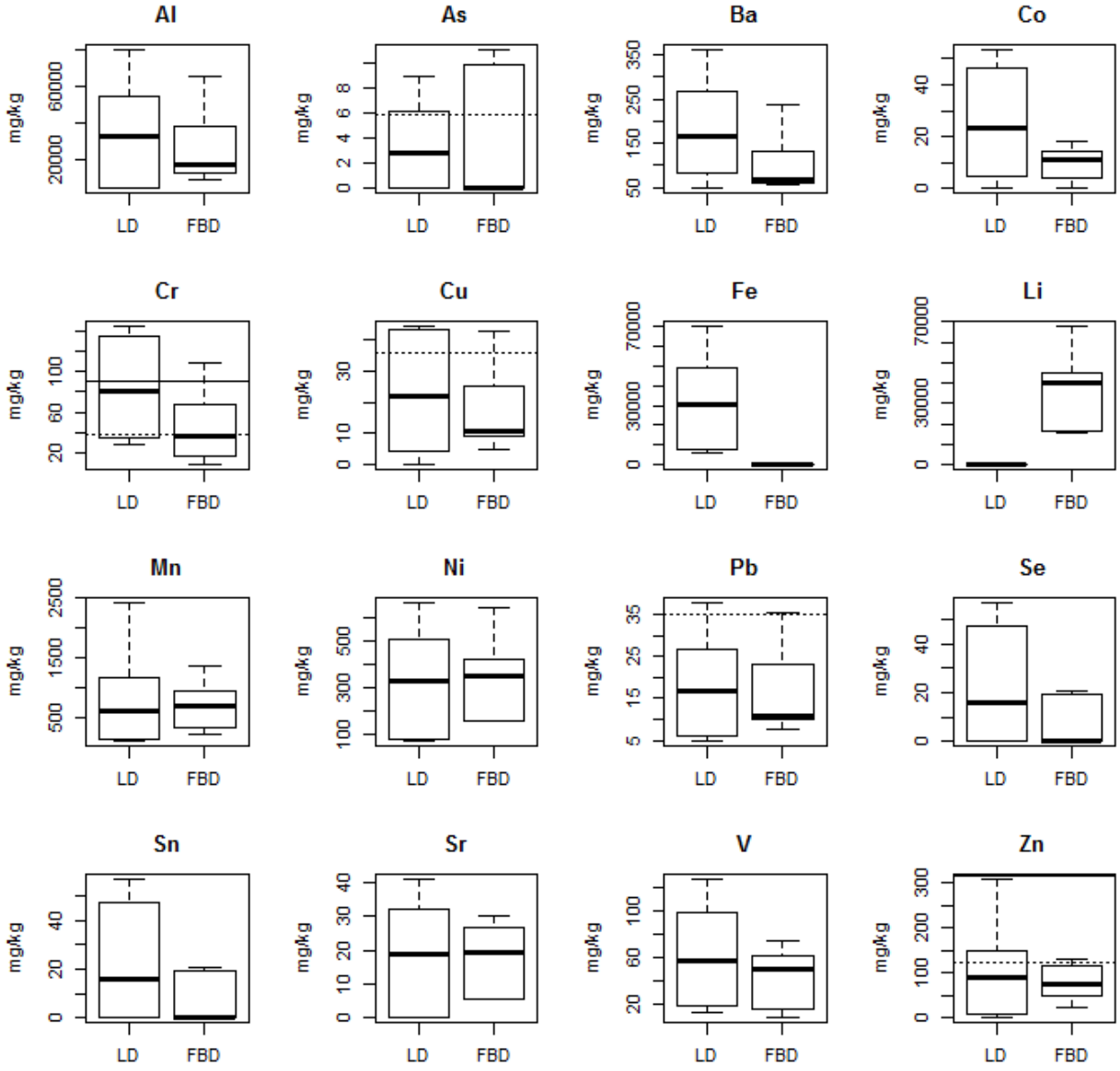

Figure 3.4 Box and whisker plots of metal concentrations recorded in sediment sampled from Loskop Dam (LD) and Flag Boshielo Dam (FBD) in 2014. Ablines: CCME (2012) threshold effect level.

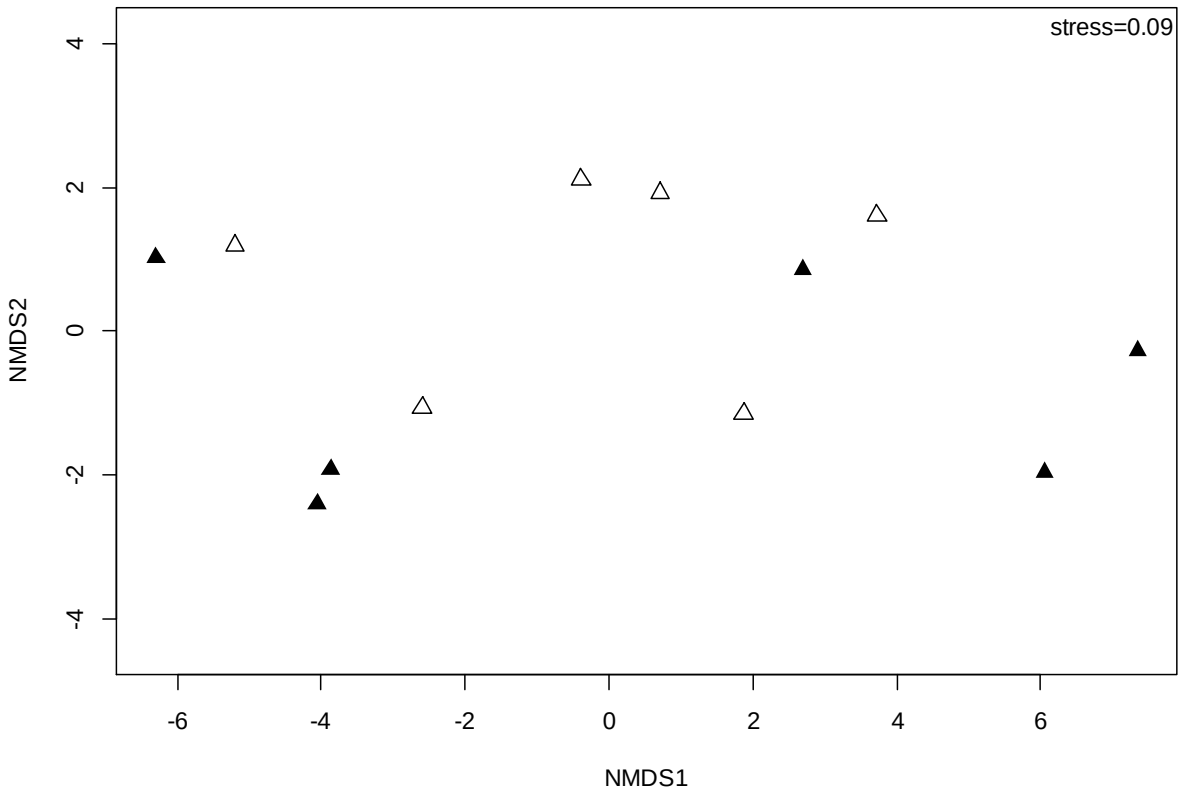

Figure 3.5 Non-metric multi-dimensional scaling plot for the metal concentration recorded in sediment at Loskop Dam (▲) and Flag Boshielo Dam (∆) during 2014 surveys.

3.4 Discussion

3.4.1 System variables

3.4.1.1 Temperature and dissolved oxygen

Dissolved oxygen is essential to all forms of aquatic life (Chapman 1996). Dissolved oxygen may vary with seasons or even over 24 hour periods, in relation to temperature, salinity and biological activity. Moreover, water turbulence and atmospheric pressure may also induce variation on the level of dissolved oxygen. The solubility of oxygen decreases as temperature and salinity increase (Dallas & Day 2004), however, this hypothesis was not supported in the present study. Factors that may explain the reported trend of dissolved oxygen, temperature and salinity in the present study may include respiration by aquatic biotas, photosynthesis by plants and aerobic decomposition of organic matter by micro-organisms (Svobodova *et al.* 1993; Dallas & Day 2004).

The water at both dams seemed to be well oxygenated with the mean percentage saturation of 162.3% and 164.38 mg/ℓ being recorded at Loskop and Flag Boshielo dams, respectively. DWAF (1996) reported that 80% to 120% of saturation may protect all life stages of most southern African aquatic biota, endemic to, or adapted to aerobic warm water habitats. Prolonged exposure to sublethal, low oxygen concentrations may lead to changes in behaviour, blood chemistry, growth rate and food intake (Dallas & Day 2004). The dissolved oxygen concentration below 5 mg/ℓ may adversely affect the functioning and survival of biological communities and below 2 mg/ℓ may lead to the death of most fish (Chapman 1996). Decreased oxygen concentration is generally associated with increased temperature which may have effect on the water chemistry by increasing the toxicity of other constituents such ammonia, cadmium, cyanide and zinc (Dallas & Day 2004).

3.4.1.2 Water pH

The pH is largely determined by the concentration of hydrogen ions (H^+), and alkalinity by the concentrations of hydroxyl (OH^-), bicarbonate (HCO_3^-) and carbonate

(CO_3^{2-}) ions in water. It is an important variable in water quality assessment as it influences many biological and chemical processes within a water body (Chapman 1996). In an aquatic ecosystem, pH determines the chemical species, and thus availability and toxicity of metals in water (Dallas & Day 2004). In areas affected by acid mine drainage, the pH tend to decrease to less than 3 (Bartram & Ballance 1996). However, acid mine drainage is one of the primary drivers of pollution in the upper catchment of the Olifants River system but alkaline pH was recorded at both dams with Loskop Dam showing the maximum pH of 10.06. The pH at Loskop Dam was within the CCME (2012) guideline for aquatic ecosystem during winter but exceeded during summer.

At Flag Boshielo Dam, the pH was within the CCME (2012) guideline for aquatic ecosystem during both seasons. DWAF (1996) has not stipulated specific guideline for aquatic ecosystem, however, it emphasises that the pH should not be allowed to vary from the range of the background pH values for a specific site and time of day by >0.5 of a pH unit or by >5%. Relatively small changes of pH are seldom lethal, although sublethal effects such as reduced growth rates and reduced fecundity may result from the physiological stress placed on the organism by increased energy requirements in acid or alkaline waters (Palmer *et al.* 2004).

pH may be influenced by the geology of the dams from which dissolution takes place with the participation of carbon dioxide (Nikanorov & Brazhnikova 2009). Moreover, alkaline pH may occur in eutrophic water bodies where the blue-green algae, green algae and higher aquatic plants take up considerable amounts of CO_2 during the day for intensive photosynthetic activity. Eutrophication affects the buffering capacity of the water and the pH can rise to over 9.0 or even higher if bicarbonate is adsorbed from waters of medium alkalinity (Svobodova *et al.* 1993). The total nitrogen and phosphorus levels have shown that Flag Boshielo Dam was oligotrophic with Loskop Dam being mesotrophic. It is therefore reasonable to conclude that geological characteristics may have had an influence on the pH of both Loskop and Flag Boshielo dams.

3.4.1.3 Total dissolved solids, conductivity and salinity

Materials dissolved in water are commonly measured as total dissolved solids, as conductivity or as salinity. Total dissolved solids represent the total amount of dissolved material, organic and inorganic, ionised and un-ionised, in a water sample. Conductivity is a measure of the ability of water to conduct an electrical current. Salinity refers to the saltiness of the water (Dallas & Day 2004; Palmer *et al.* 2004). Total dissolved solids, conductivity and salinity usually correlate closely in an aquatic ecosystem (Dallas & Day 2004). In the present study, the three constituents have shown a correlation with strong positive correlation being observed for total dissolved solids and conductivity.

Total dissolved solids may be influenced by the geology of the area, however, anthropogenic activities such as industrial effluents, irrigation and water re-use may increase the level of total dissolved solids (Dallas & Day 2004). It is therefore, probable that the agricultural and mining activities dominating the upper and middle Olifants River catchment may be the driver for the increased total dissolved solids and conductivity. There is no guideline for total dissolved solids set for aquatic ecosystem, however, DWAF (1996a) emphasised that the level should not be changed by >15% from the normal cycles of the water body under natural conditions at any time of the year.

3.4.1.4 Total water hardness

Water hardness was originally described as the soap-destroying power of water, caused by the presence of Calcium and magnesium salts and measured by titration against a standard soap solution (DWAF 1996b). Total water hardness is now defined the sum of the calcium and magnesium concentrations expressed as milligrams per litre (mg/ℓ) of calcium carbonate ($CaCO_3$) (Chapman 1996). Slightly and moderately hard waters were observed at Loskop and Flag Boshielo dams, respectively. The effects of water hardness are mostly felt in domestic use and there is no guidelines stipulated by DWAF (1996) or CCME (2012) for aquatic ecosystem. DWAF (1996b) reported that water hardness should be limited to between 50-100

mg/ℓ as $CaCO_3$ where possible. Water hardness is known to decrease the toxicity of metals by forming insoluble carbonates or by providing precipitating calcium carbonate that acts as a surface for adsorption of metal ions (Dallas & Day 2004).

3.4.2 Major ions

3.4.2.1 Calcium

Calcium dissolves out of almost all rocks and is present in all waters. Low calcium concentration may be recorded in waters associated granite or siliceous sand. Elevated calcium concentrations may be recorded in waters associated with limestone and gypsum. Moreover, industrial activities, water and waste water treatment processes may increase calcium concentration in surface waters. Together with magnesium, calcium contribute to the total hardness of the water (Bartram & Ballance 1996; Chapman 1996). Elevated calcium concentrations were observed at both dams with Flag Boshielo Dam showing significantly higher concentration throughout the study. In an aquatic ecosystem, very little is known about the effect of calcium, however, Dallas & Day (2004) reported that water with low calcium concentration may be unable to support molluscs and crustaceans. There is no guidelines set by DWAF (1996a) or CCME (2012) for aquatic ecosystems, however, Jooste *et al.* (2005) emphasised that the concentration up to 250 mg/ℓ is acceptable for all users.

3.4.2.2 Magnesium

Magnesium is regarded as one of the common constituents of natural waters (Bartram & Ballance 1996). The main sources of magnesium include rocks containing ferromagnesium minerals and carbonate rocks. Although many industrial processes use magnesium, the contribution is relatively low to the total magnesium is surface waters (Chapman 1996). Significant magnesium concentrations were observed at Loskop and Flag Boshielo dams. Magnesium is involved in several physiological processes in living organisms, hence essential element (Dallas & Day 2004). In freshwater ecosystems, natural calcium concentration may range from 1 to

>100 mg/ℓ depending on the rock types within the catchment (Chapman 1996). Therefore, magnesium concentration at Loskop and Flag Boshielo dams was in an acceptable level.

3.4.2.2 Sodium and potassium

Sodium and potassium are the major cations involved in ionic, osmotic and water balance in all organisms. Sodium is very common in natural waters. Although potassium occurs in lower concentrations as compared to sodium, it sometimes acts as nutrients for plant growth (Dallas & Day 2004). Sewage, industrial effluents agricultural activities may increase the concentration of sodium and potassium in surface waters (Chapman 1996). The present study supported the hypothesis that sodium concentration is always higher than potassium in surface waters. Sodium may become a problem in drinking water (Bartram & Ballance 1996); however, the concentration was within the world health organisation (WHO 2006) guideline for drinking water at both dams. As for potassium, natural waters are expected to exhibit the concentration of <10 mg/ℓ (Chapman 1996). Both dams have therefore, not deviated from the expected concentrations for natural waters.

3.4.2.3 Chloride

In surface waters, chlorine occurs mostly as chloride ions. Natural sources of chloride may be atmospheric deposition of oceanic aerosols and weathering of some sedimentary rocks (mostly rock salt deposits). Anthropogenic sources may include industrial and sewage effluents, stream inflow, and agricultural and road run-off (Chapman 1996; Diamantino *et al.* 2001). The chloride ions commonly occur as salt i.e. NaCl, $MgCl_2$, KCl, $AlCl_2$ etc. and is highly soluble in water (Diamantino *et al.* 2001). Elevated concentration of chloride containing compounds may be to toxic to freshwater organisms (Wepener *et al.* 2000). Although the chloride concentration was significantly higher at Flag Boshielo Dam, it was within the CCME (2012) guideline set for aquatic ecosystems at both Loskop and Flag Boshielo dams. The middle Olifants River catchment is dominated by extensive irrigation practices

(Claassen *et al.* 2005) which may be linked to the increased chloride concentration at Flag Boshielo Dam.

3.4.2.4 Carbonate and bicarbonate

Bicarbonate and carbonate are the forms of carbon dioxide that dissolves in water, and may influence the hardness and alkalinity of the water. Carbon dioxide sources may include atmosphere and biological respiration. However, geological properties may contribute to the presence of bicarbonates and carbonate in surface waters (Chapman 1996; DWAF 1996). In aquatic ecosystems, carbon dioxide dissolve in water to form carbonic acid which readily dissociates to bicarbonate and carbonate ions depending on pH (Castro *et al.* 2004). Bicarbonate is dominant at pH range of 6.4 to 8.6 whereas carbonate becomes dominant at pH of >10.3 (DWAF 1996; Dallas & Day 2004). Coinciding with the reported bicarbonate-carbonate proportion, the present study has shown relatively high bicarbonate concentration at a pH range of 8.17 to 10.06 throughout the study. Together with carbon dioxide, bicarbonate and carbonate ions form the major buffering system in most natural surface waters. No toxicity has been associated with carbonate and bicarbonate in aquatic ecosystems (Dallas & Day 2004).

3.4.2.5 Sulphate

Sulphate is an abundant ion in the earth's crust and its concentration in water can range from a few milligrams to several thousand milligrams per litre (Bartram & Ballance 1996). Elevated sulphate concentrations are indicative of pollution related to acid mine drainage (De Villiers & Mkwelo 2009). In the present study, mean sulphate concentrations of >100 mg/ℓ were recorded at Loskop and Flag Boshielo dams. However, the pH was alkaline throughout the study. The dissolved sulphate derives from the oxidation of metal sulphides such as pyrite, abundant in, for example, coal-rich lithology and precious metal-rich deposits (Anderson *et al.* 2000; De Villiers & Mkwelo 2009). Industrial and mining discharges, and atmospheric precipitation can also add significant amounts of sulphate to surface waters (Chapman 1996; Oberholster *et al.* 2010). There is no sulphate guideline stipulated by CCME (2012)

and DWAF (1996) for aquatic ecosystems, however, elevated sulphate concentration may form sulphuric acid, which is a strong acid that reduces pH and can have devastating effects on aquatic biota (Dallas & Day 2004).

3.4.3 Nutrients

Nutrients are elements required for plant growth and are generally not toxic (exceptions include nitrite and ammonia) but excess level result in eutrophication which may have a significant impact on the structure and functioning of aquatic ecosystem. The major nutrients that contribute to eutrophication in aquatic ecosystem are phosphorus as phosphate ions (PO_4^{3-}) and nitrogen as nitrate, nitrite and ammonium ions and inorganic phosphorus and sulphate (DWAF 1996; Dallas & Day 2004; Palmer *et al.* 2004).

3.4.3.1 Nitrogen

Inorganic nitrogen occurs abundantly in nature and is an essential constituent of proteins, which include the enzymes that catalyse all biochemical processes, and is therefore a major component of all living organisms (Dallas & Day 2004). In South Africa, inorganic nitrogen concentrations in natural aerobic surface waters are usually below 0.50 mg/ℓ but may increase to above 5-10 mg/ℓ in highly enriched waters. Anthropogenic sources of nitrogen in aquatic ecosystem may be of the point-source type which include sewage treatment works and industries, or nonpoint-source which include agricultural runoff, urban runoff, atmospheric deposition (Davies & Day 1998; Dallas & Day 2004). Moreover, agricultural activities such as land clearing and fertilizer application are considered significant contributors to eutrophication of aquatic ecosystems (Palmer *et al.* 2004).

Inorganic nitrogen in aquatic environment may be present in many forms including ammonia, ammonium ion, nitrite and nitrate (Dallas & Day 2004). These nitrogen forms are readily oxidised or reduced both chemically and biochemically by bacteria. Therefore, concentrations of ammonia, ammonium ion, nitrite and nitrates in surface waters are usually low because of their instability. In surface waters the ratio of ammonia and ammonium depends on the pH and temperature of the water

(Svobodova *et al.* 1993). 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 (DWAF1996a). However, the present study has shown ammonium ion concentration being higher than un-ionised ammonia in pH values of >8 at both dams. Ammonium ion does contribute to eutrophication, however, it has little or no toxicity to aquatic biota (Dallas & Day 2004).

Although ammonia concentration was lower than that of ammonium ion, it has exceeded the DWAF (1996a) guideline set for aquatic ecosystem at both dams. Furthermore, change in water temperature and pH might increase the ammonia concentration by reducing the ammonium ions. Other factors that may affect the availability and toxicity of ammonia include dissolved oxygen, carbon dioxide and total dissolved solids, and the presence of other toxicants such as metal ions (Dallas & Day 2004; Palmer *et al.* 2004).

Inorganic nitrogen may also be present as nitrite which is the intermediate in the inter-conversion between ammonia and nitrate (Dallas & Day 2004). The concentration of nitrite in freshwaters is usually very low, rarely above 1 mg/ℓ because of its instability. Nitrite is readily oxidized to nitrate or reduced to ammonia, both chemically and biochemically by bacteria (Svobodova *et al.* 1993; Chapman 1996). In the present study, nitrite concentration was lower as compared to other forms of nitrogen and below detection level at Flag Boshielo Dam. In aquatic ecosystems, nitrite can be closely associated with ammonia concentrations. In normal aerobic conditions, ammonia is oxidised to nitrite and then to nitrate by two separate bacterial actions. If the second stage of oxidation is inhibited or the first stage of induced by bactericidal chemicals in the water, nitrite concentrations increase (Svobodova *et al.* 1993). Therefore, bacterial activity as the driver for low concentration of nitrite at Flag Boshielo Dam may not be dismissed.

The nitrite concentration of 0.1 mg/ℓ was recorded at Loskop Dam. There is no nitrite guideline set for aquatic ecosystem. Svobodova *et al.* (1993) reported that the toxicity of nitrite in fish is incompletely known. Toxicity of nitrite may be affected by water

chemistry, particularly by chloride concentration. There is an inverse linear relationship between nitrite toxicity and chloride concentration (Svobodova *et al.* 1993; Dallas & Day 2004).

Nitrates are the final product of the aerobic decomposition of organic nitrogen compounds and are the common form of combined nitrogen found in natural waters (Chapman 1996; Dallas & Day 2004). Sources of nitrate include municipal and industrial wastewaters, and agricultural runoff (Chapman 1996; DWAF 1996). In spite of their many sources, nitrates are seldom abundant in natural surface waters (normally <0.1 mg/ℓ), because photosynthetic action is constantly converting them to organic nitrogen in plant cells (Dallas & Day 2004). In the current study, a significantly high concentration was reported at Loskop Dam. There is no exact guideline for nitrate set for aquatic ecosystems, however, DWAF (1996a) designed one to ensure that the trophic status of water bodies does not change in a negative direction e.g. from oligotrophic to eutrophic status. Nitrate is generally not toxic but elevated concentrations may be toxic to very young infants because nitrate binds with foetal haemoglobin to form a non-functional molecule, methaemoglobin (Dallas & Day 2004).

The total nitrogen categorised Flag Boshielo Dam as oligotrophic with Loskop Dam showing mesotrophic conditions. Other recent studies have reported eutrophic to hypertrophic conditions at Loskop Dam (Oberholster *et al.* 2010; Dabrowski *et al.* 2013). Although Flag Boshielo Dam is located about 80 km downstream of Loskop Dam, the total nitrogen concentration classified it as oligotrophic. The trophic status of the two dams reaffirms that Loskop Dam act as a repository for effluents from industries and mines operating in the upper Olifants River catchment. Coinciding with the current study, Madanire-Moyo *et al.* (2012) reported oligotrophic condition at Flag Boshielo Dam.

3.4.3.2 Phosphorus as orthophosphate

Phosphorus is an essential nutrient for living organisms and exists in water bodies as both dissolved and particulate species (Chapman 1996). In natural waters and in

wastewaters, phosphorus occurs mostly as dissolved orthophosphates and polyphosphates, and organically bound phosphates. Phosphorus is seldom found in high concentration (<0.01) in natural waters since it is used by plants and sequestered in cells. Elevated concentrations are likely to occur in waters receiving sewage and leaching or runoff from agricultural practices (Dallas & Day 2004). In the present study, phosphorus was below detection limit (<0.05) at both dams. Oberholster *et al.* (2010) recorded phosphorus concentration ranging from 0.129 mg/ℓ to 0.711 mg/ℓ at Loskop Dam. Furthermore, Dabrowski *et al.* (2013) recorded orthophosphate concentrations ranging from 0.0021 mg/ℓ to 0.185 mg/ℓ at Loskop Dam with Dabrowski *et al.* (2014) recording dissolved inorganic phosphorus ranging from 0.005 mg/ℓ to 0.084 mg/ℓ at Flag Boshielo Dam.

Phosphate is extremely reactive under oxidising conditions and it interacts with many cations (e.g. Ca) to form relatively insoluble compounds that precipitate and adsorb to organic compounds e.g. humics and particulate materials e.g. clay. Settlement of particulate materials results in removal of phosphorus from the water column to the sediments. Therefore, during periods of low discharge, bottom sediment act as a sink for phosphorus (DWAF 1996). Recent studies have recorded notable dissolved inorganic phosphorus or orthophosphate concentrations at both dams (Oberholster *et al.* 2010; Oberhoster *et al.* 2012; Dabrowski *et al.* 2013; Dabrowski *et al.* 2014), therefore, phosphorus precipitation to the bottom sediment may be the explanation for the low concentration in the water column at Loskop and Flag Boshielo dams.

3.4.4 Metals

3.4.4.1 Aluminium

Aluminium is one of the most toxic trace metals and is not an essential nutrient in any organism. The solubility of aluminium depends on pH and its bioavailabity and toxicity depends on the chemical species involved (DWAF 1996; Dallas & Day 2004; Atkinson *et al.* 2007). Under acidic condition, aluminium occurs as soluble, bioavailable and toxic hexahydrate species ($Al^{6+}.H_2O$ or aquo-Al) (Dallas & Day 2004). Aluminium precipitates as hydroxide, which flocculate in water under alkaline pH (Svobodova *et al.* 1993). In most cases, precipitated aluminium tends to sink down and adsorb to sediment. When water quality changes aluminium may be reintroduced back into the surface water column in a bioavailable form (Coetzee *et al.* 2002). In the present study, the aluminium concentration in water was very low and thus undetectable. However, elevated concentrations were recorded in sediment at Loskop and Flag Boshielo dams.

Oberholster *et al.* (2010) reported high concentration of aluminium in slightly acidic water which has decreased with increasing pH. The alkaline pH reported in the present study may be the explanation for low concentration in the water column and high in sediment. There is no TEL or PEC set by MacDonald *et al.* (2000) for aquatic ecosystem, however, elevated concentration of aluminium may affect the success of aquatic organisms and communities in acidic environment (Atkinson *et al.* 2007). Although aluminium concentration was below detection level, it may not be dismissed as one of the potential stressors at both dams since it can still be remobilised from the sediment to the water column if the pH drops to less than 6.

3.4.4.2 Arsenic

Arsenic is a carcinogenic metalloid toxic to marine and freshwater aquatic life (DWAF 1996). It enters aquatic ecosystem through combination of natural processes such as weathering reactions, biological activity, and volcanic emissions, as well as a result of anthropogenic activities. Excessive use of arsenic-based pesticides and

indiscriminate disposal of domestic sewage and industrial wastes, as well as mining activities, have resulted in widespread arsenic contamination of soils and waterways (Mahimairaja *et al.* 2005). The toxicity of arsenic depends on numerous interacting factors such pH and redox potential (DWAF 1996; Magellan *et al.* 2014). The two most common forms are arsenic (III) and arsenic (V), both of which form stable compounds with carbon, resulting in numerous organo-arsenical compounds (DWAF 1996). Arsenic adsorbs readily to sediment and combines with dissolved organic carbon.

In the present study, arsenic was not detected in the water column. However, concentrations exceeding threshold effect level were recorded in sediment during summer and winter at Flag Boshielo Dam and during winter at Loskop Dam. Mahimairaja *et al.* (2005) reported that the significant proportion of arsenic in aquatic ecosystem is derived from the sediments. The mobility of arsenic in lake sediment and its release to the water column is related partly to seasonal changes. In areas that become stratified in summer, arsenic released from sediments accumulates in the hypolimnion until turnover, when it is mixed with epilimnetic waters. The mixing may result in a 10% to 20% increase in arsenic concentration in the water column (Grobler *et al.* 1987). Although arsenic concentration was below detection limit in the water column, its effect on the aquatic biota of the two dams may not be neglected since sediment may serve as the secondary source when the pH drops or during turnover.

3.4.4.3 Barium

Barium is a dense alkaline earth metal that occurs in nature as a divalent cation in combination with other elements (Choudhury & Cary 2001). It is a naturally occurring component of minerals that are found in small but widely distributed amounts in the earth's crust, especially in igneous rocks, sandstone, shale and coal. Naturally barium is stable in the +2 valence state and is found primarily in the form of inorganic complexes. However, factors such pH, redox potential, cation exchange capacity, and the presence of sulphate, carbonate, and metal oxides (e.g. oxides of aluminium,

manganese, silicon, and titanium) will affect the partitioning of barium and its compounds in the environment (ATSDR 2007c). Anthropogenic sources of barium are primarily industrial. Burning of coal, fossil fuels and waste releases barium into the atmosphere (Choudhury & Cary 2001).

Notable concentration of barium was reported in the water column at Loskop and Flag Boshielo dams. There is guideline for barium concentration in the water column set by DWAF (1996) or CCME (2012) for aquatic ecosystem. The biogeochemical cycle of barium include wet and dry deposition to land and surface water, adsorption to soil and sediment particulates, and biomagnification in terrestrial and aquatic food chain. Barium in sediments is found largely in the form of barium sulphate (barite) which is seldom toxic (Choudhury & Cary 2001; ATSDR 2007c). Therefore, the increased barium concentration in sediment at both Loskop and Flag Boshielo dams may not pose any deleterious threat to the aquatic biota. However, change in pH and other physico-chemical properties may remobilise into the water column. Barium concentration of 5.8 mg/ℓ in the water column may impair reproduction and growth of aquatic biota (Choudhury & Cary 2001).

3.4.4.4 Chromium

Chromium is a relatively scarce metal and the occurrence and amounts thereof in aquatic ecosystems are usually very low (DWAF 1996). In the aquatic environment chromium occur as chromium (III) and chromium (VI) as water soluble complex anions. Chromium toxicity to aquatic biota is significantly influenced by abiotic factors such as hardness, temperature, pH, and salinity of water (Grobler *et al.* 1987; DWAF 1996). Chromium toxicity increases with the increase in temperature but decrease with an increase of salinity and sulphate concentration. In an aquatic ecosystem, chromium readily adsorb to sediment but when the physico-chemical properties becomes favourable i.e. acidic pH, sediment becomes a source by supplying chromium to the interstitial water (Popa 2008).

Chromium concentration was below detection limit in the water at Loskop and Flag Boshielo dams. However, chromium concentrations above TEL were recorded in

sediment at both dams. The hazards associated with exposure to chromium are dependent on its oxidation state, ranging from the low toxicity of the metal form to the high toxicity of the hexavalent form (Liu *et al.* 2014). Under acidic conditions, the toxicity of chromium tends to increase since the concentration in water will increase with sediment serving as a source (Grobler *et al.* 1987).

3.4.4.5 Cobalt

Cobalt is an essential micronutrient required for the formation of vitamin B_{12} and for its function in enzymatic processes. It is a naturally occurring metal but its concentration in aquatic ecosystems may be elevated by anthropogenic activities such as burning of fossil fuels, sewage sludge, phosphate fertilizers, mining and smelting of cobalt-containing ores and industrial processes that use cobalt compounds (Diamond *et al.* 1992; CCME 2013). Cobalt binds strongly with sediment and suspended particulate matter, and it has been reported that cobalt will remain for the most part in bottom sediments after entering aquatic ecosystem (Dave & Nilsson 2004; CCME 2013).

In the present study, cobalt concentration was below detection level in the water column at Loskop and Flag Boshielo dams. However, sediment exhibited significant cobalt concentration at both dams. Jooste *et al.* (2015) reported significant cobalt concentrations in sediment at Flag Boshielo Dam. Coinciding with these results, Dave & Nilsson (2004) reported significantly low cobalt concentration in the water column and substantially higher in bottom sediment at Lake Molnbyggen in Sweden. Cobalt is in most cases associated with nickel, silver, lead, copper and iron ores, and its solubility is strongly dependent on pH (Nagpal 2004). The alkaline pH at both dams may be the explanation for elevated cobalt concentrations in the bottom sediment.

3.4.4.6 Copper

Copper is a common metal in the rocks and minerals of the earth's crust and it enters the aquatic environment naturally as a result of weathering processes or from

dissolution of copper minerals and native copper (DWAF 1996). Copper occurs in three oxidation states, as metallic copper(0), cuprous copper(I) and cupric copper(II). The major processes that control the chemical speciation of copper in freshwater systems include precipitation, formation of complexes with inorganic or organic ligands and adsorption by particulate material. These processes can be affected by the concentration of copper, pH, alkalinity and concentration of copper-binding sites associated with dissolved organic material and suspended particulates (McKnight *et al.* 1983). Copper toxicity depends on the solubility and chemical species of the copper present in water. Free cupric copper ions (Cu^{2+}) are considered most toxic, whereas complex forms are the least toxic to aquatic organisms (DWAF 1996c). The mobility and solubility of copper is high in water with an acidic pH and it precipitates in alkaline water and is thus not toxic. Furthermore, copper toxicity may be reduced as water hardness increases and in the presence of zinc, molybdenum and sulphate (Dallas & Day 2004).

According to DWAF (1996a), copper is correlated with water hardness as follows:

Water hardness as $CaCo_3$ (mg/ℓ)	< 60 (soft)	60 – 119 (medium)	120 – 180 (hard)	> 180 (very hard)
TWQR	0.0003	0.0008	0.0012	0.0014

Loskop Dam exhibited medium to hard water with Flag Boshielo Dam showing hard to very hard water. The copper concentration in water was below detection limit at both dams. However, elevated copper concentrations were observed in sediment at Loskop and Flag Boshielo dams. Concentrations of copper in sediment were in most cases within the TEL set by MacDonald *et al.* (2000) for aquatic ecosystem at both dams. In alkaline waters, copper forms hydroxides of low solubility, and in water with a high bicarbonate/carbonate concentration copper precipitates as poorly soluble or insoluble cupric carbonate (Svobodova *et al.* 1993). Recent studies have reported alkaline pH and significant copper concentration in the water column at Loskop and Flag Boshielo dams (Oberholster *et al.* 2010; Lebepe *et al.* 2016). Therefore, physico-chemical properties as the explanation for low copper concentration in the water column and high concentration in sediment may not be dismisses.

3.4.4.7 Iron

Iron is an essential micronutrient in all organisms, forming part of haeme-containing respiratory pigments (e.g. haemoglobin), catalases, cytochromes and peroxidases (Dallas & Day 2004; Kraemer 2004). In aquatic ecosystem, iron occurs in ferrous state II (soluble compounds) or ferric state III (mostly insoluble compounds). The ratio of these two forms of iron depends on the oxygen concentration in the water, pH and on other chemical properties of the water (Svobodova *et al.* 1993). The minimum solubility of iron is observed in the neutral to alkaline pH range (Kraemer 2004). Iron may be toxic in poorly oxygenated waters with a low pH where it is present mainly in soluble form (Svobodova *et al.* 1993).

Although sediment exhibited extremely high iron concentration at Loskop and Flag Boshielo dams, concentrations in the water column were within the CCME (2012) limit for aquatic ecosystem at both dams. Iron concentrations reported in the water column in this study was comparable to those reported in other studies conducted at Loskop and Flag Boshielo dams (Oberholster *et al.* 2010; Oberhoster *et al.* 2012; Dabrowski *et al.* 2013; Dabrowski *et al.* 2014). Iron precipitates and adsorb to sediment under alkaline condition remobilise back into the water column under acidic conditions (Bartram & Ballance 1996; Fonseca *et al.* 2011). Oberhoster *et al.* (2012) reported acidic pH in Blesbok stream which is located upstream of Loskop Dam, however, the stream has not had an impact on the pH at Loskop Dam.

3.4.4.8 Lead

Lead is a common and toxic trace metal which has the capability of being accumulated in living tissues, and other vertebrates (Dallas & Day 2004). It is defined by the United States Environmental Protection Agency (USEPA) as potentially hazardous to most forms of life (DWAF 1996). Sources of lead include industrial and municipal wastewaters discharge, mining, combustion of fossil fuel etc. Lead toxicity to aquatic biota is significantly influenced by the pH and water hardness (Svobodova *et al.* 1993; DWAF 1996).

Lead guideline for aquatic ecosystem was set with respect to water hardness and the table below shows how these two constituents correlate:

Water hardness as $CaCo_3$ (mg/ℓ)	< 60 (soft)	60 – 119 (medium)	120 – 180 (hard)	> 180 (very hard)
TWQR	0.0002	0.0005	0.001	0.0012

Lead enters the aquatic ecosystem and largely accumulates at the bottom sediment at concentration about four times greater than in the water (Svobodova *et al.* 1993). In the present study, lead concentration was below detection level in the water with significant concentration being recorded in sediment at both dams. Recent studies reported similar trend on lead concentration at Loskop and Flag Boshielo dams at alkaline pH (Lebepe 2012; Kekana 2013). Lead may be removed from water column by association with sediment and suspended particulates of inorganic and organic material, such as hydrous oxides and clays and humic acids, respectively (DWAF 1996). Lead concentration recorded in sediment was within the TEL set by MacDonald *et al.* (2000) for aquatic ecosystem. Low concentration of lead may affect fish by forming a film of coagulated mucous over the gills and subsequently over the entire body (DWAF 1996a). Significant lead concentration in the water column may interacts with iron and therefore interferes with haemoglobin synthesis (Dallas & Day 2004).

3.4.4.9 Lithium

Lithium is the lightest metal in its elemental form and is highly reactive as a pure element. Due to its reactivity, lithium does not occur naturally as a pure element, but in stable minerals and salts. Lithium concentration in aquatic ecosystems is often overlooked due to its significantly low concentration recorded in various freshwater ecosystem in the United States (Kszos & Stewart 2003). However, notable concentrations were recorded in the water column and sediment at Loskop and Flag Boshielo dams. Kekana (2013) also reported significant concentrations (0.001 to 0.009 mg/ℓ) in the water column at Flag Boshielo Dam. There is no guideline for lithium concentration set by DWAF (1996), CCME (2012) or MacDonald *et al.* (2000)

for aquatic ecosystem, however, concentration ranging from 33 to 197 mg/ℓ has been reported to have acute environmental effect in aquatic ecosystems (Aral & Vecchio-Sadus 2008).

3.4.4.10 Manganese

Manganese is a transition metal which is widespread in the environment, occurring in all rocks and soils. It is a micronutrient, required by plants for photosynthesis and by animals for neural development. However, in excess manganese may be toxic to fish and humans, and impair drinking water delivery and quality (Heal 2001). Manganese is the eighth most abundant metal in the Earth's crust (Heal *et al.* 2002). It occurs in two main forms in an aquatic environment: soluble manganese(II) and insoluble manganese(IV). The mobility of manganese is largely governed by pH and redox conditions (Heal 2001). Reduction and oxidation reactions govern the behaviour of manganese in soil and water, favouring formation of manganese(II) in groundwater and also in reducing, acidic soil conditions (Heal *et al.* 1997).

Manganese mean concentrations were within the DWAF (1996) guideline in the water column at Loskop and Flag Boshielo dams. The concentration was below detection limit in the water column at Flag Boshielo Dam during winter. However, elevated concentrations were reported in sediment throughout the study at both dams. Abesser & Robinson (2010) and Heal (2001) reported that biochemical and physical processes, and change in pH and redox conditions influence the solubility of manganese in the water column. Although manganese concentrations in water were within the TWQR at both dams, these dams remain to be at risk of high manganese concentrations which might be resuspended into the water column at any time.

3.4.4.11 Nickel

Nickel is ubiquitous and known as a nutritionally essential trace metal on several animal species, micro-organisms and plants. However, increased concentration in some areas from both anthropogenic release and naturally varying levels may be toxic to living organisms (Cempel & Nikel 2006). Anthropogenic activities contribute

to the nickel loading in aquatic and terrestrial ecosystems include mining, smelting, refining and waste incineration (Vandenbrouck *et al.* 2008). Bioavailability and toxicity of nickel may strongly be influenced by its salts, chemical and physical form, pH and water hardness (Eisler 1998; Karthikeyan *et al.* 2007). Moreover, harmful effects of nickel are mostly due to the interference with the metabolism of essential metals, such as iron(II), manganese(II), calcium(II), zinc(II), copper(II) or magnesium(II), which can suppress or modify the toxic and carcinogenic effects of nickel (Cempel & Nikel 2006). Nickel ions is soluble at pH of <6.5 and form insoluble hydroxide at pH of >6.7. Most of the nickel present in freshwaters is in the ionic form and about half in the form of stable organic complexes, many of which readily adsorb onto clay particles and/sediment (Dallas & Day 2004).

In the present study, nickel concentration in water column was below detection limit but concentrations exceeding MacDonald *et al.* (2000) TEL and PEC were recorded in sediment at both dams. Nickel and its compounds have been regarded as dangerous substance and they are included in the Priority Substances List under the Canadian Environmental Protection Act (Eisler 1998). Although nickel was below detection level in the water column, its solubility is highly influenced by physico-chemical properties (Eisler 1998; Karthikeyan *et al.* 2007). It is therefore reasonable to deduce that Loskop and Flag Boshielo dams are at risk of experiencing increased nickel concentrations in the water column without contribution from external sources.

3.4.4.12 Strontium

Strontium is a soft, silvery metal with physical and chemical properties similar to those of calcium (Irwin *et al.* 1997; Crafford & Avenant-Oldewage 2010). It has not shown to be essential for growth and development of aquatic biota. Increased strontium concentration contributes to the total water harness. There are relatively few organometallic compounds of strontium and their industrial uses are few; therefore their toxicology is of limited concern. Strontium may sometimes be a marker for oil industry contamination as well as pollution from cattle feedlots. Pure strontium

are seldom toxic, however, many strontium compounds are hazardous to fish and wildlife with strontium chromate being carcinogenic (Irwin *et al.* 1997).

The present study has shown elevated concentration on strontium in the water column and sediment at both dams. Industrial wastes and mining effluents may to a greater extent influence strontium concentration in rivers (Seymore *et al.* 1995; Chowdhury & Blust 2012). There are no guidelines for strontium concentration in the water column and sediment set for aquatic ecosystems, however, strontium accumulates in the vertebrae and opercula of fish, and has in most cases been associated with skeletal abnormalities in freshwater fishes (Crafford & Avenant-Oldewage 2010; Chowdhury & Blust 2012). In an aquatic ecosystem, strontium has an analogous character with calcium and its uptake is reported to occur through calcium transport systems located in the chloride cells of gills and enterocytes of the intestine in fish (Chowdhury & Blust 2002). Therefore, in calcium-rich waters, calcium competes with strontium for uptake processes and result in lower strontium accumulation, hence low strontium toxicity (Seymore *et al.* 1995).

3.4.4.13 Zinc

Zinc is an essential nutritional trace element for plants and animals. Humans have a high tolerance level to elevated zinc concentrations, while fish are highly susceptible to zinc poisoning (DWAF 1996b). Zinc occurs in rocks and ores and is readily refined into a pure stable metal. It can enter aquatic ecosystems through both natural processes such as weathering and erosion, and through industrial activity. Zinc occurs in two oxidation states in aquatic ecosystems, namely as the metal and as zinc(II). Zinc(II) ion is toxic to aquatic ecosystems particularly to fish even at a relatively low concentration (DWAF 1996a). The solubility, bioavailability and toxicity of zinc is influenced by physico-chemical properties such as pH, alkalinity, ionic strength and calcium (Svobodova *et al.* 1993; DWAF 1996).

In the present study, zinc was below detection level in the water column at Flag Boshielo Dam; however, significant concentration was recorded in sediment. Loskop Dam exhibited zinc concentration which was within the CCME (2012) guideline in the

water column and a concentration exceeding MacDonald *et al.* (2000) guideline in sediment. Zinc concentrations recorded in the present study were comparable to those reported in other studies in the Olifants River system (Dabrowski *et al.* 2013; Dabrowski *et al.* 2014; Lebepe *et al.* 2016). However, zinc concentration in the water column at Flag Boshielo Dam was reduced, hence non-detectable. Under alkaline condition, zinc precipitates and become adsorbed by hydrous metal oxides, onto clay minerals and other organic materials, thus forming a complex which may then become fixed in the bottom sediment (DWAF 1996). The pH may thus, be the explanation for non-detectable zinc concentration in the water column and elevated concentration in sediment at Flag Boshielo Dam.

CHAPTER 4

METAL BIOACCUMULATION IN *Oreochromis mossambicus* AND *Labeo rosae* FROM LOSKOP AND FLAG BOSHIELO DAMS

4.1 Introduction

The enrichment of metals in freshwater ecosystems has become a global concern since they are not biodegradable. Metals enter aquatic ecosystems through industrial and mining effluents, domestic sewage and runoff (Perumalsamy & Arumugam 2013). Organisms in aquatic ecosystems assimilate metals from the environment and bioaccumulate them within their tissues (Coetzee *et al.* 2002; Jabeen *et al.* 2012). Bioaccumulation occurs throughout the food chain, and organisms at the top of the food chain may accumulate lethal dose (Davies & Day 1998; US-EPA 2000). Fish are often found near or at the top of the food chain (Khan *et al.* 2012); and they can accumulate metals million times more than organisms at the bottom of the food chain (Davies & Day 1998). Fish are therefore considered important indicators of heavy metal enrichment of the aquatic ecosystem (Jabeen *et al.* 2012; Khan *et al.* 2012). Moreover, metal levels in fish may be used as an index of pollution in an aquatic ecosystem, which is considered as an important tool for highlighting health condition of fish (Qadir & Malik 2011).

Metal accumulation and distribution between the different tissues depend on the mode of exposure, which may be through food ingestion, suspended particulate matters, metal ion exchange through gills and skin, and water intake (Qadir & Malik 2011). Jezierska & Witeska (2006) reported that the metal concentration in fish tissues is directly proportional to the concentration in the external environment. Although this relationship was observed in both laboratories and field studies, it only occurs if the metals are waterborne. But if food is the main source of metal exposure, such a relationship does not necessarily occur (Jezierska & Witeska 2006).

Several studies on metal accumulation in fish inhabiting polluted ecosystems have shown that a considerable level of metals may accumulate in the fish without causing

mortality (Akan *et al.* 2012; Liu *et al.* 2012; El-Moselhy *et al.* 2014). However, numerous health effects such as oxidative damage to living tissues, alteration in sensory reception, reduced responses to normal olfactory function (feeding, mating, selection, or homing), reduction in swimming performance, gill purge, ventilation, coughs, learning impairment, loss of equilibrium that lapsed into paralysis, loss of reproductive efficiency, and irregular metamorphosis may be prominent (Kaoud & El-Dahshan 2010; Qadir & Malik 2011). Metals in fish become deleterious when concentrations exceed permissible levels or threshold limits which vary from one species to another. Some metals, such as iron, chromium, copper, zinc and manganese are essential since they play important roles in biological systems; however, they become toxic at higher concentrations and result in adverse ecological effects. Other metals such as lead and cadmium have no documented role in living organism and can be toxic even in trace amounts (Nhiwatiwa *et al.* 2011; Qadir & Malik 2011).

Although no mortalities have been reported from considerable levels of metals in fish tissues, numerous health effects such as oxidative damage to living tissues, alteration in sensory reception, reduced responses to normal olfactory function (feeding, mating, selection, or homing), reduction in swimming performance, gill purge, ventilation, coughs, learning impairment, loss of equilibrium that lapsed into paralysis, loss of reproductive efficiency, and irregular metamorphosis have been prominent (Kaoud & El-Dahshan 2010; Qadir & Malik 2011).

Olifants River system is considered to be one of the most polluted river systems in Southern Africa with acid mine drainages emanating from the abandoned and operational mines in the upper catchment being the primary driver of pollution (De Villiers & Mkwelo 2009; Oberholster *et al.* 2010). Given rising concerns over the long term effect of increasing metal concentration on the health of fish populations, this section aimed to evaluate metal concentration trend across three fish tissues (liver, gills and muscle) of *L. rosae* and *O. mossambicus* from Loskop and Flag Boshielo dams.

4.2 Materials and methods

4.2.1 Fish sampling

Fish were collected using gill nets: single net 50 m long, 3 m drop, composed of 10 m panels of 50 to 150 mm mesh (Fig. 4.1a). A minimum of 10 specimens of each fish species were collected from Loskop and Flag Boshielo dams during winter (May/Jun) and summer (Nov/Dec) 2014 survey. Fish were kept in holding tanks filled with dam water and transported to the field laboratory (Fig. 4.2) for processing. In the field laboratory, air pump was used to aerate tanks filled with dam water to increase the oxygen level, hence, minimise fish stress (Fig. 4.1b).

Figure 4.1 a. Gill net; **b.** Aerated water tank with air pump pointed with an arrow.

4.2.2 Fish processing and analysis

Fish were weighed using a balance scale, and the total, fork and standard lengths were measured using a measuring board (Fig. 4.3a&b). Immediately after taking measurements, fish were euthanised by severing the spinal cord just behind the head. Fish were then dissected ventrally. A skinless sample of muscle tissue (±15 g), liver and gills were collected from each fish specimen, and wrapped with aluminium foil. Samples were frozen on site, and stored at -20°C prior to analysis at a SANAS

accredited laboratory in Pretoria. In the laboratory, tissue samples were freeze dried, digested according to the methods of Bervoets & Blust (2003) and analysed for metals using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Metal concentrations were measured in mg/kg dry weight. All samples were subjected to the same QC/QA as the water and sediment samples. Recoveries were within 10% of the certified values.

Figure 4.2 Field laboratory at Loskop Dam.

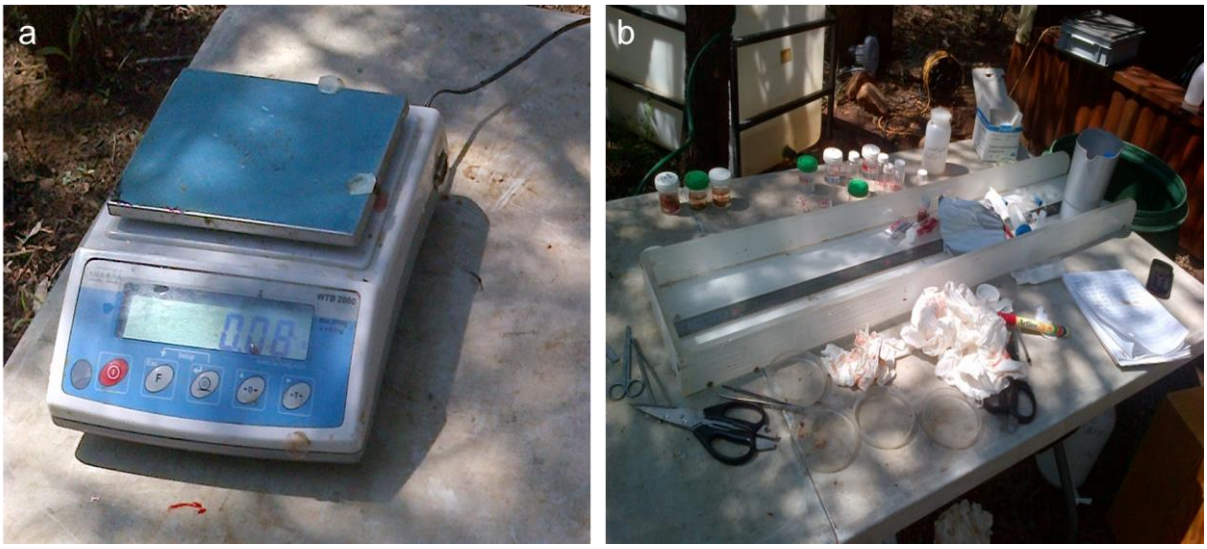

Figure 4.3a Scale used to weigh fish; **b.** measuring board used to measure the total, fork and standard lengths of fish.

4.2.3 Bioaccumulation factor

The bioaccumulation factor (BAF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in the medium (Mohamed 2008). The BAF was calculated as used by Authman & Abbas (2007) and adapted by Mohamed (2008) as follows:

$$\text{BAF} = \frac{\text{Concentration in organ}}{\text{Concentration in medium (water or sediment)}} \quad \text{Equation 4.1}$$

4.2.4 Statistical analyses

The mean and standard deviation were calculated for the metal concentrations in liver, gills and muscle tissue of fish for each dam. For box and whisker plots, metal concentrations were 4th root transformed to reduce the confounding effect of metals present at high concentrations, e.g. iron and aluminium, hence, making the trend clearly visible. Normality of the data was tested using Shapiro-Wilk test whereas Levene's test was used to evaluate the homogeneity of variance. An independent t-test or Wilcoxon-Mann-Whitney U-test was used to evaluate whether the metal concentrations in tissues varied between the two dams using R-3.1.1 (R

Development Core Team 2014). Data was considered statistically significance at $p<0.05$. Box and whisker plots were prepared for the metal concentrations in fish tissues at both dams using R-3.1.1. For correlation test, specimens from Loskop and Flag Boshielo dams of each species were pooled together and treated as one group. Correlation test was carried out using PerformanceAnalytics package and chart.correlation function in R-3.1.1 (Peterson *et al.* 2014).

To evaluate differences in the multivariate metal concentrations between the two species, the procedures described by Jooste *et al.* (2014) were followed. Non-metric multi-dimensional scaling plot was prepared to visualise the metal concentrations data of the two species from both Loskop and Flag Boshielo dams. A distance-based test of homogeneity of multivariate dispersion and a multiple analysis of variance (Anderson 2001a, b) were performed to determine whether there was a significant difference on metal concentrations in each species between the two dams using the betadisper and adonis functions in VEGAN, hereafter DISPER and MANOVA.

4.3 Results

4.3.1 Metal concentrations in fish species

Metal concentrations recorded during summer and winter in each tissue were pooled and presented in box and whisker plots. Concentrations recorded in *L. rosae* tissues are presented in Figure 4.4 to Figure 4.6 whereas for *O. mossambicus* are presented in Figure 4.8 to Figure 4.10. Mean concentrations are presented in Table 4.1. Fish tissues in both species have shown to accumulate metals differently with liver exhibiting higher concentrations for most metals followed by gills and muscle, respectively (liver>gills>muscle). Metals deviated from the popular trend included boron, zinc, barium, manganese, lead, strontium and chromium. Boron, zinc, barium, manganese and lead have shown gill>liver>muscle pattern whereas strontium showed gill>muscle>liver trend for both species at both dams. Different trends, gill>liver>muscle and liver>gill>muscle were observed for chromium on *L. rosae* and *O. mossambicus*, respectively. The NMDS plot has also shown clear separations for metal concentrations between different tissues on both *L. rosae* and *O. mossambicus* (Fig. 4.12).

4.3.1.1 Metal concentrations in *Labeo rosae* tissues

Mean metal concentrations in the liver, gills and muscle at Loskop Dam were in the following descending orders: Fe> Al> Cu> Zn> Mn> Se> Ni> V> As> Ag> Ba> B> Sr> Cr> Co> Cd> Sb> Pb> Sn; Sr> Fe> Al> Zn> Mn> Ba> Se> Ni> As> V> B> Ag> Cu> Cr> Pb> Co> Sb> Sn> Cd; Al> Fe> Zn> Sr> Se> Mn> Ba> As> Ag> Cr> Cu> Ni> V> B> Sb> Pb> Co> Sn> Cd, respectively. At Flag Boshielo Dam, the mean metal concentrations in the liver, gills and muscle were in the following orders: Fe> Al> Cu> Zn> Ni> Se> Mn> Ag> V> As> Sr> Ba> Cd> Cr> B> Sb> Pb> Co> Sn; Fe> Sr> Al> Zn> Ba> Mn> Ag> Se> Ni> Cu> As> V> B> Cr> Pb> Co> Sb> Sn> Cd; Al> Zn> Fe> Sr> Ag> Se> Mn> Ba> As> Cu> Ni> Cr> B> Pb> V> Sb> Sn> Co> Cd, respectively.

The muscle of *L. rosae* populations have shown significant difference ($p<0.05$) for cadmium, tin, vanadium and zinc concentrations between the two dams (Fig. 4.4).

The NMDS plot has shown no clear separation on metal concentrations in the muscle of *L. rosae* between the two dams (Fig. 4.7). The gills of *L. rosae* exhibited significant differences ($p<0.05$) for aluminium, barium, cobalt, iron, lead, selenium, tin, strontium, vanadium and zinc concentrations between the two dams (Fig. 4.5). The NMDS plot has shown a clear separation on the metal concentration in the gill tissues of *L. rosae* between the two dams (Fig. 4.7). DISPER results have shown no significant difference ($p=0.80$), however, significance difference ($p<0.001$) was observed in MANOVA confirming that the separation observed in the NMDS plot was statistically significant.

In the liver of *L. rosae,* significant differences ($p<0.05$) were observed for aluminium, boron, cadmium, cobalt, copper, iron, nickel and vanadium concentrations between the two dams (Fig. 4.6). The NMDS plot exhibited clear separation for metal concentrations in the liver of *L. rosae* populations between the two dams (Fig. 7). DISPER results were significant ($p=0.01$), indicating that there was a statistical difference in dispersion between the two dams. MANOVA has also shown significant results ($p<0.001$) confirming that the separation on the NMDS plot was statistically significant.

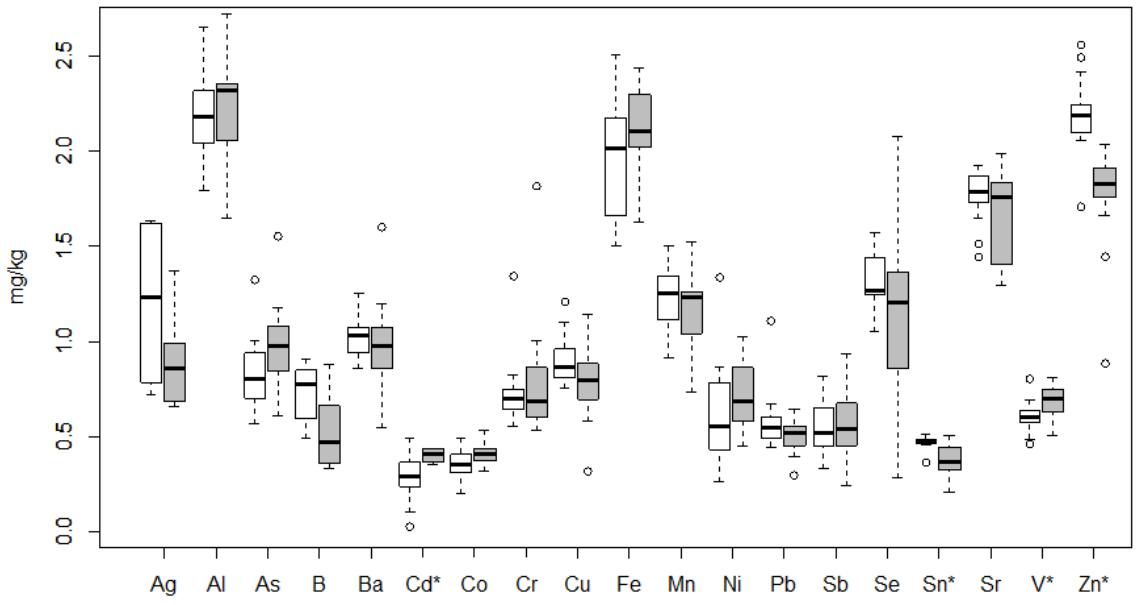

Figure 4.4 Metal concentrations (mg/kg dry weight) recorded in the muscle tissue of *Labeo rosae* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

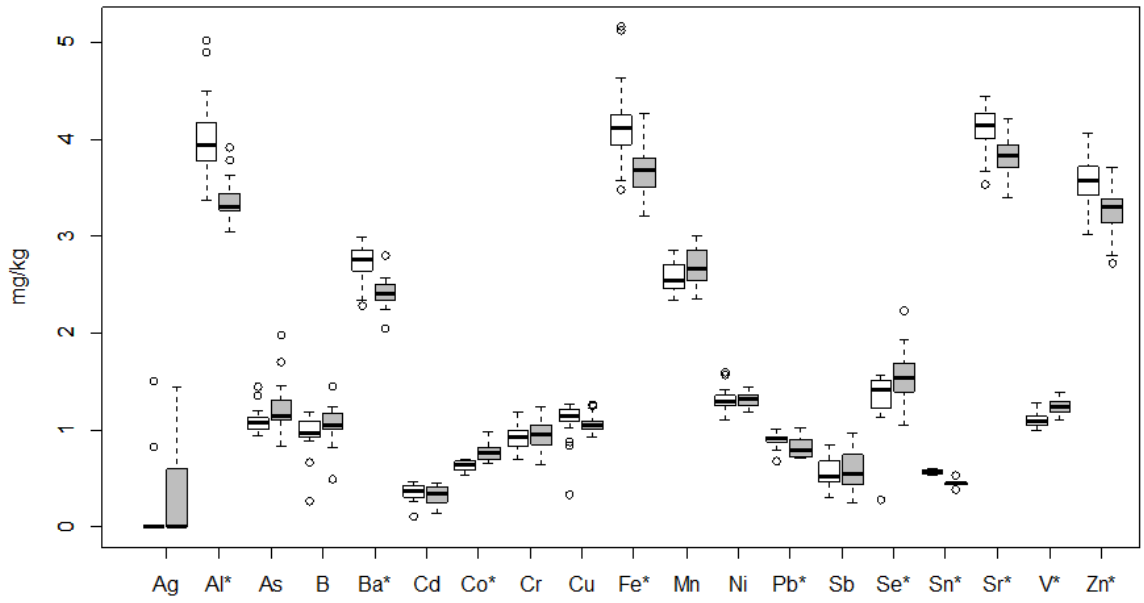

Figure 4.5 Metal concentrations (mg/kg dry weight) recorded in the gill tissue of *Labeo rosae* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

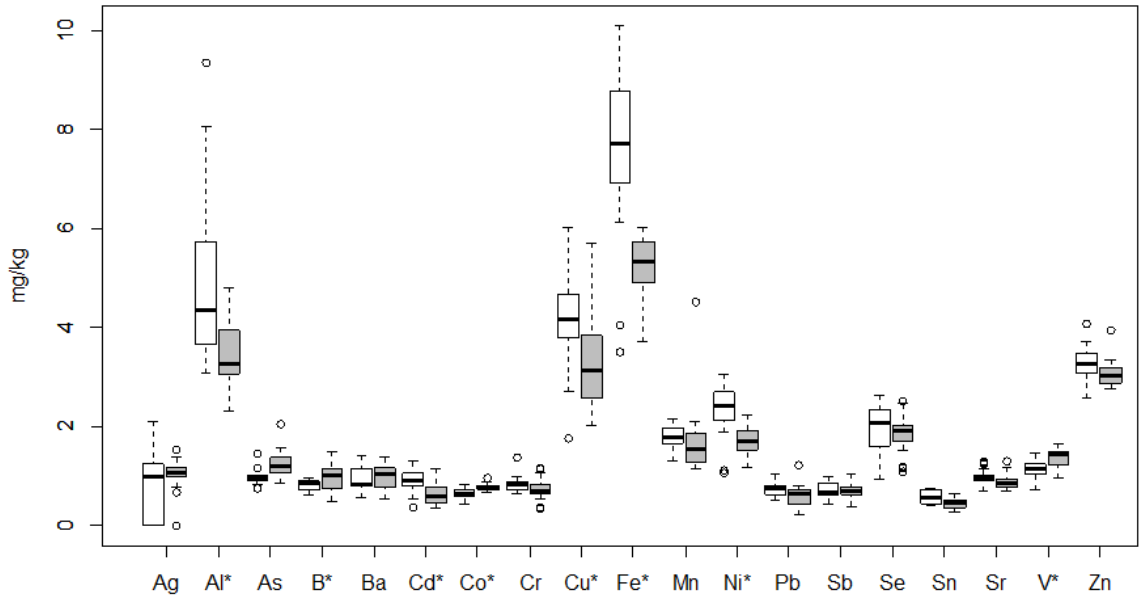

Figure 4.6 Metal concentrations (mg/kg dry weight) recorded in the liver tissue of *Labeo rosae* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

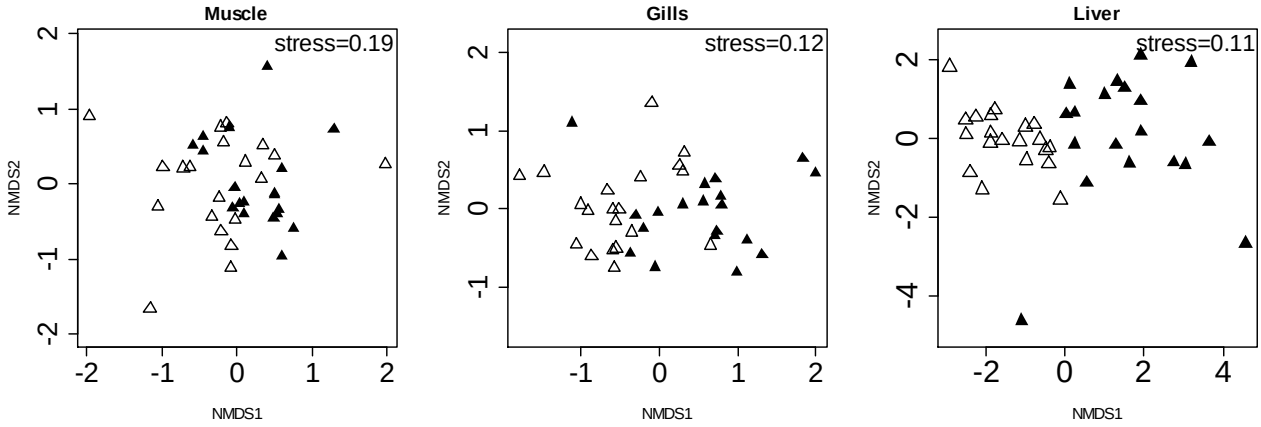

Figure 4.7 Non-metric multi-dimensional scaling plots for metal concentrations in the muscle, gills and liver tissues of *Labeo rosae* from Loskop Dam (∆) and Flag Boshielo Dam (▲).

4.3.1.2 Metal concentrations in *Oreochromis mossambicus* tissues

There were some similarities on the trends of metal concentrations between the two species. The first four metals in each tissue were in most cases similar for both species. At Loskop Dam, mean metal concentrations in the liver, gills and muscle were in the following descending trends: Fe> Al> Zn> Cu> Mn> Ni> Co> V> Sr> Se> As> Ag> Ba> Cr> B> Pb> Sb> Cd> Sn; Fe> Al> Sr> Mn> Zn> Ba> Se> As> Ni> V> Ag> Cu> Co> B> Pb> Cr> Sb> Sn> Cd; Fe> Zn> Al> Se> As> Mn> Sr> Ag> Cu> B> Ni> Ba> Cr> Sb> Co> V> Pb> Sn> Cd, respectively. Flag Boshielo Dam population exhibited the following descending trends: Fe> Al> Cu> Zn> V> Ni> Se> Ag> Mn> Co> As> Ba> B> Cd> Cr> Pb> Sb> Sr> Sn; Al> Fe> Sr> Mn> Zn> Ba> V> Se> Ni> Ag> Cu> Pb> B> As> Cr> Co> Sb> Sn> Cd; Zn> Al> Fe> Mn> Se> Sr> Cu> Ag> As> B> Ni> Cr> Ba> Sb> V> Co> Pb> Sn> Cd in the liver, gills and muscle, respectively.

Oreochromis mossambicus have shown a significant difference ($p<0.05$) for arsenic, cobalt, iron, lead, tin and zinc concentrations in the muscle tissue between the two dams (Fig. 4.8). The NMDS plot exhibited no separation on metal concentrations in the muscle of *O. mossambicus* between the two dams (Fig. 4.11). In the gills, metal concentrations exhibited a significant difference ($p<0.05$) between Loskop and Flag Boshielo populations except silver, boron, cadmium, antimony, selenium and tin (Fig. 4.9). The NMDS has shown a clear separation on the metal concentrations in the gills between the two populations (Fig. 4.11). DISPER analysis has shown no significant difference ($p=0.12$) but MANOVA exhibited significance difference ($p<0.001$) confirming that the separation on the NMDS plot was statistically significant.

In the liver, significant differences ($p<0.05$) were observed for boron, cadmium, copper, nickel, lead, selenium, vanadium and zinc concentrations between Loskop and Flag Boshielo dams (Fig. 4.10). The NMDS plot has shown a clear separation between the two dams (Fig. 4.11). DISPER analysis has shown significant difference ($p=0.004$) between the two dams. Moreover, MANOVA has shown significant results ($p<0.001$) confirming that the separation observed in the NMDS plot was significant.

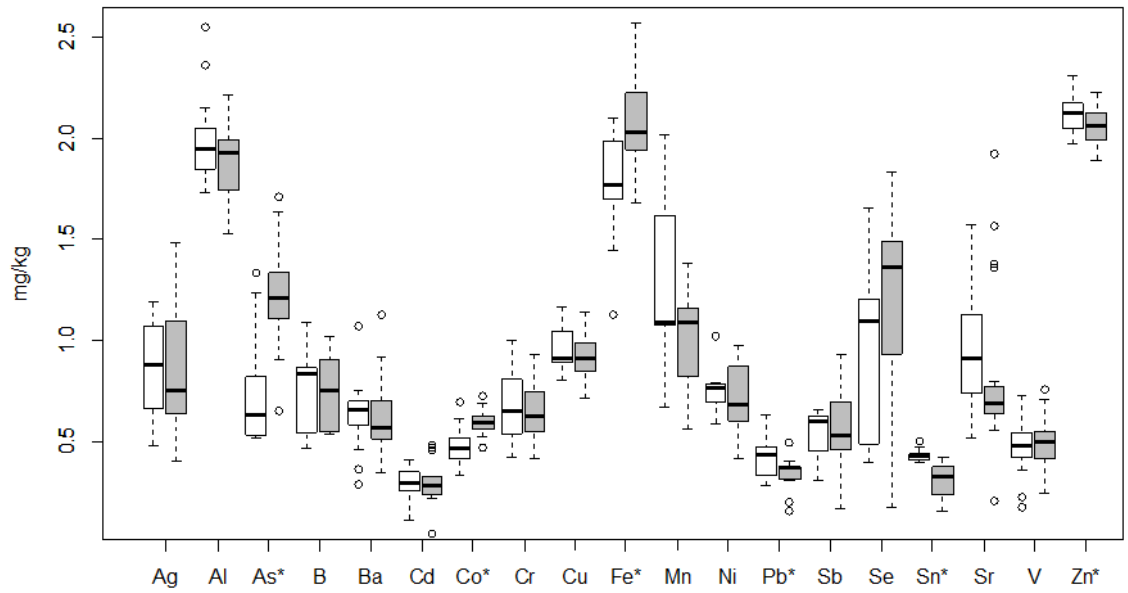

Figure 4.8 Metal concentrations (mg/kg dry weight) recorded in the muscle tissue of *Oreochromis mossambicus* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

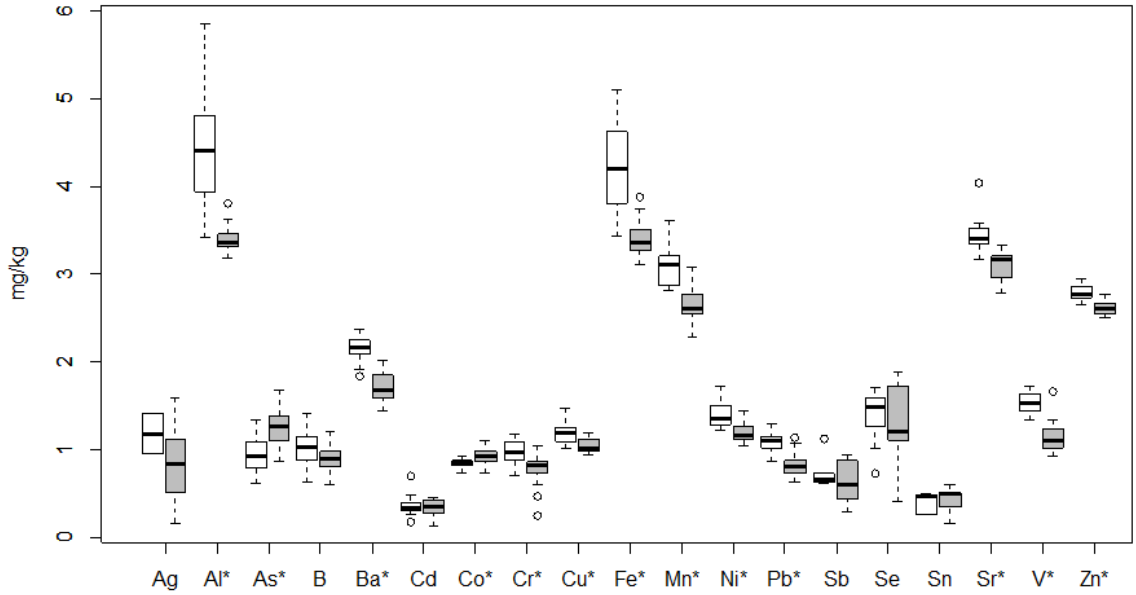

Figure 4.9 Metal concentrations (mg/kg dry weight) recorded in the gill tissue of *Oreochromis mossambicus* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

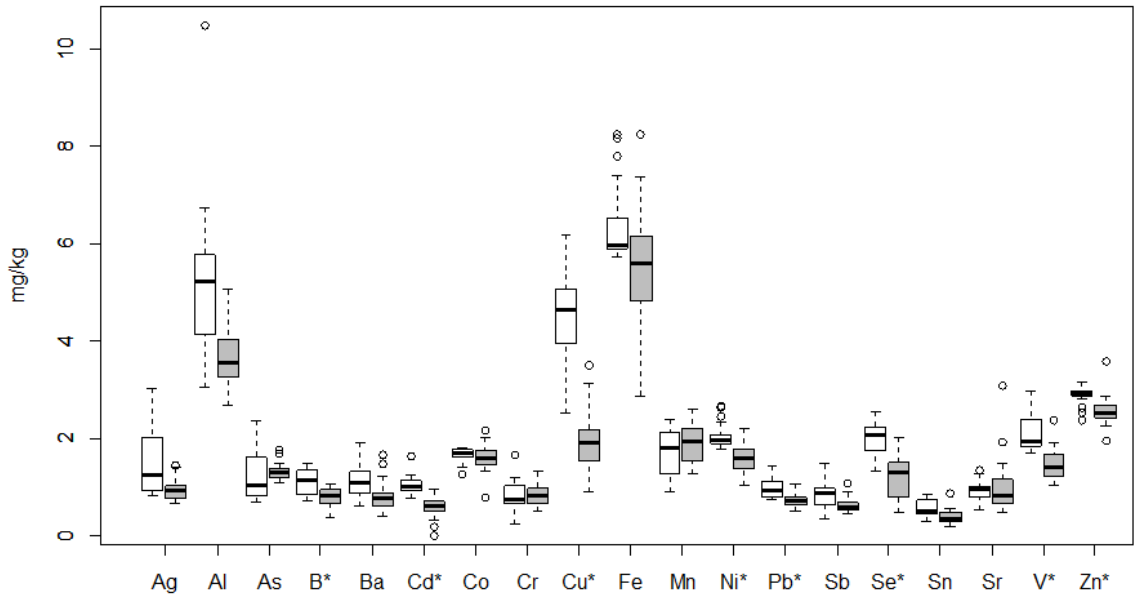

Figure 4.10 Metal concentrations (mg/kg dry weight) recorded in the liver tissue of *Oreochromis mossambicus* from Flag Boshielo Dam (white) and Loskop Dam (grey). * denote significant difference.

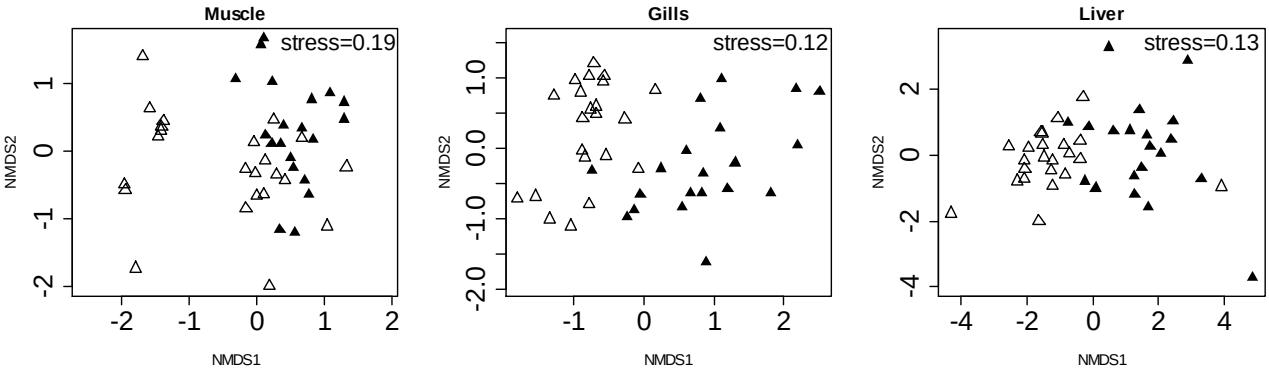

Figure 4.11 Non-metric multi-dimensional scaling plots for metal concentrations in the muscle, gills and liver tissues of *Oreochromis mossambicus* from Loskop Dam (∆) and Flag Boshielo Dam (▲).

Table 4.1 Mean concentration of metals (mg/kg dry weight) recorded in different tissues of *Labeo rosae* and *Oreochromis mossambicus* at Loskop and Flag Boshielo dam presented as mean ± standard deviation.

Metals	***Labeo rosae***						***Oreochromis mossambicus***					
	Loskop Dam (N=20)			Flag Boshielo Dam (N=20)			Loskop Dam (N=23)			Flag Boshielo Dam (N=20)		
	Liver	Gill	Muscle	Liver	Gill	Muscle	Liver	Gill	Muscle	Liver	Gill	Muscle
Ag	1.65 ± 1.21	1.41 ± 1.43	0.88 ± 1.01	4.23 ± 5.26	4.07 ± 2.64	3.70 ± 3.30	1.09 ± 1.22	1.48 ± 1.89	1.06 ± 1.43	17.63 ± 29.95	2.42 ± 1.60	0.83 ± 0.73
Al	186.66 ± 144	132.81 ± 35	26.60 ± 9.97	1128 ± 1808	286.25 ± 125.7	34.11 ± 43.17	224.32 ± 153.10	134.41 ± 23	13.01 ± 5.21	1302.84 ± 2506	438.03 ± 260	16.33 ± 7.75
As	3.18 ± 3.65	2.92 ± 3.32	1.16 ± 1.25	1.39 ± 1.46	1.61 ± 0.89	0.73 ± 0.86	3.20 ± 1.96	3.20 ± 2.09	2.63 ± 1.91	6.30 ± 10.28	1.14 ± 1.02	0.62 ± 0.98
B	1.30 ± 1.24	1.48 ± 1.02	0.17 ± 0.21	0.52 ± 0.36	1.02 ± 0.56	0.38 ± 0.21	0.50 ± 0.35	0.73 ± 0.54	0.43 ± 0.37	1.79 ± 1.61	1.32 ± 1.00	0.52 ± 0.49
Ba	1.32 ± 1.09	34.03 ± 8.7	1.25 ± 1.35	1.09 ± 1.12	58.16 ± 11.76	1.16 ± 0.47	0.94 ± 1.74	8.95 ± 3.16	0.26 ± 0.37	2.61 ± 3.26	21.79 ± 5.63	0.23 ± 0.27
Cd	0.32 ± 0.42	0.02 ± 0.01	0.02 ± 0.01	0.97 ± 0.67	0.02 ± 0.01	0.01 ± 0.01	0.20 ± 0.18	0.02 ± 0.01	0.01 ± 0.02	1.41 ± 1.38	0.03 ± 0.05	0.01 ± 0.01
Co	0.41 ± 0.15	0.40 ± 0.22	0.03 ± 0.02	0.20 ± 0.11	0.16 ± 0.05	0.02 ± 0.02	7.47 ± 4.62	0.74 ± 0.28	0.13 ± 0.05	7.63 ± 2.25	0.51 ± 0.11	0.06 ± 0.05
Cr	0.49 ± 0.54	0.88 ± 0.49	0.87 ± 2.34	0.64 ± 0.74	0.80 ± 0.42	0.42 ± 0.71	0.71 ± 0.73	0.45 ± 0.28	0.21 ± 0.19	1.16 ± 2.03	1.02 ± 0.58	0.29 ± 0.29
Cu	178.94 ± 231	1.35 ± 0.50	0.49 ± 0.37	421.9 ± 309	1.68 ± 0.70	0.73 ± 0.43	24.11 ± 34.46	1.27 ± 0.41	0.75 ± 0.34	522.88 ± 420.4	2.09 ± 0.93	0.90 ± 0.43
Fe	775.51 ± 325	188.11 ± 56	20.97 ± 7.72	4368 ± 2673	329.35 ± 140	18.11 ± 11.23	1253.53 ± 1055	134.76 ± 34	21.64 ± 14.35	1832.45 ± 1112	343.52 ± 158	11.20 ± 4.84
Mn	28.06 ± 91.03	51.94 ± 15.03	2.18 ± 1.34	11.85 ± 5.26	45.38 ± 12.19	2.42 ± 1.08	15.72 ± 11.51	51.63 ± 15.00	1.37 ± 0.99	11.81 ± 10.30	93.63 ± 28.4	5.41 ± 6.40
Ni	9.72 ± 6.06	3.05 ± 0.69	0.34 ± 0.27	38.58 ± 23.2	3.12 ± 1.22	0.58 ± 1.10	7.96 ± 5.59	2.09 ± 0.75	0.32 ± 0.24	19.77 ± 11.69	4.14 ± 1.74	0.34 ± 0.21
Pb	0.29 ± 0.50	0.49 ± 0.23	0.07 ± 0.04	0.35 ± 0.25	0.67 ± 0.22	0.17 ± 0.32	0.34 ± 0.26	0.54 ± 0.36	0.02 ± 0.01	1.03 ± 0.93	1.49 ± 0.61	0.04 ± 0.04
Sb	0.32 ± 0.29	0.24 ± 0.30	0.15 ± 0.20	0.39 ± 0.31	0.16 ± 0.16	0.13 ± 0.13	0.23 ± 0.32	0.27 ± 0.28	0.17 ± 0.22	1.03 ± 1.33	0.43 ± 0.54	0.11 ± 0.07
Se	14.32 ± 10.30	7.22 ± 5.53	3.33 ± 4.64	20.91 ± 14.2	3.69 ± 2.06	3.26 ± 1.56	3.88 ± 4.32	4.76 ± 4.29	3.89 ± 3.37	18.91 ± 10.75	4.67 ± 2.54	2.02 ± 2.37
Sn	0.06 ± 0.05	0.05 ± 0.02	0.03 ± 0.02	0.18 ± 0.13	0.11 ± 0.02	0.06 ± 0.01	0.08 ± 0.16	0.05 ± 0.04	0.01 ± 0.01	0.14 ± 0.15	0.03 ± 0.02	0.04 ± 0.01
Sr	0.73 ± 0.64	215.44 ± 42.20	8.72 ± 4.42	1.13 ± 0.73	292.58 ± 50.79	10.36 ± 2.66	5.51 ± 18.64	93.53 ± 18.76	1.33 ± 3.00	0.96 ± 0.79	144.3 ± 33.2	1.09 ± 1.30
V	3.67 ± 2.01	2.46 ± 0.61	0.26 ± 0.11	1.96 ± 1.11	1.43 ± 0.27	0.14 ± 0.05	6.03 ± 6.50	1.96 ± 1.42	0.09 ± 0.08	26.05 ± 21.80	5.77 ± 1.69	0.07 ± 0.07
Zn	94.62 ± 40.50	115.29 ± 35.00	10.84 ± 3.77	132.5 ± 60.8	173.93 ± 45.31	24.79 ± 6.69	46.84 ± 27.75	47.07 ± 5.21	18.04 ± 3.13	70.14 ± 15.01	60.76 ± 7.27	20.42 ± 3.50

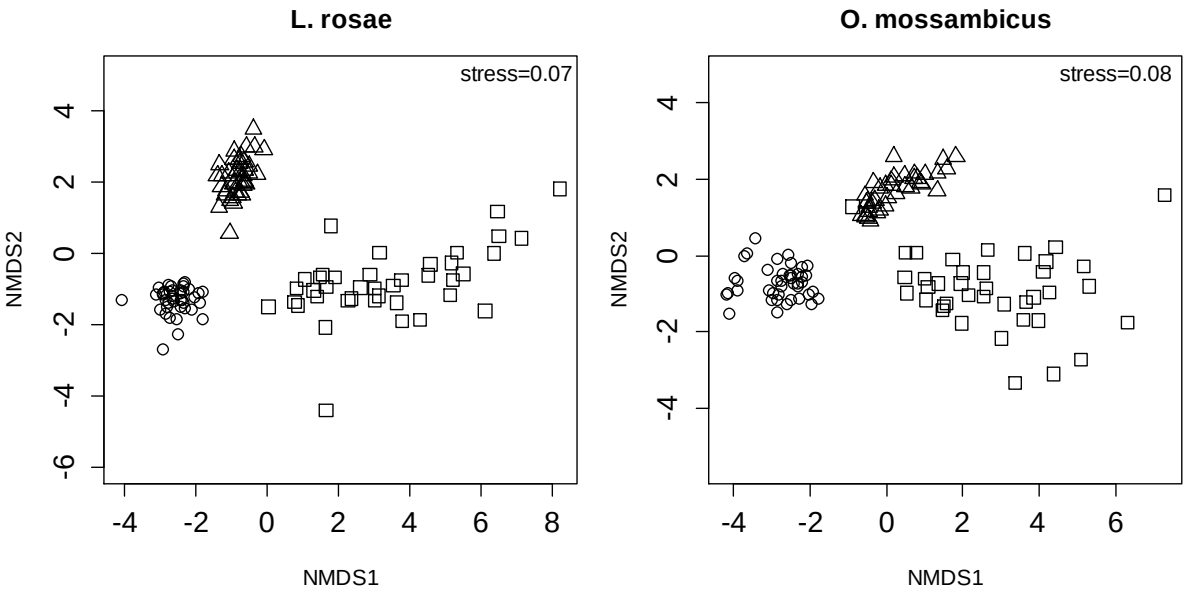

Figure 4.12 Non-metric multi-dimensional scaling plot for metal concentrations in the three tissues, muscle (○), gills (Δ) and liver (□).

4.3.2 Inter-metal relationship

Results for inter-metal relationship in *Labeo rosae* tissues are presented in Figure 4.13 (muscle), Figure 4.14 (gills) and Figure 4.15 (liver). Arsenic has shown a highly significant strong positive relationships ($p<0.05$) with cobalt, chromium, antimony and selenium in the muscle of *L. rosae* (Fig. 4.13). Other metals which have shown a highly significant strong positive relationships ($p<0.05$) in the muscle of *L. rosae* include, Ni-Pb, Cr-Sb, Cr-Se, Sb-Se and Mn-Sr (Fig. 4.13). Silver has shown a highly significant positive relationship with arsenic and antimony in the gill of *L. rosae* (Fig. 4.14). Aluminium showed highly significant positive relationship ($p<0.05$) with iron and nickel (Fig. 4.11). Other metals which have shown highly significant positive relationship ($p<0.05$) in the gills of *L. rosae* include, Fe-Ni, As-Sb, Ba-Sr and Ba-Zn (Fig. 4.14). In the liver, highly significant positive relationships ($p<0.05$) were observed for Cd-Cu, Fe-Ni, Ag-Sb, As-Sb, Cu-Se, Pb-Sr, Co-V and Se-Zn (Fig. 4.15). Antimony has shown a significant positive relationship ($p<0.05$) with silver and arsenic across the three tissues.

Positive inter-metal relationships were more evident in *O. mossambicus* as compared to *L. rosae*. Silver has shown a highly significant strong positive relationship ($p<0.05$) with arsenic, cadmium and antimony in the muscle of *O. mossambicus* (4.16). Moreover, arsenic exhibited a highly significant strong positive relationship ($p<0.05$) with cadmium, antimony and selenium (Fig. 4.16). Other metals which have shown highly significant strong positive relationship include Ba-Sr, Ba-V, Sb-Se and Sr-V (Fig. 4.16). In the gills, barium has shown a highly significant positive relationship ($p<0.05$) with manganese, lead, strontium and vanadium (Fig. 4.17). Significant positive inter-metal relationships were more evident in the gills as compared to muscle and liver. Significant strong positive relationships in the liver of *O. mossambicus* were observed for Ag-As, Ag-Pb, Ag-Sb, Ag-Se, Al-B, As-Sb, Cd-V, Fe-Ni, Ni-V and Sb-Se (Fig. 4.18).

4.3.3 Metal-fish length relationship

Most metal concentrations have shown no relationship with fish lengths with few showing negative relationships in all tissues for both species. Metals which showed negative relationships in *L. rosae* were: zinc in the muscle; aluminium, barium, iron, strontium, vanadium and zinc in the gills; manganese and nickel in the liver (Figs 4.13 – 4.18). No metal has shown a relationship with fish length in the muscle of *O. mossambicus* whereas significant negative relationships were observed for aluminium, barium, chromium, iron, manganese, nickel, lead, strontium, vanadium and zinc in the gills. Nickel, lead and selenium have also shown significant negative relationships with the length of *O. mossambicus* in the liver (Figs 4.13 – 4.18).

4.3.4 Bioaccumulation Factor

The bioaccumulation factor (BAF) between the concentration of metals in tissues and the environment are presented in tables below (Table 4.2 & 4.3). Concentrations of silver, aluminium, arsenic, cadmium, cobalt, chromium, copper, nickel, lead, antimony, tin and vanadium were below detection limit in the water at both dams, hence; their bioaccumulation factors could not be calculated. The tissue-water bioaccumulation factor (BAF_w) was notably higher as compared to the tissue-

sediment bioaccumulation factor (BAF_s). Metal concentrations were in most cases higher in the sediment than in tissues, hence the BAF_s was greater than 1 except for copper, strontium and zinc (Table 4.2 & 4.3).

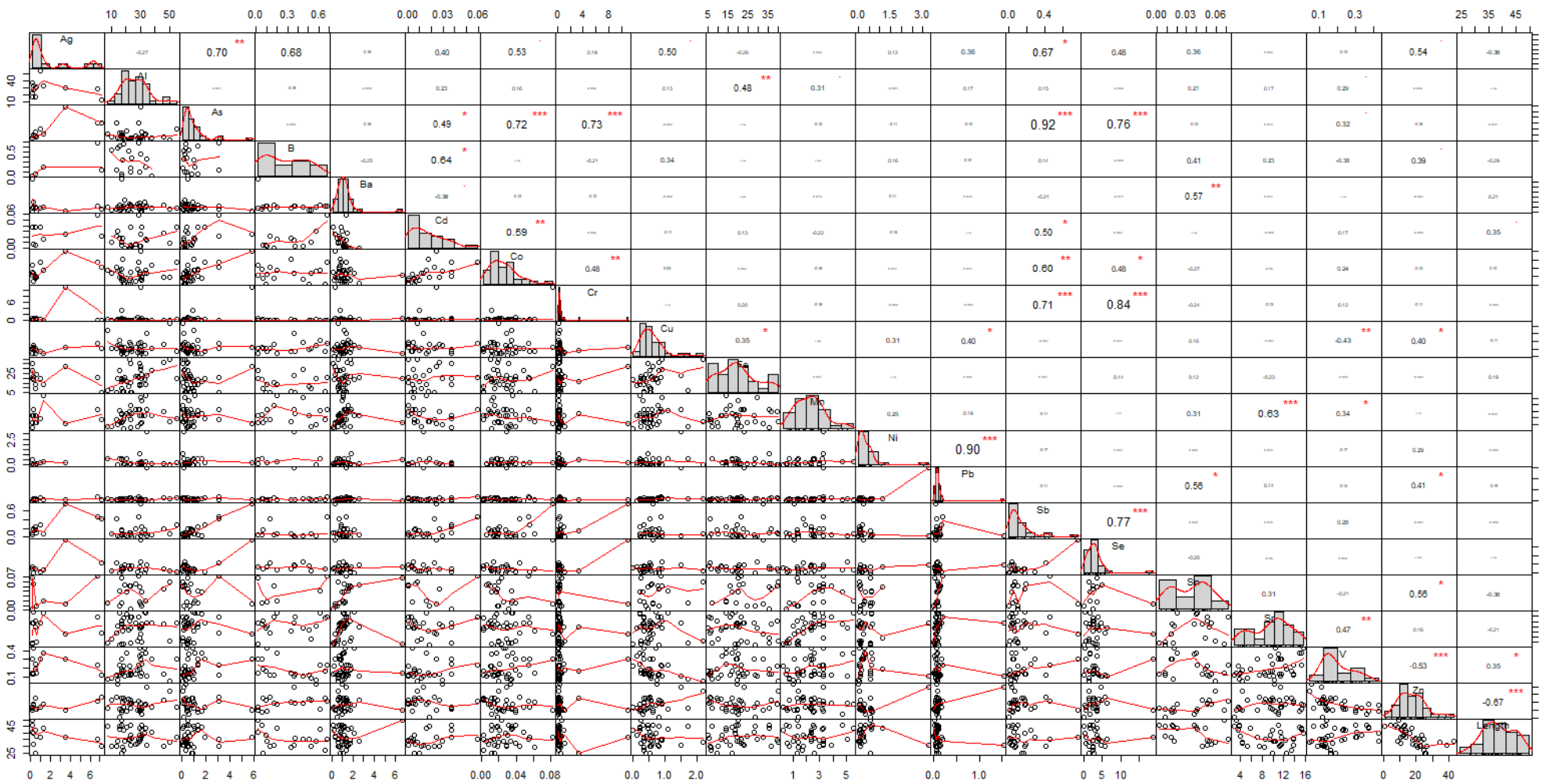

Figure 4.13 Inter-metals and metal-length relationships observed in the muscle of *Labeo rosae* from Loskop and Flag Boshielo dams.

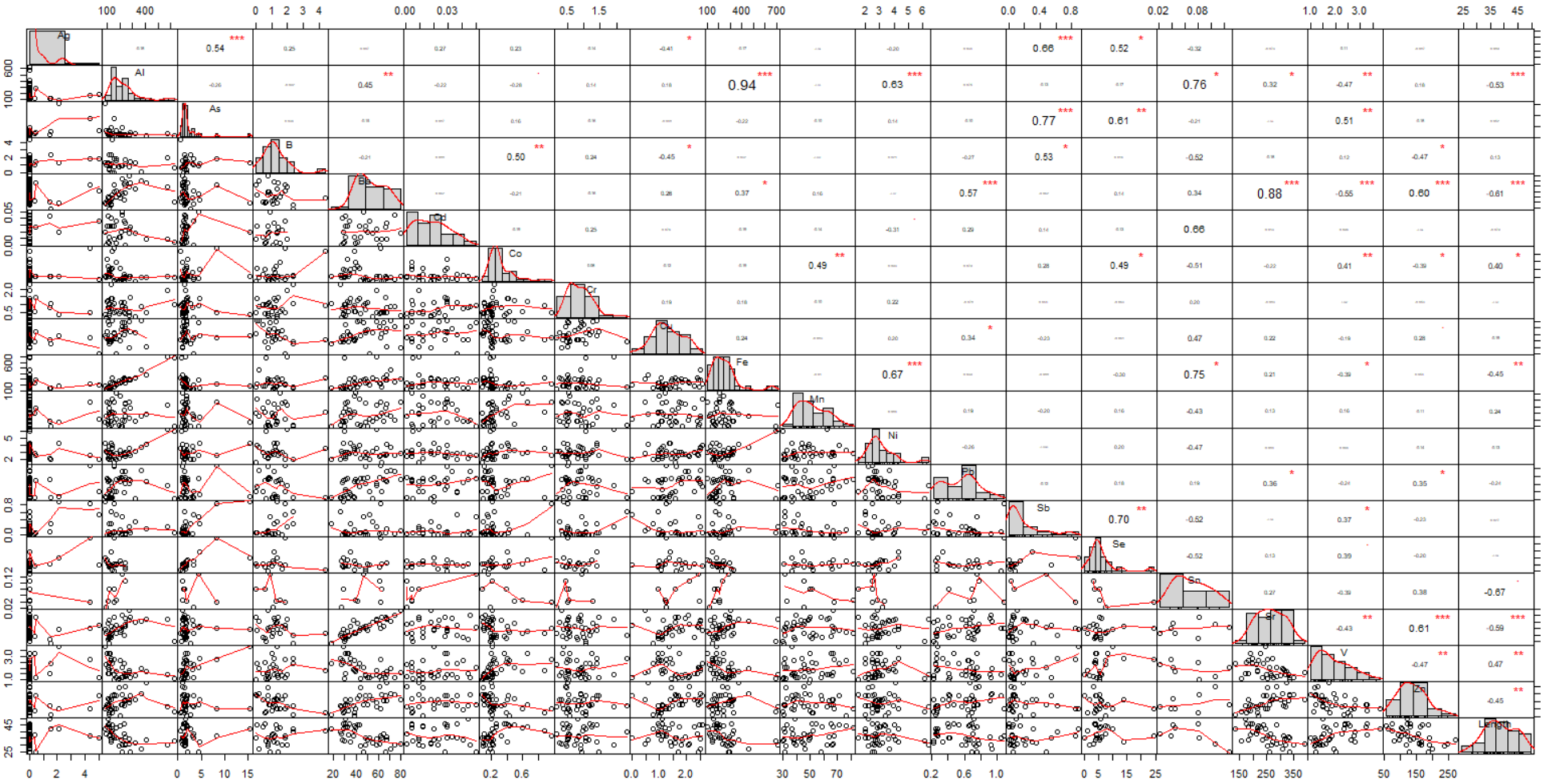

Figure 4.14 Inter-metals and metal-length relationships observed in the gills of *Labeo rosae* from Loskop and Flag Boshielo dams.

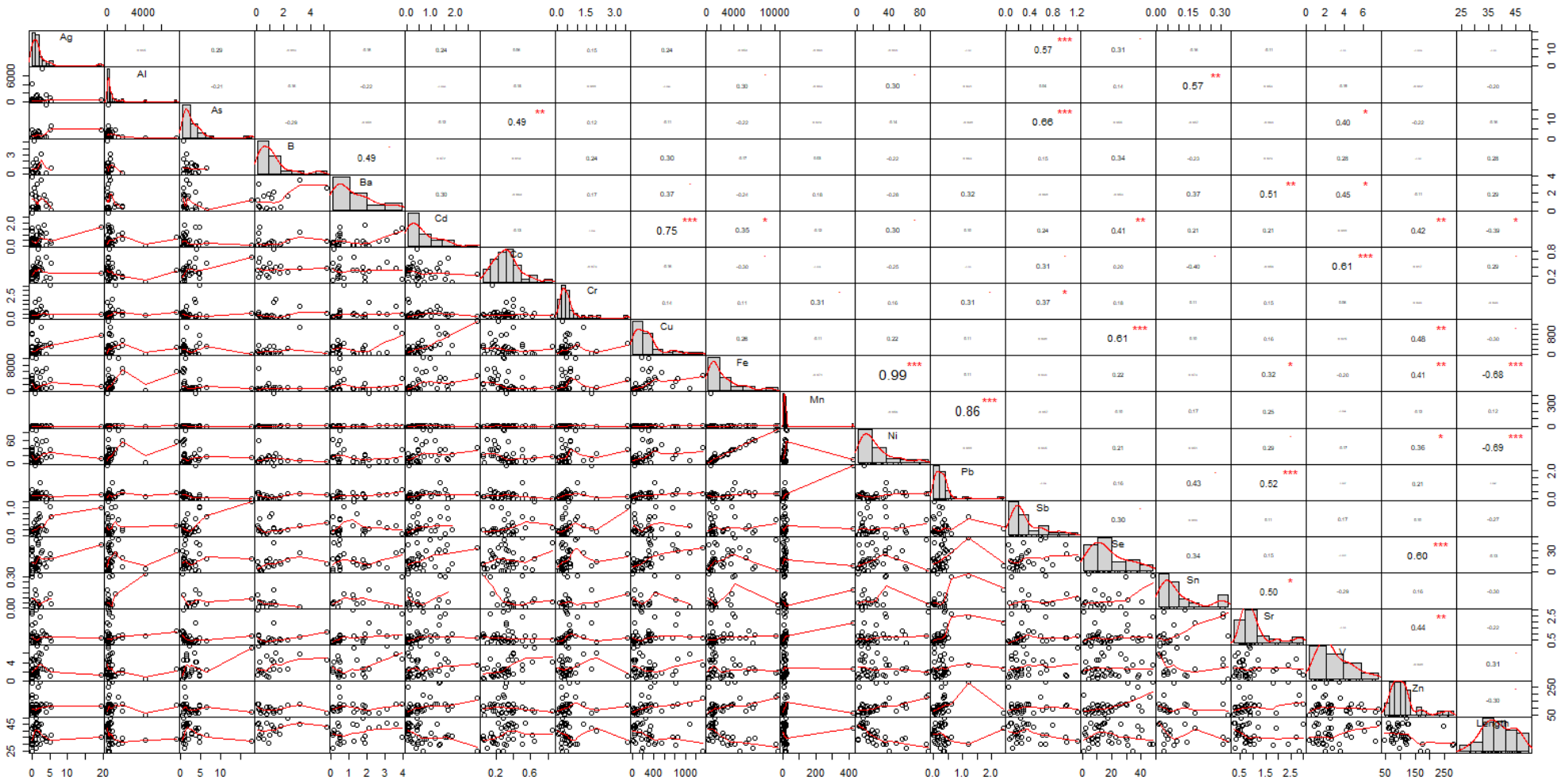

Figure 4.15 Inter-metals and metal-length relationships observed in the liver of *Labeo rosae* from Loskop and Flag Boshielo dams.

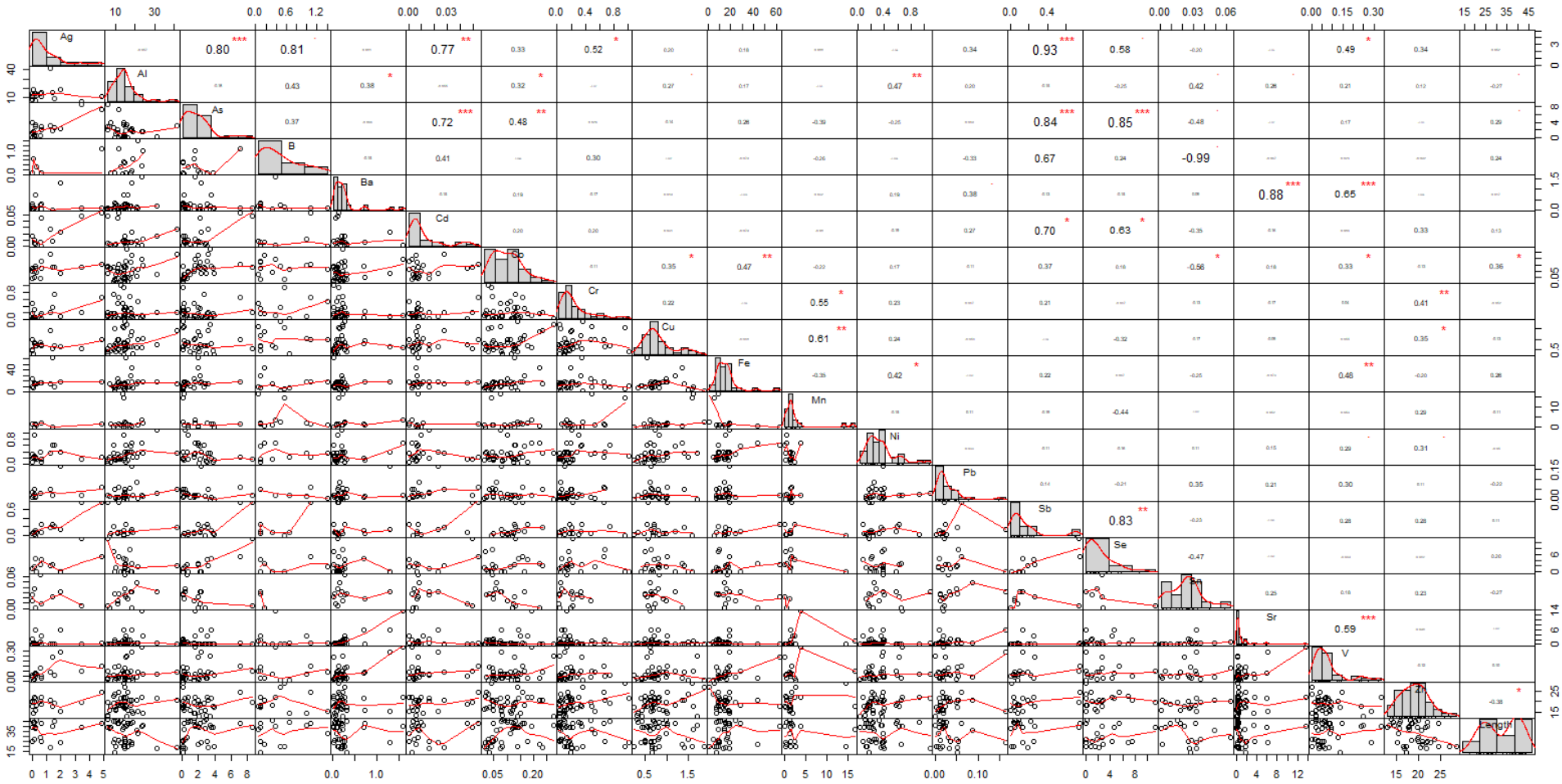

Figure 4.16 Inter-metals and metal-length relationships observed in the muscle of *Oreochromis mossambicus* from Loskop and Flag Boshielo dams.

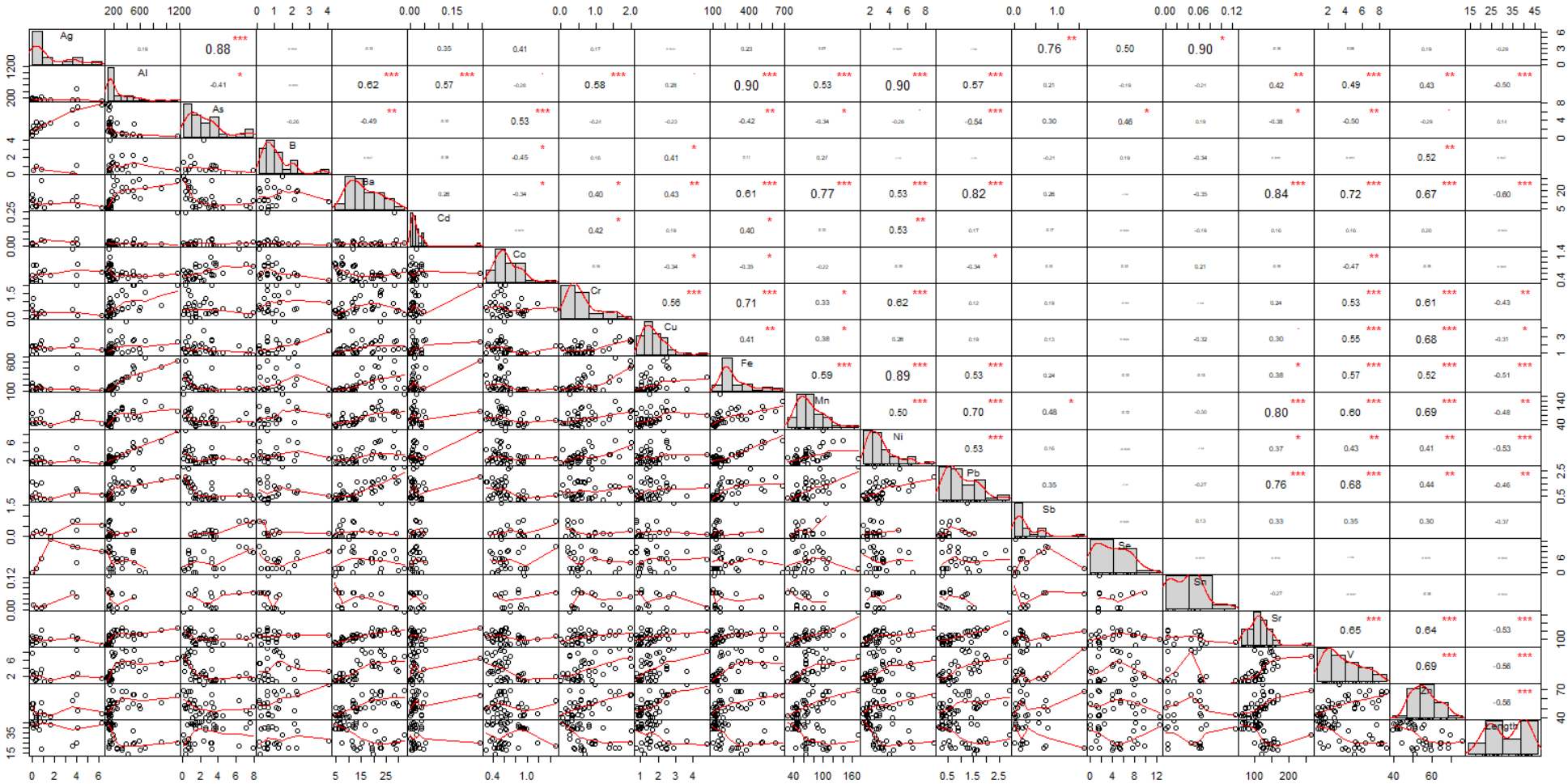

Figure 4.17 Inter-metals and metal-length relationships observed in the gills of *Oreochromis mossambicus* from Loskop and Flag Boshielo dams.

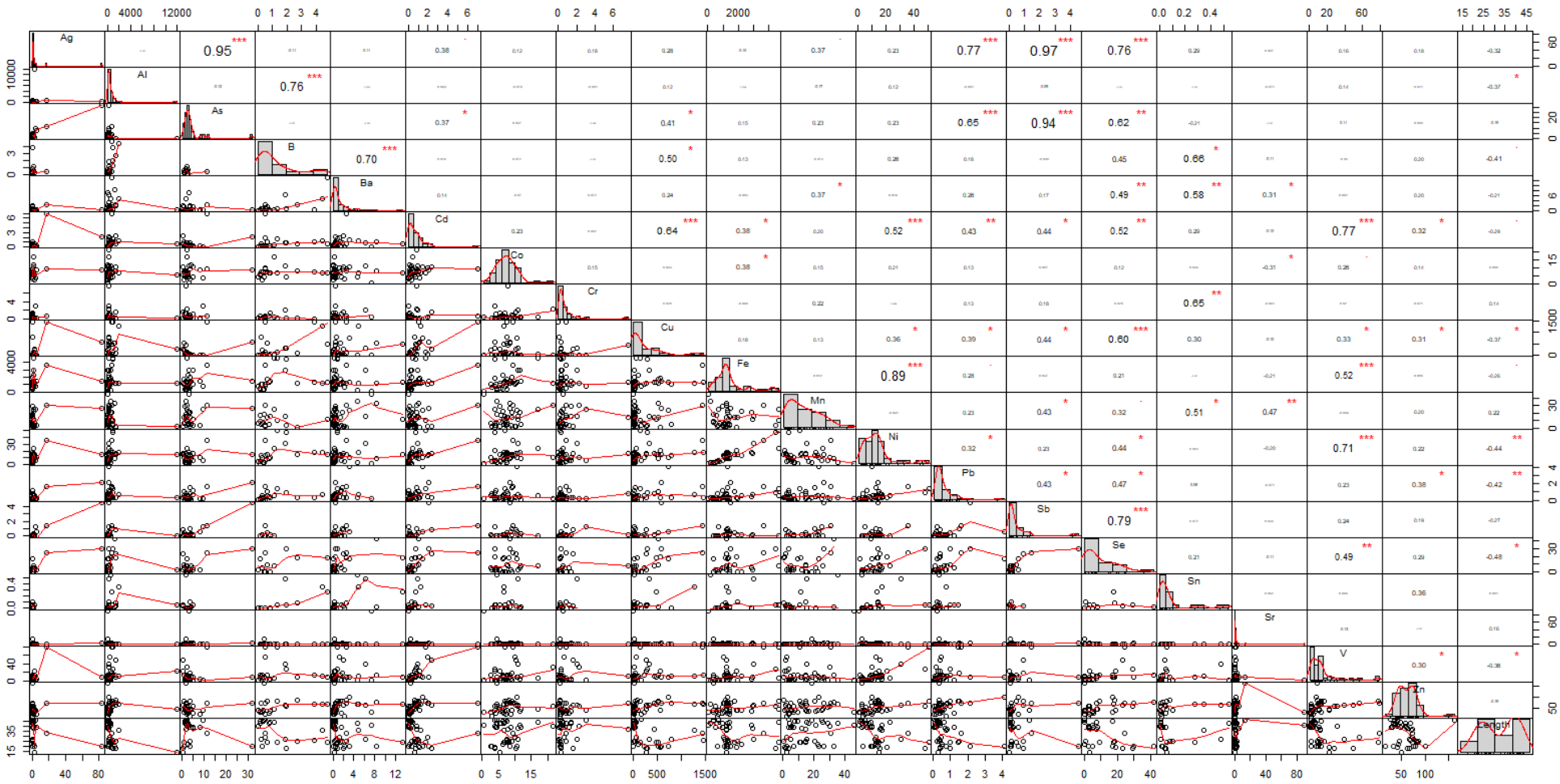

Figure 4.18 Inter-metals and metal-length relationships observed in the liver of *Oreochromis mossambicus* from Loskop and Flag Boshielo dams.

Table 4.2 The tissue-water bioaccumulation factor (BAF_w) recorded for *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during winter and summer surveys in 2014.

Metals	Loskop Dam			Flag Boshielo Dam		
	Liver	Gill	Muscle	Liver	Gill	Muscle
Labeo rosae						
Ag	N.C	N.C	N.C	N.C	N.C	N.C
Al	N.C	N.C	N.C	N.C	N.C	N.C
As	N.C	N.C	N.C	N.C	N.C	N.C
B	53.6802	59.95751	7.19213	17.26667	29.87607	12.59387
Ba	23.40764	756.1521	27.69476	18.09	969.3859	19.2671
Cd	N.C	N.C	N.C	N.C	N.C	N.C
Co	N.C	N.C	N.C	N.C	N.C	N.C
Cr	N.C	N.C	N.C	N.C	N.C	N.C
Cu	N.C	N.C	N.C	N.C	N.C	N.C
Fe	45618.27	11065.27	1233.352	N.C	16467.54	905.3665
Mn	1403.015	2596.818	109.1933	N.C	2268.837	120.9892
Ni	N.C	N.C	N.C	N.C	N.C	N.C
Pb	N.C	N.C	N.C	N.C	N.C	N.C
Sb	N.C	N.C	N.C	N.C	N.C	N.C
Se	N.C	N.C	N.C	1045.232	184.4255	162.9642
Sn	N.C	N.C	N.C	N.C	N.C	N.C
Sr	5.213388	1538.89	62.29282	6.257602	1625.444	57.55567
V	N.C	N.C	N.C	N.C	N.C	N.C
Zn	9461.616	11528.83	1029.883	N.C	N.C	N.C
Oreochromis mossambicus						
Ag	N.C	N.C	N.C	N.C	N.C	N.C
Al	N.C	N.C	N.C	N.C	N.C	N.C
As	N.C	N.C	N.C	N.C	N.C	N.C
B	21.58308	30.47718	15.11587	210.0869	44.12337	17.19053
Ba	21.259	198.644	5.646822	32.24112	363.0976	3.59725
Cd	N.C	N.C	N.C	N.C	N.C	N.C
Co	N.C	N.C	N.C	N.C	N.C	N.C
Cr	N.C	N.C	N.C	N.C	N.C	N.C
Cu	N.C	N.C	N.C	N.C	N.C	N.C
Fe	72869	8020.279	1285.769	26144.06	N.C	559.9305
Mn	827.6913	2588.272	49.21125	91622.73	4681.445	270.567
Ni	N.C	N.C	N.C	N.C	N.C	N.C
Pb	N.C	N.C	N.C	N.C	N.C	N.C
Sb	N.C	N.C	N.C	N.C	N.C	N.C
Se	N.C	N.C	N.C	51.41134	233.4215	93.7702
Sn	N.C	N.C	N.C	N.C	N.C	N.C
Sr	44.49675	655.347	9.622121	0.789784	801.5887	6.079011
V	N.C	N.C	N.C	N.C	N.C	N.C
Zn	4840.424	4685.612	1828.192	N.C	N.C	N.C

N.C = not calculated due to low concentration in the water (below detection limit)

Table 4.3 The tissue-sediment bioaccumulation factor (BAF_s) recorded for *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during winter and summer surveys in 2014.

Metals	***Labeo rosae***					
	Loskop Dam			Flag Boshielo Dam		
	Liver	Gill	Muscle	Liver	Gill	Muscle
Ag	N.C	N.C	N.C	N.C	N.C	N.C
Al	0.00529	0.003766	0.00075	0.03199	0.00812	0.00097
As	0.46041	0.422984	0.16783	0.20128	0.2327	0.10603
B	0.16798	0.187622	0.02251	0.06204	0.10734	0.04525
Ba	0.00577	0.18636	0.00683	0.00594	0.31854	0.00633
Cd	N.C	N.C	N.C	N.C	N.C	N.C
Co	0.01352	0.01311	0.00105	0.00656	0.00526	0.00068
Cr	0.00577	0.01048	0.01026	0.00797	0.00944	0.00503
Cu	6.57927	0.04974	0.01793	15.5114	0.06171	0.027
Fe	0.02389	0.00579	0.00065	0.13454	0.01014	0.00056
Mn	0.03372	0.0624	0.00262	0.01429	0.05452	0.00291
Ni	0.02962	0.00931	0.00104	0.11721	0.0095	0.00178
Pb	0.01601	0.02723	0.00399	0.01927	0.03709	0.00963
Sb	N.C	N.C	N.C	N.C	N.C	N.C
Se	0.41912	0.21126	0.09741	0.61202	0.10799	0.09542
Sn	0.00137	0.00164	0.00094	N.C	N.C	N.C
Sr	0.0005	0.14819	0.006	0.04054	10.5291	0.37283
V	0.05971	0.04002	0.00424	0.03197	0.02323	0.0022
Zn	0.73006	0.88957	0.07947	1.02217	1.34202	0.19129
	Oreochromis mossambicus					
Ag	N.C	N.C	N.C	N.C	N.C	N.C
Al	0.006269	0.003814	0.000361	0.036942	0.01242	0.000463
As	0.464553	0.423878	0.370943	0.913534	0.153906	0.083602
B	0.067539	0.095371	0.047301	0.231673	0.158527	0.061762
Ba	0.005239	0.048956	0.001392	0.014274	0.119315	0.001182
Cd	N.C	N.C	N.C	N.C	N.C	N.C
Co	0.252241	0.023527	0.004526	0.25199	0.016911	0.002025
Cr	0.009403	0.005454	0.002566	0.013742	0.012106	0.003471
Cu	0.94924	0.049025	0.028627	19.22483	0.076968	0.033042
Fe	0.038155	0.0042	0.000673	0.056441	0.010581	0.000345
Mn	0.01989	0.062198	0.001183	0.014979	0.112499	0.006502
Ni	0.024176	0.005982	0.001028	0.060265	0.012607	0.001025
Pb	0.018102	0.03076	0.000921	0.056642	0.082228	0.002397
Sb	N.C	N.C	N.C	N.C	N.C	N.C
Se	0.116593	0.150602	0.091189	0.553516	0.136676	0.054905
Sn	0.003817	0.002169	0.000284	N.C	N.C	N.C
Sr	0.004285	0.063108	0.000927	0.034399	5.192431	0.039378
V	0.106266	0.033694	0.001431	0.423837	0.09383	0.000929
Zn	0.37349	0.361544	0.141064	0.541241	0.468801	0.157593

N.C = not calculated due to low concentration in the water (below detection limit).

4.4 Discussion

4.4.1 Metal concentration in tissues

Metals are not biodegradable, and can accumulate and magnify up the food chain until the organism at the top of the food chain accumulate lethal dose (Davies & Day 1998; Khan *et al.* 2012). Fish are often near or at the top of the food chain (Khan *et al.* 2012) and can accumulate metals million times more than organisms at the bottom of the food chain (Davies & Day 1998). Metal accumulation in fish may occur through food ingestion, and metal ion exchange through gills and skin (Akan *et al.* 2012). In the present study, most metals exhibited relatively higher metal concentrations in the liver followed by gills and muscle for both species at Loskop and Flag Boshielo dams. This trend corroborates findings reported by other bioaccumulation studies in South Africa and elsewhere (Crafford & Avenant-Oldewage 2011; Qadir & Malik 2011; Marr *et al.* 2017). The increased metal concentrations in the liver may be explained by the fact that it is a primary site for detoxification (Zhao *et al.* 2012). However, other metals such as copper, iron, selenium, zinc etc. are essential for proper functioning of various physiological processes (Sauliutė & Svecevičius 2015) and were found in a significantly high concentration in the liver of both *L. rosae* and *O. mossambicus* in the present study.

Increased concentration of copper and zinc are associated with natural binding proteins such as metallothioneine proteins (MTs) which act as metal store (i.e. copper and zinc) to fulfil metabolic demands whereas iron is associated with blood cells and haemoglobin synthesis (El-Moselhy *et al.* 2014). Selenium is the most important component of antioxidant enzymes (Olmedo *et al.* 2013) and it was also reported to promote elimination of methyl mercury from freshwater fish tissues (Bjerregaard *et al.* 2011). Moreover, selenium was reported to enhance the activities of enzymes, acting as a protective agent against the toxicity of heavy metals such cadmium, lead, mercury etc. (Can *et al.* 2012). Substantially higher concentrations of copper, iron, selenium and zinc in the liver of *L. rosae* and *O. mossambicus* coincided with those reported for *L. umbratus* and *C. gariepinus* in the upper Olifants River (Coetzee *et al.* 2002); *Labeobarbus kimberleyensis*, *L. umbratus* (Gilbert *et al.*

2017) and *C. gariepinus* (Crafford & Avenant-Oldewage 2011) in the Vaal River system.

Gills have also shown to accumulate significant concentrations of metals. Gill is in direct contact with the external water environment and it is described as the main route of metal ion exchange from water (Khan *et al.* 2012; El-Moselhy *et al.* 2014). Moreover, the large surface area characterised by thin epithelium facilitate rapid diffusion in the gills (El-Moselhy *et al.* 2014). In the present study, lead, manganese and strontium were found to have accumulated more in the gills than in other tissues for both species. Gills are the primary site of accumulation of waterborne metals and most metals taken up directly from the water column are found to accumulate more in the gills than in the liver (Wepener *et al.* 2001). It is therefore, reasonable to infer that these metals were accumulated directly from the water column at both dams. Given that sediment exhibited significantly high metal concentrations, water may still experience metal concentration without the pollutants input from external sources. In this regard, further increase of lead, manganese and strontium concentrations in the gills of fish at Loskop and Flag Boshielo dams is inevitable. The trend of lead, manganese and strontium accumulating more in the gills than in the liver corroborated the finding observed for *Barbus marequensis* in the lower Olifants River (Seymore *et al.* 1995), *Labeo umbratus* and *C. gariepinus* in the upper Olifants River (Coetzee *et al.* 2002), *C. gariepinus* in the Vaal River system (Crafford & Avenant-Oldewage 2010) and *Cyprinus carpio* in Pakistan (Khan *et al.* 2012).

It is well known that muscle is not an active site for metal biotransformation and accumulation (Diamond *et al.* 1992; El-Moselhy *et al.* 2014). Metal concentration in the muscle may thus, not be used as a reflection of metal levels in aquatic ecosystems (Avenant-Oldewage & Marx 2000b). However, muscle may be included in a bioaccumulation study in a sense that it is the tissue consumed by human. Muscle exhibited the lowest metal concentrations as compared to the liver and gills for both species at both dams. Metal concentrations recorded in the muscle of *O. mossambicus* and *L. rosae* were comparable to those recently reported in *L. rosae* (Lebepe *et al.* 2016), *O. mossambicus* (Addo-Bediako *et al.* 2014b) and *C. gariepinus*

(Jooste *et al.* 2015); and significantly higher compared to those reported two decades ago in the muscle of *O. mossambicus* (Robinson & Avenant-Oldewage 1997; Kotze *et al.* 1999), *C. gariepinus* (Kotze *et al.* 1999) and *Barbus marequensis* (Seymore *et al.* 1995) in the Olifants River system. Moreover, recent studies have shown that some metals have now exceeded the international threshold for safe consumption in the muscle tissues (Addo-Bediako *et al.* 2014a, b; Jooste *et al.* 2014, 2015; Lebepe *et al.* 2016).

4.4.2 Inter-metal and metal-fish length relationships

Metals vary greatly with regard to their biological availability or ability to enter organisms and cause toxicity (Caussy *et al.* 2003). Water hardness, pH, temperature, oxygen content, the availability of other metals etc. are known to influence metal bioavailability, hence, bioaccumulation (Coetzee *et al.* 2002). Caussy *et al.* (2003) reported that adequate stores of dietary iron, and zinc inhibit the absorption of cadmium whereas dietary calcium, zinc, and other cations are reported to inhibit the absorption of iron. In the present study, inter-metal relationship was not clear but zinc was in most cases showing positive relationship with Fe and Cu. The positive relationship between iron, copper and zinc was also observed for *Schilbe intermedius* (Addo-Bediako *et al.* 2014a) and *O. mossambicus* (Addo-Bediako *et al.* 2014b) in the Olifants River.

Metal accumulation in fish may also be influenced by the size (Coetzee *et al.* 2002). However, in the present study, there was poor relationship between metal concentrations and fish length on both species. Coinciding with the current study, Arantes *et al.* (2016) and Canli & Atli (2003) reported poor relationship between fish length and metal concentrations in Brazil and Turkey, respectively. Despite the fact that fish growth can influence metal accumulation (Ward *et al.* 2010), the overall relationship remains unclear. Coetzee *et al.* (2002) reported decreasing metal concentrations with increasing fish size in freshwater fish in the Olifants River. Kraemer *et al.* (2006) emphasised that if the growth rate of the fish is faster than the rate of accumulation, the metal concentration will decrease due to somatic growth

dilution. This fact was supported for some metals on both species in the present study.

4.4.3 Bioaccumulation Factor

The BAF can be described as the ratio of metal concentration in the organism and the environment; either water or sediment (Arnot & Gobas 2006; Jakimska *et al.* 2011). The primary objective of BAF is to determine if biomagnification has taken place within the ecosystem with the factor of greater than 1 indicating bioaccumulation as well as biomagnification (Rashed 2001). Bioaccumulation factor is widely considered as a useful tool for assessing the extent to which metals have transferred from the environment to the organisms (Jakimska *et al.* 2011).

In the present study, the BAF_w of greater than 1 was observed for all metals which were detected in the water. In contrast, BAF_s exhibited values of less than 1 except for copper, strontium and zinc. This trend entails that metal concentrations were higher in sediment than in fish tissues (BAF_s) and lower in the water than in the fish tissues (BAF_w). These results were comparable to those reported by other studies in the Olifants River system for *Labeo* species (Coetzee *et al.* 2002; Lebepe 2012). Generally, high BAF_w may infer that fish have accumulated metals from the water rather than from the sediment (Abdel-Baki *et al.* 2013).

In aquatic ecosystem, sediment act both as sink, and as a carrier and future source of metals (Chau 2006). Upon entering aquatic ecosystems, metals are either taken up by aquatic biota such as fish or settle to the bottom sediment. These metals are not permanently fixed in sediment, they may under some circumstances such as change in physico-chemical properties be remobilised back into the water column (Coetzee *et al.* 2002; Chau 2006). Although metals seem to have been assimilated from the water column, sediment may have indirectly contributed to the metal increase in fish tissues.

Metal such as copper has shown higher concentrations in the liver as compared to sediment, hence, a BAF_s of greater than 1 was recorded for both species at both dams. These results coincided with those reported from Hanifah River at Al-Hair,

Saudi Arabia for tilapia species (Abdel-Baki *et al.* 2013) and Klein Olifants River in Mpumalanga for *Labeo umbratus* (Coetzee *et al.* 2002) and *L. rosae* (Lebepe 2012).

Strontium has also shown BAF_s of greater than 1 for gills in both species at Flag Boshielo Dam. Gills are described as the primary target organ for strontium accumulation due to its analogy with calcium which has its transport system in the chloride cells of the gills. However, strontium accumulation depends on its bioavailability which is controlled by factors such as pH and salinity (Chowdhury & Blust 2002; Diouf *et al.* 2006). The trend of gill showing higher strontium BAF_s than liver and muscle was also observed for *Clarias gariepinus* in the Olifants River system in Kruger National Park (Avenant-Oldewage & Marx 2000a).

Zinc exhibited relatively high concentration in the liver and gills of *L. rosae* as compared to the sediment at Flag Boshielo Dam, hence, BAF_s was greater than 1. These results are comparable to those reported in the Klein Olifants River on *L. umbratus* (Coetzee *et al.* 2002) and Flag Bioshielo Dam on *L. rosae* (Lebepe 2012). The increase of metal concentrations in fish tissues may depend mainly on the particular physiological compounds synthesised in the tissue. For example, zinc participate in the synthesis of metallothionein, metalloenzymes and the stable 5- or 6- membered ring chelates (Liu *et al.* 2014). As a result, significantly elevated zinc concentrations may be expected in the liver.

CHAPTER 5

ENZYMATIC BIOMARKERS AS INDICATORS OF ENVIRONMENTAL POLLUTION IN FRESHWATER FISH

5.1 Introduction

Management practices of aquatic ecosystems and regulatory practices with regard to contaminants discharge were primarily directed to the population level of biological organisation (Buckler & Tillitt 1996). The use of biomarkers, physiological, biochemical and histopathological parameters has received increasing attention during the recent few decades (Buckler & Tillitt 1996; Adedeji *et al.* 2012). The use of biomarkers was reported to be effective and reliable because effects at higher levels of biological organisations are always preceded by earlier changes in biological processes (Van der Oost *et al.* 2003). Biomarkers are now widely used in aquatic biomonitoring to provide early warning signals of potential ecosystem degradation caused by contaminants (Viarengo *et al.* 2007; Adedeji *et al.* 2012; Ayoola *et al.* 2014).

Biomarkers can be subdivided into three classes:

- Biomarkers of exposure which covers the detection and measurement of an exogenous substance.
- Biomarkers of effect which include measurable biochemical, physiological or other responses within tissues.
- Biomarkers of susceptibility which indicate the inherent or acquired ability of an organism to respond to the challenge of exposure to specific contaminants (Van der Oost *et al.* 2003).

Several enzymatic biomarkers may be employed to assess the exposure and effect of contaminants in aquatic ecosystems e.g. acetylcholinesterase (AChE) for pesticides exposure (Wepener *et al.* 2011), catalase and glutathione S-transferase (GST) for oxidative stress induced by metals (Mohamed *et al.* 2008; Saliu & Bawa-Allah 2012).

The use of biomarkers in biomonitoring has considerable advantages. Biomarkers provide early warning signals of adverse biological effects and it may provide an understanding of the specific mechanisms of toxicity of a chemical. For example, induction of certain detoxification enzymes (e.g. cytochrome P450 1A) occurs through activation of the aryl hydrocarbon receptor (AhR), which in itself may result in further changes. Biomarker may also be used to measure the effectiveness of remedial actions (Long *et al.* 2004; Adedeji *et al.* 2012). Like other biomonitoring tools, the use of biomarkers may however, have some limitations.

Limitations in the use of biomarkers include expertise and experience requirements because improper interpretation of biomarker responses may lead to false conclusions. Moreover, some responses established for one species may not necessarily be valid for other species (Van der Oost *et al.* 2003). Most biomarkers have limited specificity, they respond to variety of environmental contaminants. Therefore, multiple biomarkers at different levels of biological organisation may be required for effective application in a biomonitoring program (Van der Oost *et al.* 2003; Hamza-Chaffai 2014).

The use of glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) as liver toxicity biomarker has emerged during the past few decades (Winzer *et al.* 2002). These enzymes are described as key components in the metabolism and are highly sensitive to pollutants (Winzer *et al.* 2002; Osman *et al.* 2010). Glutathione-S-transferases is an intracellular enzyme of the second stage of xenobiotic metabolism and has widely been used as an oxidative stress biomarker (Rudneva *et al.* 2010; Saliu & Bawa-Allah 2012). Acetylcholinesterase (AChE) is the primary target for the action of pesticides in fish and has extensively been used as biomarker of carbamates and organophosphorus exposure (Mahboob *et al.* 2014). In the present study, LDH, G6PDH and GST were measured in the liver and AChE in the brain to assess the effect of environmental contaminants on *Labeo rosae* and *Oreochromis mossambicus* at Loskop and Flag Boshielo dams in the Olifants River system.

5.2 Materials and methods

5.2.1 Fish and tissue sampling

Sampling of fish was carried out as using gill nets with different mesh sizes (50 - 120 mm). A minimum of ten fish specimens were collected for each species during winter (May/Jun) and summer (Nov/Dec) sampling in 2014 at Loskop and Flag Boshielo dams. Fish were euthanised and dissected ventrally using a dissecting scissor. Liver samples were dissected out, put in plastic vials and immediately stored in liquid nitrogen. Brain samples were put in plastic vials containing phosphate buffer and stored in liquid nitrogen. All samples were later transferred to -80°C biofreezer at the University of Limpopo, Biotechnology Unit.

5.2.2 Biomarkers

5.2.2.1 Lactate dehydrogenase

Liver tissue samples were pulverised using cold mortar and about 100 mg of ground tissue powder was added to 5 volume of 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM Ethylenediaminetetraacetic acid (EDTA) and 2 mM $MgCl_2$. Lactate dehydrogenase was assayed following Vassault (1983) protocol. Samples were homogenised and homogenates were centrifuged for 15 min at 10 000 x g and 4ºC. Supernatants were used for the enzyme activity assays using a Beckman Coulter DTX 880 Multimode Detector microplate reader, at a wavelength of 340 nm and at 37 ºC. A 15 µℓ aliquot of diluted sample (1/40 dilution in all species) was mixed with 75 µℓ NADH (300 mM) and 25 µℓ pyruvate (4.5 mM) and the decrease in absorbance measured over 3 min at 340 nm. The assay was performed in triplicate. Activity was expressed in $\mu mol.min^{-1}.mg^{-1}$ protein. The protein concentration was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin in homogenisation buffer as a standard.

5.2.2.2 Glucose-6-phosphate dehydrogenase

About 100 mg of ground tissue powder was added to 5 volume of buffer (50 mM Tris, pH 7.4, 1 mM EDTA and 2 mM $MgCl_2$). The activity of G6PDH was assayed following

Zaheer *et al.* (1967) method. Samples were homogenised and homogenates were centrifuged for 15 min at 10 000 x g and 4ºC. Supernatants were used for the enzyme activity assays using a microplate reader, Beckman Coulter DTX 880 Multimode Detector at a wavelength of 340 nm and at 37ºC. The reaction mixture in a total volume of 150 µℓ consisted of 0.05 M Tris–HCl buffer, pH 7.6, 30 µℓ 0.1 mM nicotinamide adenine dinucleotide phosphate (NADP, Sigma, St Louis, USA), 0.8 mM glucose-6-phosphate (Sigma), 8 mM $MgCl_2$ (Merck, Mumbai), 10% (15 µℓ) post-mitochondrial supernatant (PMS) and 105 µℓ distilled water (Pandey *et al.* 2003). The assay was performed in triplicate. The change in absorbance at 25°C was recorded at 340 nm and enzyme activity was expressed in as $\mu mol.min^{-1}.mg^{-1}$ protein. The protein concentration was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin in homogenisation buffer as a standard.

5.2.2.3 Glutathione S-Transferase

Ground tissue samples were homogenised in chilled phosphate buffer (0.1M, pH7.4), and centrifuged at 10500 x g for 20 min at 4°C. The total GST activity was assayed according to Habig *et al.* (1974). The reaction mixture was made up of 165 µℓ (0.1 mM phosphate buffer, pH 7.4), 10 µℓ (1 mM GSH), 5 µℓ [1 $mmol.ℓ^{-1}$ 1-chloro-2-dinitrobenzene (CDNB)] and 10% (20 µℓ) post mitochondrial supernatant of a total volume of 200 µℓ. The assay was performed in triplicate. Absorbance variation was read at 340 nm wavelength at 25°C. The change in absorbance was measured using a microplate reader (Beckman Coulter DTX 880 Multimode). The total GST catalytic activity was expressed in $\mu mol.min^{-1}.mg^{-1}$ protein. The protein concentration was estimated according to the method of Lowry *et al.* (1951) using bovine serum albumin in homogenisation buffer as a standard.

5.2.2.4 Acetylcholinesterase

Ground tissue samples were homogenized in 0.1 M phosphate buffer, pH 8.0 (20 mg tissue in 1 mℓ of the buffer) and centrifuged at 12000 rpm. The supernatant was decanted for acetylcholine esterase analysis. Acetylcholinesterase activity was determined following the method of Ellman *et al.* (1961). The assay was performed in

triplicate whereby the supernatant (75 µℓ) was added to 0.1 M phosphate buffer pH 8.0 and 75 µℓ of 0.01M 5.50-dithiobis-2-nitrobenzoic acid in a microtiter plate wells. The reaction was started with the addition of 15 µℓ of 0.075M acetylthiocholine iodide. The change in absorbance was measured using a microplate reader (Beckman Coulter DTX 880 Multimode). The absorbance was read at 412 nm for four minute at 25°C and the AChE activity was expressed in $\mu mol.min^{-1}.mg^{-1}$ protein. The protein concentration was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin in homogenization buffer as a standard.

5.2.3 Data analysis

Enzyme activities were presented as mean and standard deviation. Box and whisker plots were made using R 3.1.1 (R Development Core Team 2014). The normality of the data was tested using Shapiro-Wilk test and Levene's test was used to test the homogeneity of variance. Depending on the distribution of the data, an independent sample t-test or Wilcoxon-Mann-Whitney U-test was used to compare results between the two dams. Differences at the 5% significance level were considered significant.

5.3 Results

5.3.1 Enzyme activities

Activities of enzymes for fish from Loskop and Flag Boshielo dams are presented as box and whisker plot (Fig. 5.1). Mean enzyme activities are presented in Table 5.1. More than 75% of *L. rosae* sampled from Loskop Dam have shown LDH activity of >30 μmol.min^{-1}.mg^{-1} protein with the activity <30 μmol.min^{-1}.mg^{-1} protein being observed for more than 75% of *L. rosae* from Flag Boshielo Dam (Fig. 5.1). There was a significant difference ($p<0.05$) in the LDH activities of *L. rosae* between the two dams. About 50% of *O. mossambicus* collected from Loskop Dam have shown LDH activities ranging from 15 to 25 μmol.min^{-1}.mg^{-1} protein. However, more than 75% of *O. mossambicus* from Flag Boshielo Dam exhibited activities ranging from 5 to 20 μmol.min^{-1}.mg^{-1} protein (Fig.5.1). The LDH activity of *O. mossambicus* has shown no significant difference ($p>0.05$) between the two dams.

Over 40% of the sampled *O. mossambicus* population exhibited G6PDH activities of >10 μmol.min^{-1}.mg^{-1} protein at Flag Boshielo Dam. At Loskop Dam, about 75% of *O. mossambicus* population have shown G6PDH activities of <5 μmol.min^{-1}.mg^{-1} protein (Fig. 5.1). Glucose-6-phosphate dehydrogenase activities of <10 μmol.min^{-1}.mg^{-1} protein were observed for about 75% of *L. rosae* population from Flag Boshielo Dam (Fig. 5.1). Over 50% of *L. rosae* sampled from Loskop Dam have shown G6PDH activities of <5 μmol.min^{-1}.mg^{-1} protein (Fig. 5.1). Both species have shown greater variability of G6PDH activities at Flag Boshielo dam as compared to activities at Loskop Dam (Fig. 5.2). Moreover, G6PHD activities have shown no significant differences ($p>0.05$) between the two dams for both species.

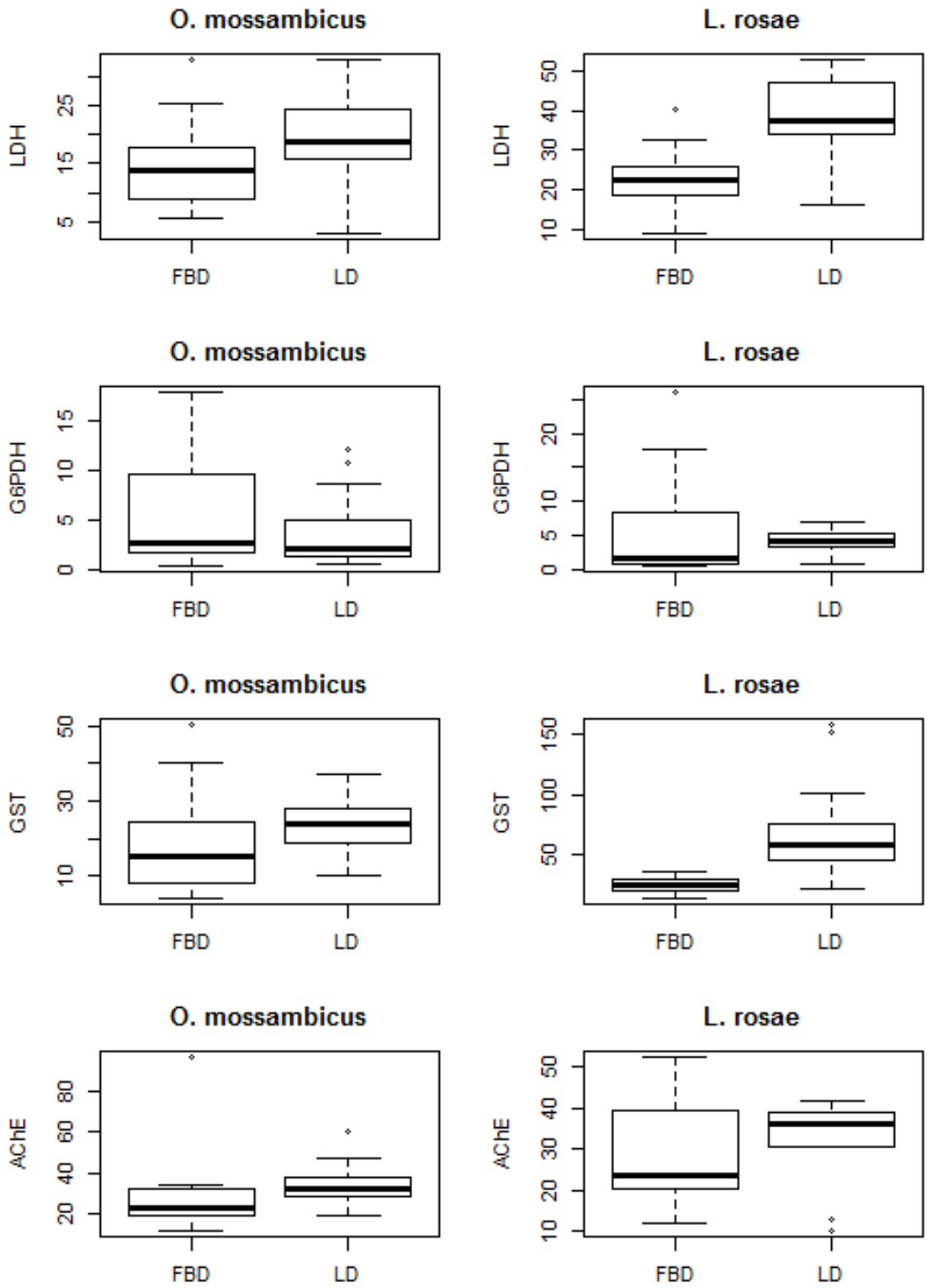

Figure 5.1 Enzyme activities recorded at Loskop Dam (LD) and Flag Boshielo Dam (FBD) for *Oreochromis mossambicus* and *Labeo rosae* during 2014 surveys.

Table 5.1 Mean enzyme activities ± standard deviation measured in the liver of *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams.

Enzymes ($\mu mol.min^{-1}.mg^{-1}$ protein)	O. mossambicus		L. rosae	
	Loskop Dam (N=20)	Flag Boshielo Dam (N=20)	Loskop Dam (N=20)	Flag Boshielo Dam (N=20)
LDH	19.60 ± 7.13	14.72 ± 6.67	37.89 ± 10.81	23.24 ± 7.15
G6PDH	3.94 ± 3.66	6.01 ± 5.84	4.24 ± 1.46	5.75 ± 7.44
GST	24.05 ± 7.43	18.84 ± 15.16	68.76 ± 36.54	24.26 ± 6.44
AChE	33.71 ± 9.81	30.01 ± 22.98	31.89 ± 10.62	28.64 ± 12.23

All sampled *L. rosae* population has shown GST activity of <50 $\mu mol.min^{-1}.mg^{-1}$ protein at Flag Boshielo Dam and about 70% of Loskop Dam population exhibited activities ranging from 50 to 100 $\mu mol.min^{-1}.mg^{-1}$ protein (Fig. 5.1). The Loskop Dam *L. rosae* population have shown great variability of GST activities. There was a significant difference ($p<0.05$) in the GST activities of *L. rosae* between the two dams. The GST activities of the sampled *O. mossambicus* population have shown no significant difference between the two dams ($p>0.05$). About 75% of *O. mossambicus* from Loskop Dam exhibited GST activities ranging from 10 to 30 $\mu mol.min^{-1}.mg^{-1}$ protein. Over 50% of *O. mossambicus* from Flag Boshielo Dam have shown GST activities of <15 $\mu mol.min^{-1}.mg^{-1}$ protein (Fig. 5.1).

Labeo rosae population from Flag Boshielo Dam has shown greater variability in AChE activities as compared to Loskop Dam population (Fig. 5.1). About 50% of the *L. rosae* from Flag Boshielo Dam have shown the AChE activities ranging from 20 – 40 $\mu mol.min^{-1}.mg^{-1}$ protein (Fig. 5.1). Over 75% of *L. rosae* from Loskop Dam have exhibited AChE activities ranging from 30 – 42 $\mu mol.min^{-1}.mg^{-1}$ protein (Fig. 5.1). However, no significant difference ($p>0.05$) was observed on the *L. rosae* AChE activities between the two dams. *Oreochromis mossambicus* has also shown no significant difference ($p>0.05$) on the AChE activities between the two dams. The variability of the AChE activities among *O. mossambicus* was lower at Flag Boshielo Dam as compared to the Loskop Dam population. About 70% of the *O. mossambicus* population exhibited AChE activities <30 $\mu mol.min^{-1}.mg^{-1}$ protein at Flag Boshielo

Dam (Fig. 5.1). Over 60% of *Oreochromis mossambicus* sampled from Loskop Dam showed AChE activities of >30 µmol.min^{-1}.mg^{-1} protein (Fig. 5.1).

5.3.2 Enzyme activities and fish length relationship

Lactate dehydrogenase has shown no correlation between the activity and fish length for both fish species at both dams (Fig. 5.2). A slight positive correlation was observed for G6PDH activity on *O. mossambicus* from Flag Boshielo Dam (Fig. 5.3). The length of *O. mossambicus* from Loskop Dam exhibited a slight negative correlation with the G6PDH activity (Fig. 5.3). Glutathione S-transferase has shown slight positive correlation with the length of *L. rosae* from Loskop Dam and no relationship was observed for *O. mossambicus* at both dams (Fig. 5.4). Acetylcholinesterase activity has shown positive correlation with the length of *O. mossambicus* from Loskop Dam (Fig. 5.5). However, a slight positive correlation was observed for *L. rosae* from both dams (Fig. 5.5).

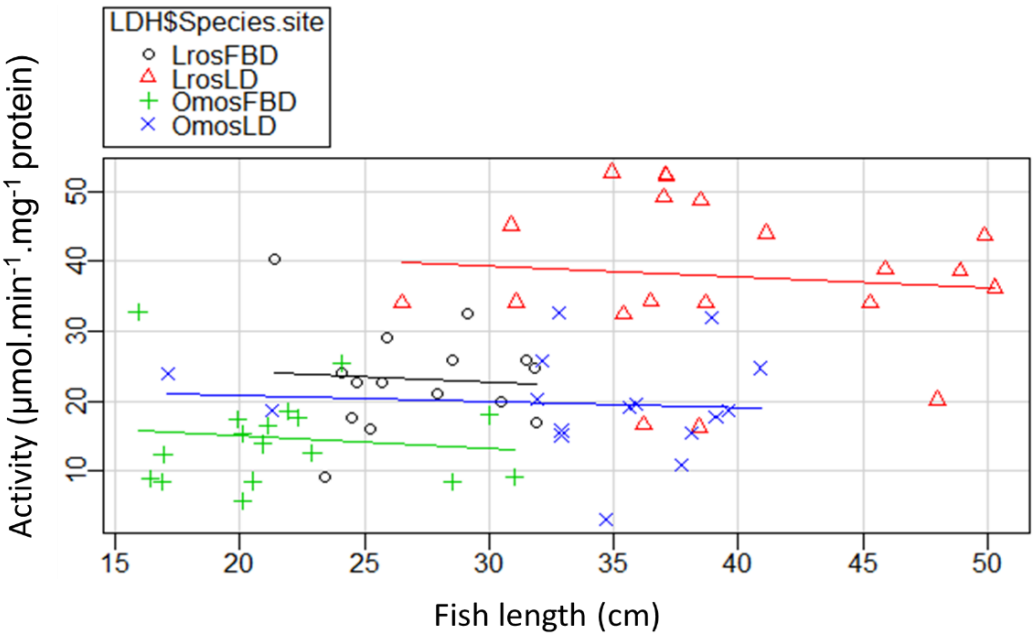

Figure 5.2 Correlation between lactate dehydrogenase activity and the length of *Oreochromis mossambicus* and *Labeo rosae* sampled from Loskop and Flag Boshielo dams during 2014 surveys.

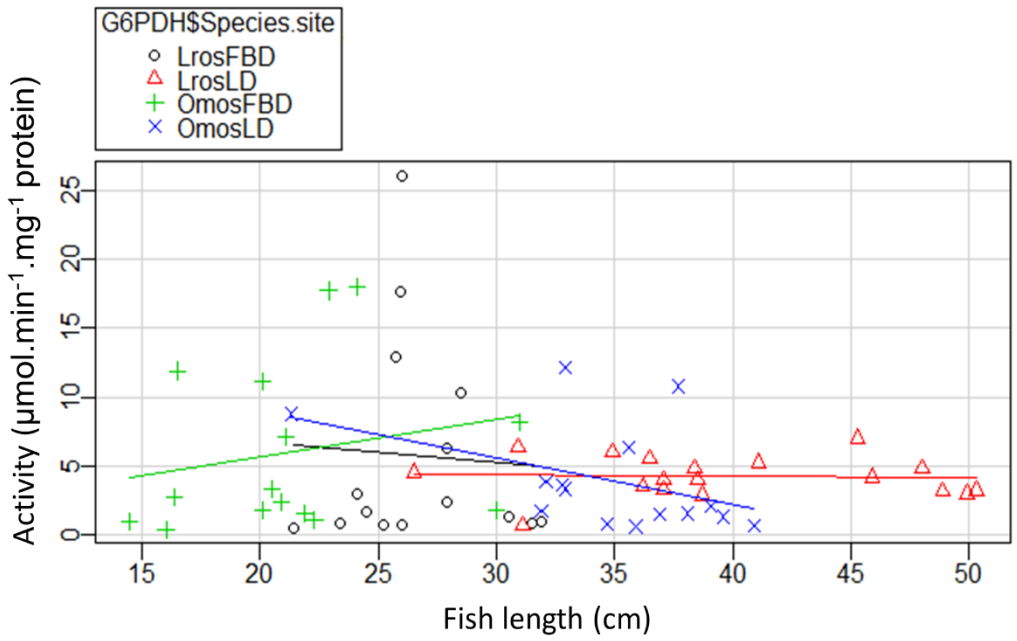

Figure 5.3 Correlation between glucose-6-phosphate dehydrogenase activity and the length of *Oreochromis mossambicus* and *Labeo rosae* sampled from Loskop and Flag Boshielo dams during 2014 surveys.

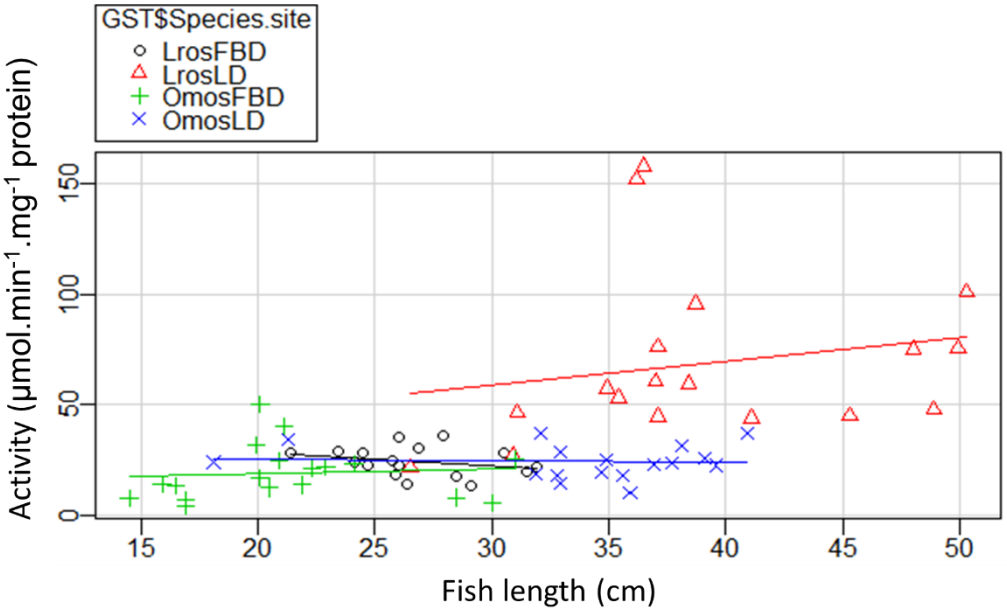

Figure 5.4 Correlation between glutathione S-transferase activity and the length of *Oreochromis mossambicus* and *Labeo rosae* sampled from Loskop and Flag Boshielo dams during 2014 surveys.

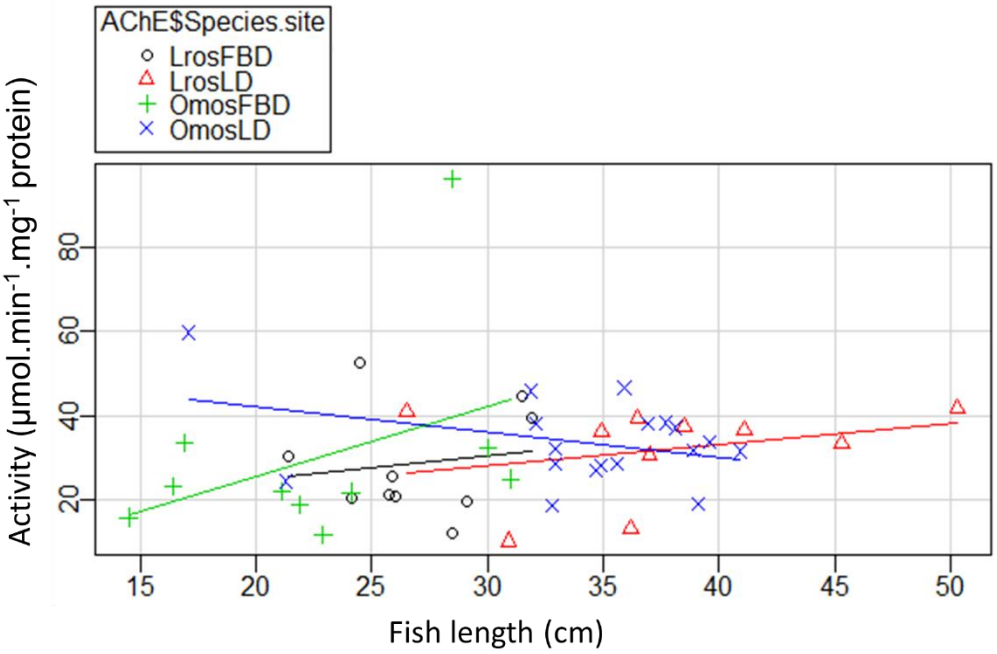

Figure 5.5 Correlation between acetylcholinesterase activity and the length of *Oreochromis mossambicus* and *Labeo rosae* sampled from Loskop and Flag Boshielo dams during 2014 surveys.

5.4 Discussion

The use of enzymatic biomarkers to assess the effects of environmental contaminants has been widely advocated in a sense that it provides early warning signals of exposure (Van der Oost *et al.* 2003; Osman *et al.* 2010). Different biomarkers respond differently based on their physiological functions (Helfman *et al.* 2009). Lactate dehydrogenase is the terminal enzyme of anaerobic glycolysis located in the cellular cytoplasm (Cohen *et al.* 2005). During glycolysis, LDH converts pyruvate from glucose to lactic acid which is produced in larger amounts during a period of stress, exercise and/or hypoxia. Lactate dehydrogenase commonly reflects the metabolic capacity of the tissue after a long term exposure to environmental contaminants (Osman *et al.* 2010).

In the present study, relatively high LDH activities were recorded in the liver of fish from Loskop Dam. In contrast, most metals exhibited higher concentrations in the liver of fish from Flag Boshielo Dam. The increased metal concentrations in the liver of fish from Flag Boshielo Dam seem to have inhibited LDH activities on both species. The inhibition of LDH by metal was also reported in *Daphnia magna* (Diamantino *et al.* 2001) and *Gambusia holbrooki* (Castro *et al.* 2004), and may be linked to the impaired carbohydrates metabolism (Ambili *et al.* 2013). The liver of fish from Loskop Dam exhibited elevated LDH activities. As mentioned earlier, LDH usually reflect the metabolic capacity of the tissue after a long term exposure to contaminants and increased activity may suggest a bias towards the anaerobic glycolytic pathway (Osman 2012). Adaptive changes taking place in the target organ as well as the sensitivity of the organ to toxicants may also influence the activity of LDH (Ambili *et al.* 2013).

In contrast with the LDH trend, G6PDH has shown relatively high activities for Flag Boshielo Dam populations. Glucose-6-phosphate dehydrogenase is a cytosolic enzyme that catalyses the first step in the pentose phosphate pathway which generates NADPH. Nicotinamide adenine dinucleotide phosphate is used by glutathione reductase to maintain the level of glutathione within the cell and protects the cell from oxidative damage. For this reason G6PDH is considered as an

antioxidant enzyme (Ursini *et al.* 1997; Gul *et al.* 2004; Osman *et al.* 2010). A relatively high G6PDH activity may have been associated with an elevated concentration of metals in the liver of fish populations from Flag Boshielo Dam. In fish exposed to elevated levels of contaminants, the activity of G6PDH increases to elevate the production of NADPH for detoxification processes (Pandey *et al.* 2003) which may be the explanation of positive correlation between metal levels and G6PDH activities in the liver of fish at Flag Boshielo Dam.

Glucose-6-phosphate dehydrogenase and LDH are described as key factors in the metabolism with high sensitivity to environmental contaminants (Osman *et al.* 2010; Osman 2012). The current study has shown negative correlation between the activities of LDH and G6PDH. A similar trend was observed for *Clarias gariepinus* (Osman *et al.* 2007; Osman *et al.* 2010), *Oreochromis niloticus* (Osman 2012) and several Cyprinids fishes (Gul *et al.* 2004) from water bodies with different contaminant levels. The trend exhibited by these two enzymes corroborates their reliability when used together in an ecotoxicology study.

Glutathione-S-transferase is a cytosolic enzyme which plays an important role in the biotransformation, detoxification and excretion of xenobiotics (i.e. metals) and their metabolites (Monteiro *et al.* 2006; Carvalho-Neta & Abreu-Silva 2013). Changes in the activity of GST may directly reflect the metabolic disturbances and cell damage in specific organs of fish. The GST activity in the liver of *O. mossambicus* showed no significant difference between the two dams. However, significantly higher GST activities were observed for *L. rosae* populations at Loskop Dam.

Oreochromis mossambicus is one of the freshwater fish species which are highly tolerant to changes in environmental conditions (DWA 2009). Therefore, slight increases in metal concentration in the liver of *O. mossambicus* at Flag Boshielo Dam did not have significant effect on the GST activity. For *L. rosae,* over 75% of the population from Loskop Dam exhibited GST activities of >45 $\mu mol.min^{1-}.mg^{-1}$ protein whereas all *L. rosae* from Flag Boshielo Dam have shown GST activities of <45 $\mu mol.min^{1-}.mg^{-1}$ protein. The increase in the activity of GST may indicate

enhancement of the defensive mechanism to counteract the effects of metals and increase the possibility of a more efficient defence against metal toxicity (Monteiro *et al.* 2006). Coinciding with Monteiro *et al.* (2006), the GST activity for *L. rosae* exhibiting higher metal concentrations in the liver was significantly elevated.

Acetylcholinesterase is a specialised enzyme and its main physiological function is hydrolysis of acetylcholine, a mediator of neurotransduction in cholinergic synapses (Jebali *et al.* 2013). Its inhibition is directly linked with the mechanisms of toxicity of organophosphorus and carbamate insecticides. These insecticides are not persistent in an aquatic ecosystem; therefore it is not easy to measure them directly from the water (Lionetto *et al.* 2003). Acetylcholinesterase allows the detection of pesticides exposure even at low concentrations (Oliveira *et al.* 2007). The present study has shown no significant difference for either species between the two dams.

However, there was a high variability in AChE activities in *L. rosae* population from Flag Boshielo Dam with more than 25% of the population exhibiting the activity of <20 $\mu mol.min^{-1}.mg^{-1}$ protein. The upper and middle Olifants catchment are characterised by extensive agricultural activities such as planting of wheat, vegetables, tobacco, peanuts, cotton and citrus fruit (Van Vuuren 2008). Previous study has shown significant concentrations of organophosphorus and carbamates in the upper Olifants (Bollmohr *et al.* 2008). Moreover, pesticides like organophosphates and carbamates are regularly applied (aerial application) to crops in the Groblersdal and Marble Hall areas which is the drainage basin feeding the Flag Boshielo Dam (Bollmohr *et al.* 2008; Kekana 2013). Therefore, pesticides as the inhibitor of AChE in *L. rosae* population at Flag Boshielo Dam may not be dismissed.

In South Africa, the use of enzymatic biomarkers in biomonitoring studies emerged in the past few decades and has proven to be reliable in detecting early warning signs of exposure and toxicity of environmental contaminants. The present study has shown GST, LDH and G6PDH activities showing relationships with metal concentrations within the fish liver. The middle Olifants catchment which feeds Flag Boshielo Dam is characterised by higher prevalence of agricultural activities than

upper and lower catchments. Coinciding with land use proportion, fish populations from Flag Boshielo Dam have shown relatively high AChE inhibition than those from Loskop Dam.

Although Loskop and Flag Boshielo dams are about 80 km apart, it is evident that there is a slight to moderate difference in the health of fish between these two dams, presumably due to differences in water chemistry which is driven by different land uses in the upper and middle Olifants catchment. Based on the results from the present study, it is reasonable to argue that the agricultural activities in the middle catchment result in organophosphates and carbamates load in the Olifants River system, particularly at Flag Boshielo Dam. These results further confirm that biomarkers are sensitive indicator of aquatic pollution such that even slight increase of environmental contaminants can be detected.

CHAPTER 6

HISTOPATHOLOGY BASED HEALTH ASSESSMENT OF *Oreochromis mossambicus* AND *Labeo rosae* AT LOSKOP AND FLAG BOSHIELO DAMS

6.1 Introduction

The health of aquatic ecosystems cannot be measured directly but rather by the health of the biota found in the system (Adams *et al.* 1993). With fish being relatively sensitive to changes in their surrounding environment, including increase in contaminants, its health may thus reflect and give a good indication of the health status of an aquatic ecosystem it lives in (El-Sayed *et al.* 2013). Feist *et al.* (2015) reported that the assessment of the health status of fish is an important approach which can be applied in an aquatic ecosystem to provide information on the ecological state of the entire ecosystem.

A regular approach which was used to assess the health of fish was the health assessment index developed by Adams *et al.* (1993) which focuses on macroscopic assessment of fish organs. The health assessment index was successfully applied in been applied in North America (Chaiyapechara *et al.* 2003) and South Africa (Avenant-Oldewage *et al.* 1995; Crafford & Avenant-Oldewage 2009) to determine the effect of environmental contaminants on the health of feral fish. Although macroscopic changes are relatively rapid and inexpensive to detect, they are preceded by changes in tissues or cellular levels. Macroscopic changes are the net result of adverse biochemical, physiological and histopathological changes within an organism (Ameur *et al.* 2012; Reddy & Waskale 2013).

Histopathological response is known to be a very sensitive biomarker which may be used to detect early signs of toxic effects of pollution in an aquatic ecosystem (Camargo & Martinez 2007). Recent fish health assessment studies in some of South Africa's freshwater systems have incorporated histopathology to detect early toxic effects of pollution in tissue or cellular levels before the significant changes can be observed on organ or fish behaviour and have proven to be effective (Van Dyk *et al.*

2009b; McHugh *et al.* 2011). The Olifants River, a tributary of the Limpopo River, has been characterised by elevated concentrations of metals which have resulted in deleterious concentrations being recorded in fish tissues (Oberholster *et al.* 2010; Lebepe *et al.* 2016). Moreover, episodic ecological disasters have been prominent at Loskop Dam and in the Olifants Gorge in KNP over the past few decades and have resulted in the establishment of the Consortium for the Restoration of the Catchment (CROC) initiative which was led by SANParks (De Villiers & Mkwelo 2009). Although high metal concentrations were also evident at Flag Boshielo Dam, no fish mortality has been observed recently (Dabrowski 2014).

Due to the increased levels of metal contaminants at Loskop and Flag Boshielo dams, as well as massive mortalities at Loskop Dam and Olifants Gorge (De Villiers & Mkwelo 2009; Heath *et al.* 2010), the health of fish populations in the Olifants River system remains a concern. Health assessment studies were conducted in this system, however, they focused on macroscopic assessment (Madanire-Moyo *et al.* 2012; Watson *et al.* 2012), with few incorporating histopathology (Lebepe 2012). Given that Flag Boshielo Dam has not experienced fish mortalities recently, the present study compared the overall condition and health of *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams. This was achieved by evaluating the length-weight relationship, condition factor (K) and hepatosomatic index (HSI), and histopathology-based fish health assessment at both dams.

6.2 Material and methods

6.2.1 Fish sampling and morphometry

Sampling of fish was carried out using gill net with mesh size ranging from 50 – 150 mm. Fish were weighed using a balancing scale; lengths were measured using the measuring board and then euthanised by severing the spinal cord just behind the head. Fish were opened ventrally and the liver was dissected out and weighed. Fish weight and length were used to calculate the condition factor following Heath *et al.* (2004) protocol (Equation 6.1). Liver weight was used to calculate (HSI) following Van Dyk *et al.* (2012) protocol (Equation 6.2). The length-weight relationship of fish were measured following Anderson *et al.* (1996) protocol (Equation 6.3).

$$CF = \frac{W \times 10^5}{L^3} \quad \text{.....Equation 6.1}$$

$$HSI = \frac{\text{Liver mass}}{\text{Body mass}} \times 100 \quad \text{.....Equation 6.2}$$

$$W = aL^b \quad \text{.....Equation 6.3}$$

For condition factor formula; W = weight (g) and L = length (cm) (Heath *et al.* 2004). For length-weight relationship; W = weight (g), a = coefficient of body shape (y intercept) and b = is the coefficient balancing the dimensions of the equation and its values can be less, greater or equal to 3 (Karachle & Stergiou 2012). When b<3, the fish grows faster in length than in weight and when b>3, the fish grows faster in weight than in length. Whereas when b=3 the rate at which fish length and weight are growing is the same (isometric) (Anderson *et al.* 1996; Karachle & Stergiou 2012).

6.2.2 Tissue fixation and processing

A gill arch and approximately a third of the liver were dissected and fixed in 10% neutral buffered formalin. The samples were dehydrated through a series of ethanol

(70%, 80%, 96%, 100%). Xylene was used to clear the samples and making them transparent. The samples were infiltrated through increasing concentrations of Tissue-Tek® III wax in a 60˚C oven. According to the methods by Humason (1962), once thoroughly infiltrated, samples were embedded in Tissue-Tek® III wax blocks with careful orientation.

Each block was sectioned at 4-5 µm using a rotary/sliding microtome (Reichert-Jung 2040). The samples were floated using gelatine and distilled water solution, and then mounted on glass microscope slides and air dried in 60˚C oven (Humason 1962). Dried samples were prepared for light microscopy analysis using standard technique for Haematoxylin and Eosin staining. The stained section was mounted with cover slips using Entellan.

6.2.3 Histopathological assessment

Qualitative histopathological assessment was carried out using a compound microscope to identify alterations in selected target organs. Alterations were semi-quantitatively assessed using Bernet *et al.* (1999) protocol as modified by Agamy (2012). Histopathological lesions were classified into four major reaction patterns, i.e. circulatory, regressive, progressive and inflammation each possessing distinctive histological features and affecting specific areas of tissue related to function. Alterations were identified and given an importance factor (1 to 3) which represents the potential of the alteration to affect the organ functioning and the overall fish health. Score values (0 to 6) were assigned depending on the degree and extent of the alteration. Score values and importance factors were used to calculate Organ index (I_{org}).

Organ index values were used to classify the severity of the lesion using classification system which is based on the following scoring scheme developed by Zimmerli *et al.* (2007):

- Histological index <10: normal tissue; no pathological changes.
- Histological index 11–20: slight modifications are present.

- Histological index 21–30: moderate modifications.
- Histological index 31–40: pronounced modifications.
- Histological index >40: severe alterations.

This scoring system was also used to classify reaction patterns.

6.2.4 Data analysis

The regression analysis was carried out to determine the length-weight relationship of fish populations. The length and weight of fish were log-transformed to determine the slope (b) and y-intercept (a). The normality of the data was tested using Shapiro-Wiki test and homogeneity of variance was tested using Levene's test. Depending on the data distribution, an independent sample t-test or Wilcoxon-Mann-Whitney U-test was used to evaluate the difference for the condition factor, hepatosomatic and organ indices between the two dams. Statistical analyses were carried out using R-3.1.1 statistical software (R Development Core Team 2014). A significant level was set at $p<0.05$.

6.3 Results

6.3.1 Fish sizes

Sampled populations have shown variability with regard to fish weight within each dam (Fig. 6.1). Each population has shown a significant difference ($p<0.05$) between the two dams with extremely large fish (max mass: 2350 g *O. mossambicus* and 1935 g *L. rosae* being recorded at Loskop Dam (Fig. 6.1). Flag Boshielo Dam populations exhibited the maximum weight of 721.35 for *L. rosae* and 899.60 for *O. mossambicus*. The total length for both fish populations has also shown a significant difference ($p<0.05$) between the two dams, with longer fish being recorded at Loskop Dam.

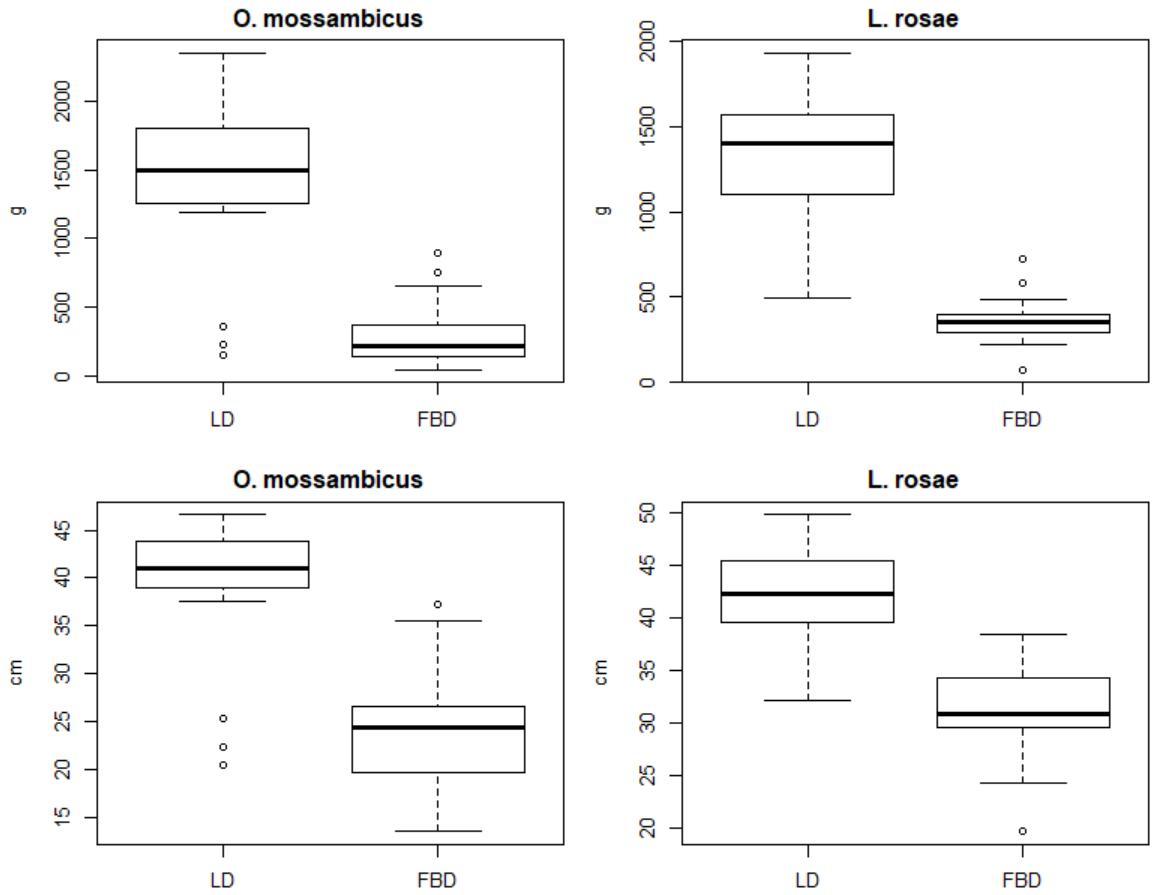

Figure 6.1 The weight and lengths of fish recorded at Loskop and Flag Boshielo dams during 2014 surveys.

6.3.2 Length-weight relationship

The length-weight relationship observed for *L. rosae* from Loskop Dam was $W=0.00004L^{2.85}$ for male population (adjusted $R^2=0.94$) and $W=0.0004L^{2.52}$ for female population (adjusted $R^2=0.22$) (Table 6.1) (Fig. 6.3). Both male and female *L. rosae* populations have shown negative allometric growth (Table 6.1), and a significant length-weight relationship ($p<0.05$) (Fig. 6.1). At Flag Boshielo Dam, the length-weight relationship observed for *L. rosae* was $W=0.29L^{1.23}$ (adjusted $R^2=0.38$) and $W=0.000002L^{3.29}$ (adjusted $R^2=0.96$) for male and female populations, respectively. The male population has shown negative allometric growth with female exhibiting positive allometric growth (Table 6.1). The length-weight relationship for both male and female *L. rosae* from Flag Boshielo Dam have shown a positive significant relationship ($p<0.05$) (Fig. 6.2).

Table 6.1 Length-weight relationship of *Oreochromis mossambicus* and *Labeo rosae* recorded at Loskop and Flag Boshielo dams during winter and summer surveys in 2014.

Dams	Species	Sex	n	a	b	Adjusted R^2	Growth
L. rosae	Loskop Dam	Male	10	0.000040	2.85	0.94	- Allometric
		Female	12	0.000400	2.52	0.22	- Allometric
		Combined	22	0.050000	1.94	0.53	- Allometric
	Flag Boshielo Dam	Male	15	0.290000	1.23	0.38	- Allometric
		Female	9	0.000002	3.29	0.96	+ Allometric
		Combined	24	0.230000	2.91	0.76	- Allometric
O. mossambicus	Loskop Dam	Male	14	0.000010	3.10	0.98	+ Allometric
		Female	9	0.000007	3.18	0.98	+ Allometric
		Combined	23	0.000010	3.12	0.73	+ Allometric
	Flag Boshielo Dam	Male	13	0.000020	2.92	0.99	- Allometric
		Female	11	0.000030	2.94	0.94	- Allometric
		Combined	24	0.000020	2.93	0.94	- Allometric

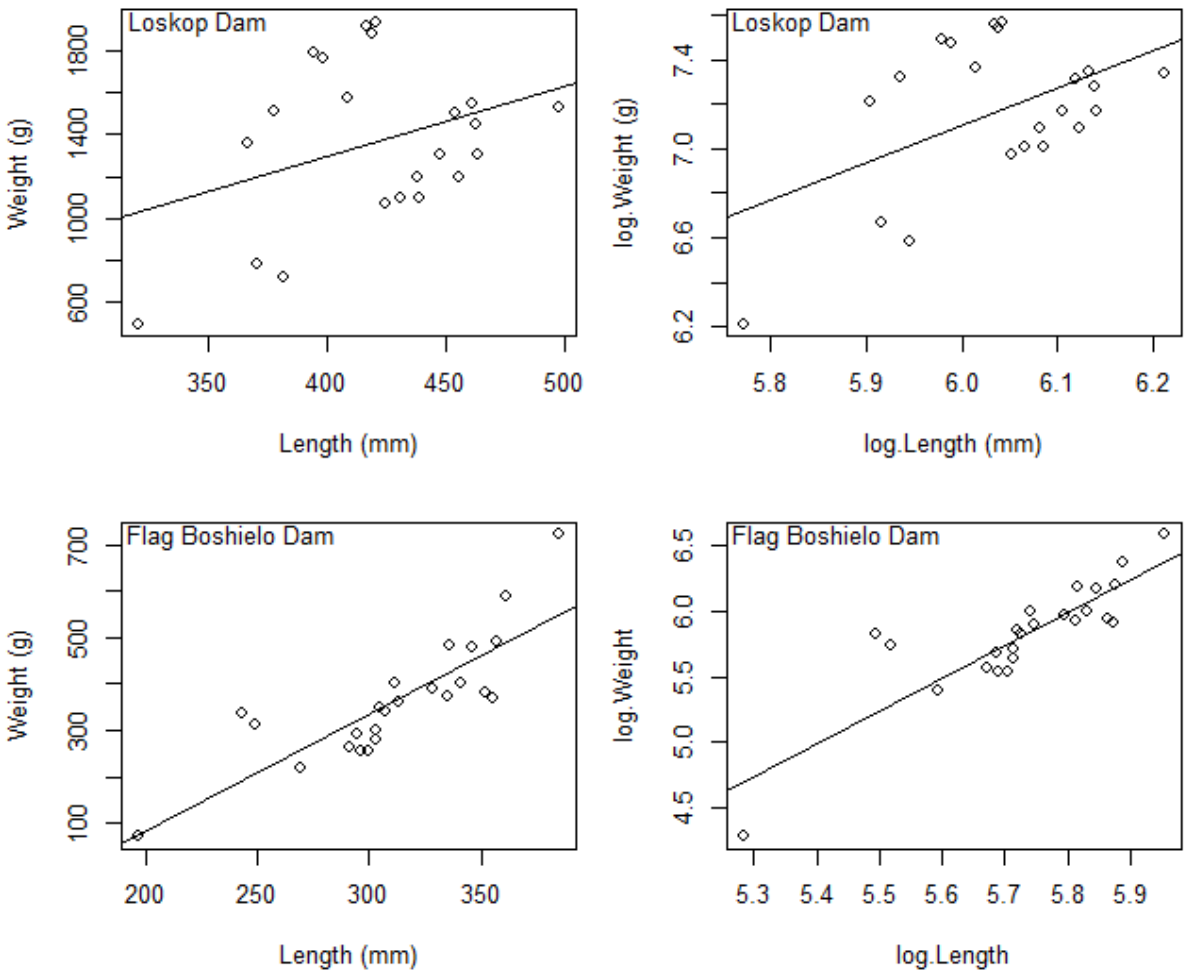

Figure 6.2 The relationship of measured length and weight (left column) and the relationship of logarithmically transformed length and weight (right column) of *L. rosae* recorded at Loskop and Flag Boshielo dams during 2014 surveys.

Oreochromis mossambicus sampled from Loskop Dam have shown positive allometric growth for both male and female populations with those sampled from Flag Boshielo Dam exhibiting negative allometric growth (Table 6.1). The length-weight relationship observed on *O. mossambicus* from Loskop Dam was $W=0.00001L^{3.10}$ (adjusted $R^2=0.98$) for males and $W=0.000007L^{3.18}$ (adjusted $R^2=0.98$) for females (Table 6.1) (Fig. 6.3). Both male and female *O. mossambicus* populations have shown a positive significant length-weight relationship at both Loskop and Flag Boshielo dams ($p<0.05$) (Fig. 6.3).

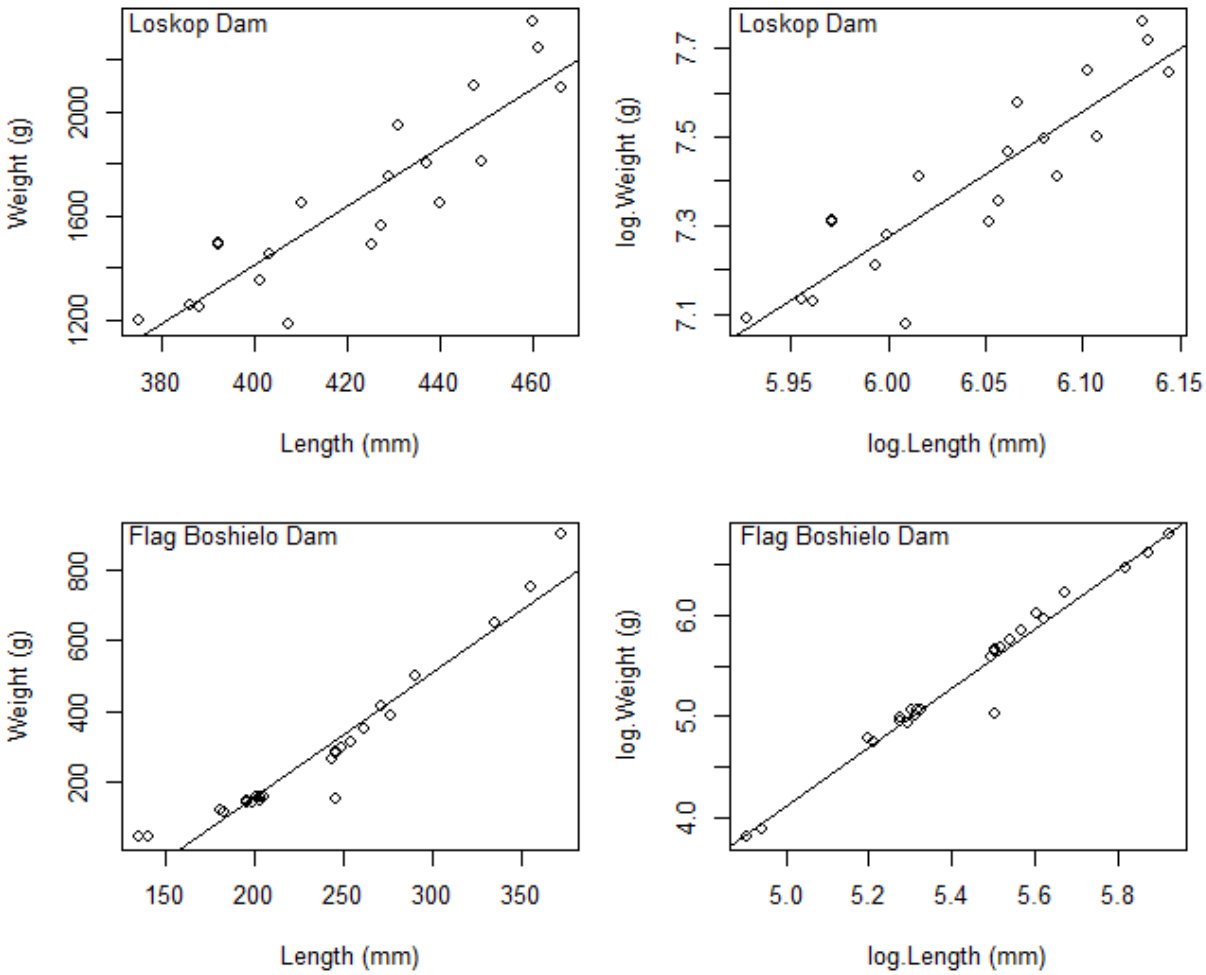

Figure 6.3 The relationship of measured length and weight (left column) and the relationship of logarithmically transformed length and weight (right column) of *O. mossambicus* recorded at Loskop and Flag Boshielo dams during 2014 surveys.

6.3.3 Overall fish conditions

6.3.3.1 Condition factor

Male *O. mossambicus* have shown significant difference ($p<0.05$) on the condition factor between the two dams with higher values being observed at Loskop Dam (Fig. 6.4). In contrast, no significant difference ($p>0.05$) was observed on condition factor for female *O. mossambicus* between the two dams. The condition factor for male *L. rosae* exhibited significant difference ($p<0.05$) between Loskop and Flag Boshielo dams. Female *L. rosae* have shown a great variability on condition factor at Loskop

Dam (Fig. 6.4) and a significant difference ($p<0.05$) between the Loskop and Flag Boshielo dams.

6.3.3.2 Hepatosomatic index

In the present study, over 60% of the sampled *O. mossambicus* exhibited HSI values ranging from 1 to 2% at Loskop Dam with over 90% of the Flag Boshielo Dam population showing HSI values below 1 (Fig. 6.5). Moreover, HSI has shown a significant difference ($p<0.05$) for *O. mossambicus* between the two dams. More than 75% of *L. rosae* population sampled from each dam showed HSI of <1 (Fig. 6.5). However, HSI values for *L. rosae* have shown a significant difference ($p<0.05$) between the two dams.

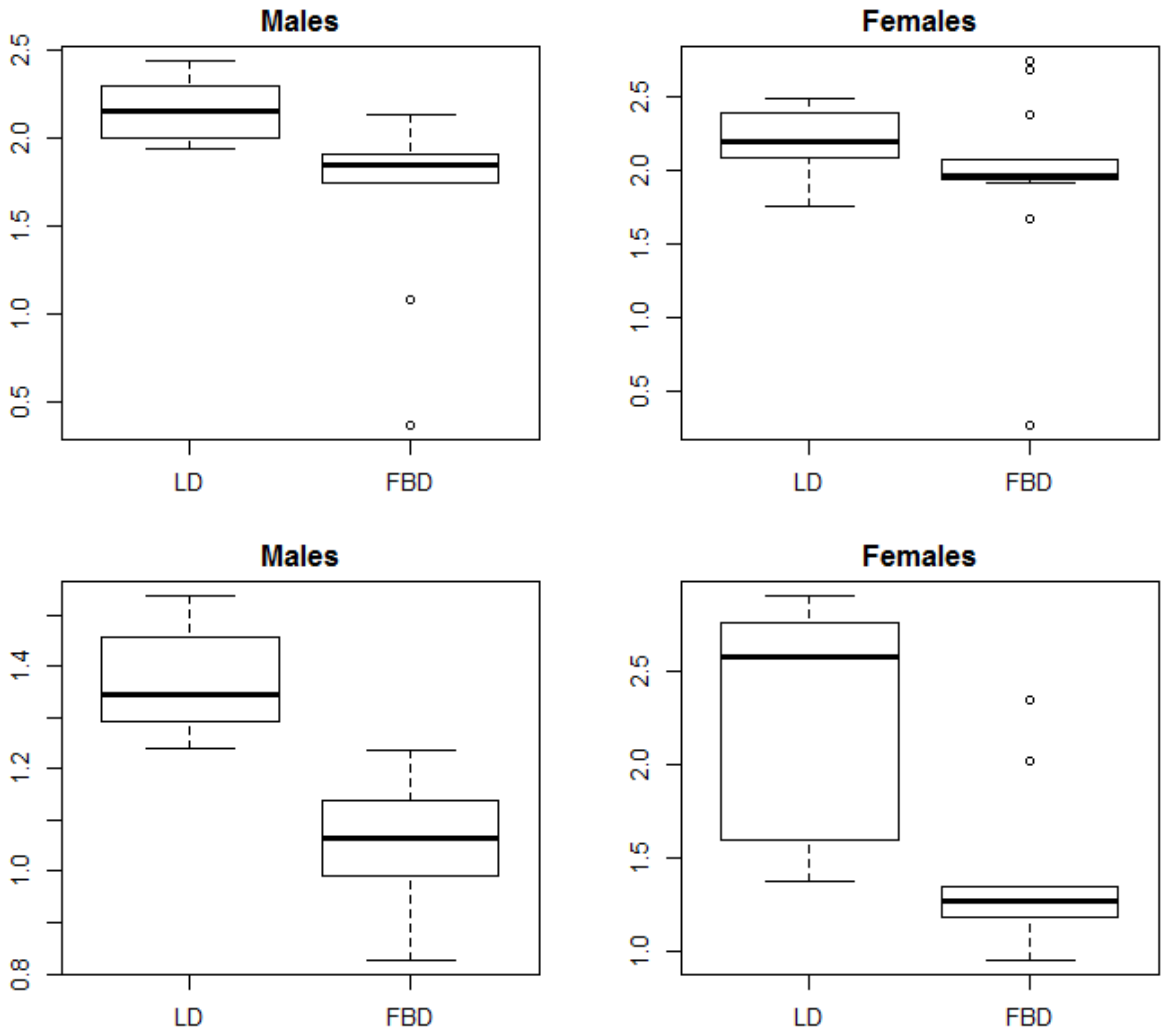

Figure 6.4 Condition factors of *Oreochromis mossambicus* (top row) and *Labeo rosae* (bottom row) populations recorded at Loskop and Flag Boshielo dams during 2014 surveys.

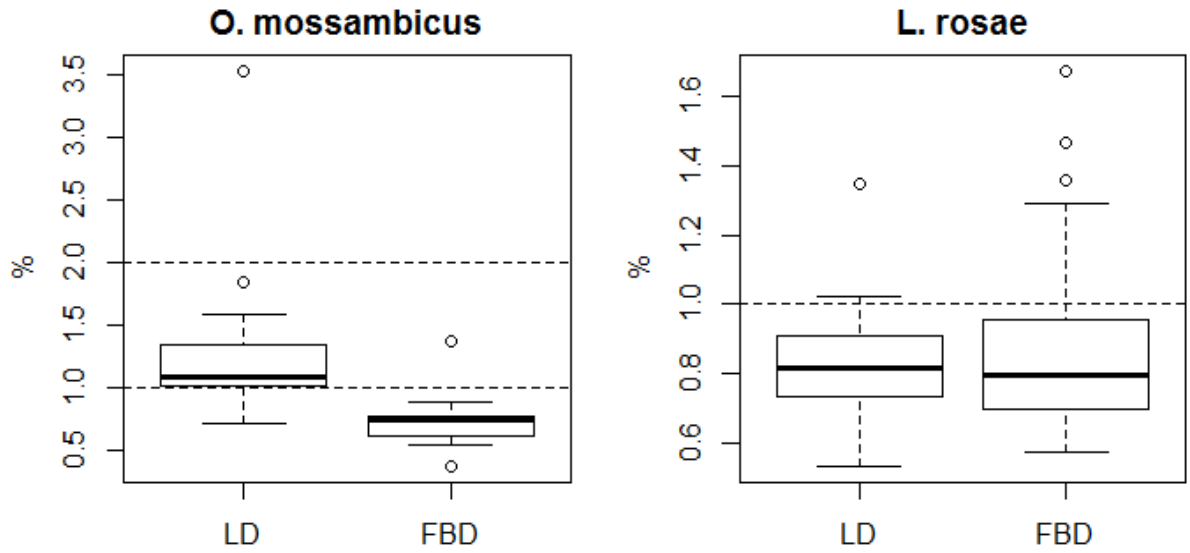

Figure 6.5 Hepatosomatic indices of *Oreochromis mossambicus* and *Labeo rosae* populations recorded at Loskop and Flag Boshielo dams during 2014 surveys.

6.3.4 Histopathological assessment

6.3.4.1 Gills

In the present study, regressive changes (epithelial lifting, nuclear alterations, necrosis, and structural alterations in the form of lamella fusion) were more prominent for both species as compared to other reaction patterns (Table 6.2, Figs 6.6 & 6.7). *Labeo rosae* have shown epithelial lifting, pillar cell rupture, epithelial cells hyperplasia which resulted in primary lamella fusion and secondary lamella fusion, nuclear alterations, necrosis, oedema and haemorrhage at both dams with aneurism being recorded at Flag Boshielo Dam (Table 6.2, Fig. 6.7).

In *O. mossambicus*, epithelial lifting, pillar cell rupture, epithelial cells hyperplasia which resulted in secondary lamella fusion, nuclear alterations, necrosis, oedema, haemorrhage and branching of secondary lamella were observed at both dams with hyperplasia of mucous cells and shortened secondary lamella being recorded at Loskop Dam (Table 6.2).

Oreochromis mossambicus have shown higher gill index at Loskop Dam with *L. rosae* showing higher gill index at Flag Boshielo Dam (Table 6.4). Zimmerli *et al.*

(2007) scoring scheme has shown that the histopathological alterations observed in *O. mossambicus* and *L. rosae* ranged from slight to moderate modification. Moderate modifications (score > 20) were observed for *O. mossambicus* at Loskop Dam with score of slight modification (score < 20) being recorded at Flag Boshielo Dam (Table 6.4). *Labeo rosae* population showed the score of 11.16 at Loskop Dam and 16.40 at Flag Boshielo Dam (Table 6.4). Both species have shown significant difference on score values between the two dams (t-test, $p<0.05$).

6.3.4.2 Liver

Regressive changes such as cell rupture, nuclear alterations, necrosis, peripheral nucleus, melanomacrophages (MMCs) and fatty vacuolisation were the most prominent alterations observed in the liver of both fish species at both dams (Table 6.3, Figs 6.8 & 6.9). Other alterations observed included hepatocellular hypertrophy and pleomorphism, hydropic degeneration, neoplasm, haemorrhage and sinusoidal congestion (Table 6.3 & Fig. 6.8).

Most alterations were observed on both species at both dams, however, they exhibited difference in the magnitude of severity. Liver index values of 16.75 and 8.2 were recorded for *O. mossambicus* at Loskop and Flag Boshielo dams, respectively (Table 6.4). *Labeo rosae* exhibited liver index values of 10.68 and 11.05 at Loskop and Flag Boshielo dams, respectively (Table 6.4). The liver index for both species has shown significant difference between the two dams (t-test, $p<0.05$). Zimmerli *et al.* (2007) scoring scheme classified alterations as mild modifications for both species at Loskop and Flag Boshielo dams (scores <20).

Table 6.2 Prevalence of histopathological alterations (%) in gills of *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during 2014 surveys.

Reaction patterns	Alterations	***Labeo rosae***		***Oreochromis mossambicus***	
		Loskop Dam (n=19)	Flag Boshielo Dam (n=20)	Loskop Dam (n=22)	Flag Boshielo Dam (n=20)
Circulatory disturbances	Haemorrhage	16	37	41	90
	Aneurism	0	32	9	10
	Oedema	37	26	59	60
Regressive changes	Epithelial lifting	90	93	92	83
	Pillar cell rupture	81	88	87	77
	Fusion of primary lamella	26	53	0	0
	Fusion of secondary lamella	11	32	27	40
	Nuclear alteration	40	55	38	60
	Shortened secondary lamella	0	0	5	0
	Branching of secondary lamella	0	0	5	60
	Necrosis	33	37	23	50
Progressive changes	Epithelial hyperplasia	37	37	50	20
	Hyperplasia of mucous cells	0	0	27	0
Inflammation	Exudate	10	5	5	0

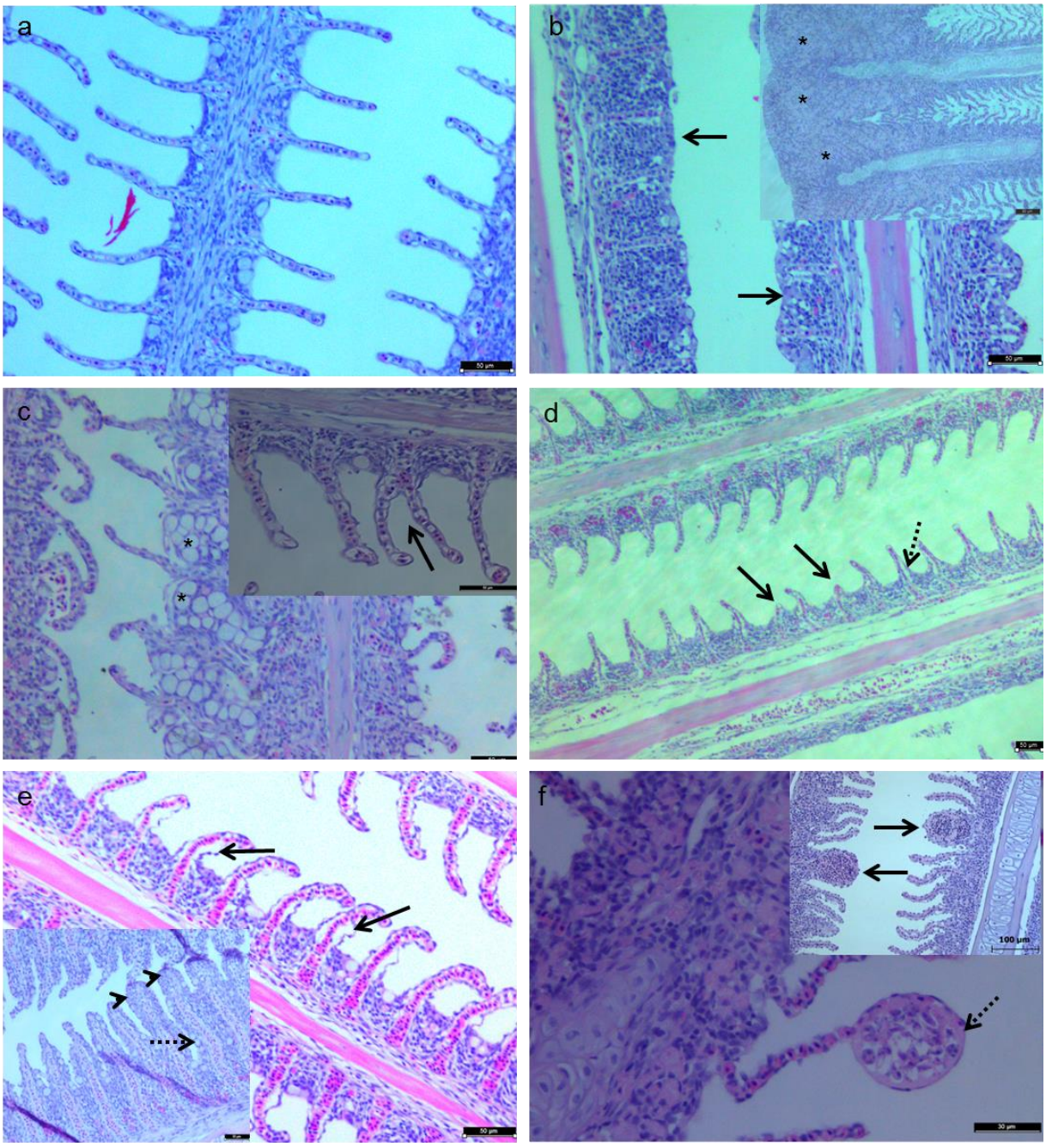

Figure 6.6 a. Normal gill tissues; **b.** fusion of secondary lamella induced by epithelial cell hyperplasia (arrows), primary lamella fusion (star); **c.** mucous cells hyperplasia (stars), branching of secondary lamellae (arrow); **d.** shorted secondary lamella (solid arrows), epithelial lifting (dotted arrow); **e.** epithelial lifting (solid arrows), mucous cells hyperplasia (dotted arrows), epithelial cells hyperplasia (arrow heads); **f.** protozoan induced aneurism (dotted arrow), blood cells induced aneurism (solid arrows).

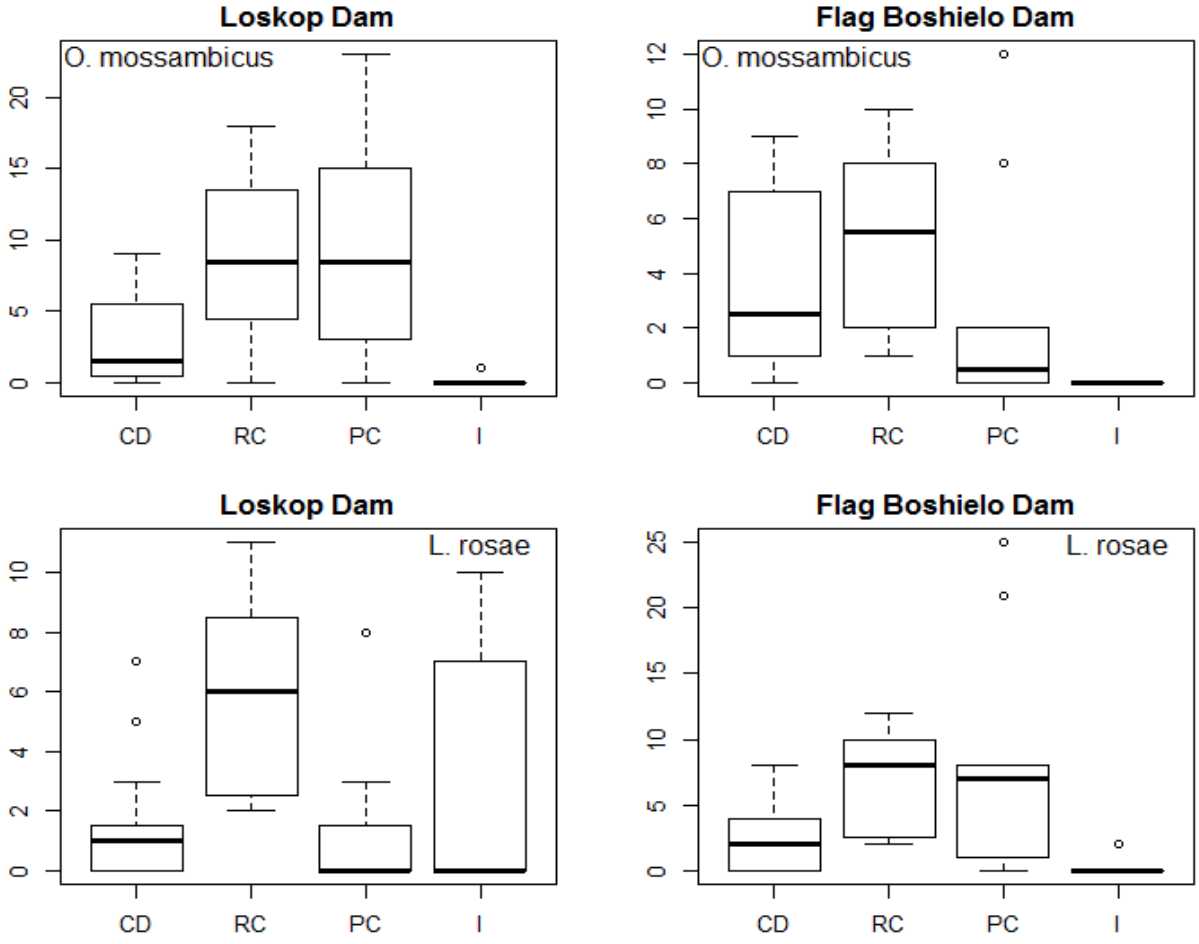

Figure 6.7 Gill reaction patterns recorded for *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during 2014 surveys. CD: circulatory disturbance; RC: regressive change; PC: Progressive change; I: Inflammation; T: Tumour.

Table 6.3 Prevalence of histopathological alterations (%) in the liver of *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during 2014 surveys.

Reaction patterns	Alterations	*L. rosae*		*O. mossambicus*	
		Loskop Dam (n=19)	Flag Boshielo Dam (n=20)	Loskop Dam (n=22)	Flag Boshielo Dam (n=20)
Circulatory disturbances	Haemorrhage	11	0	5	0
	Sinusoidal congestion of blood cells	11	0	5	0
Regressive changes	Hydropic degeneration	53	21	18	60
	MMCs	84	74	41	0
	Fatty vacuolization	89	0	59	80
	Hepatocellular pleomorphism	79	53	45	40
	Nucleus at a peripheral position	47	53	41	40
	Cell rupture	42	32	59	80
	Nuclear alteration	84	90	83	89
	Necrosis	81	88	87	90
Progressive changes	Hepatocellular hypertrophy	68	53	50	20
	Hepatocellular hyperplasia	20			13
Inflammation	Exudate	26	5	28	15

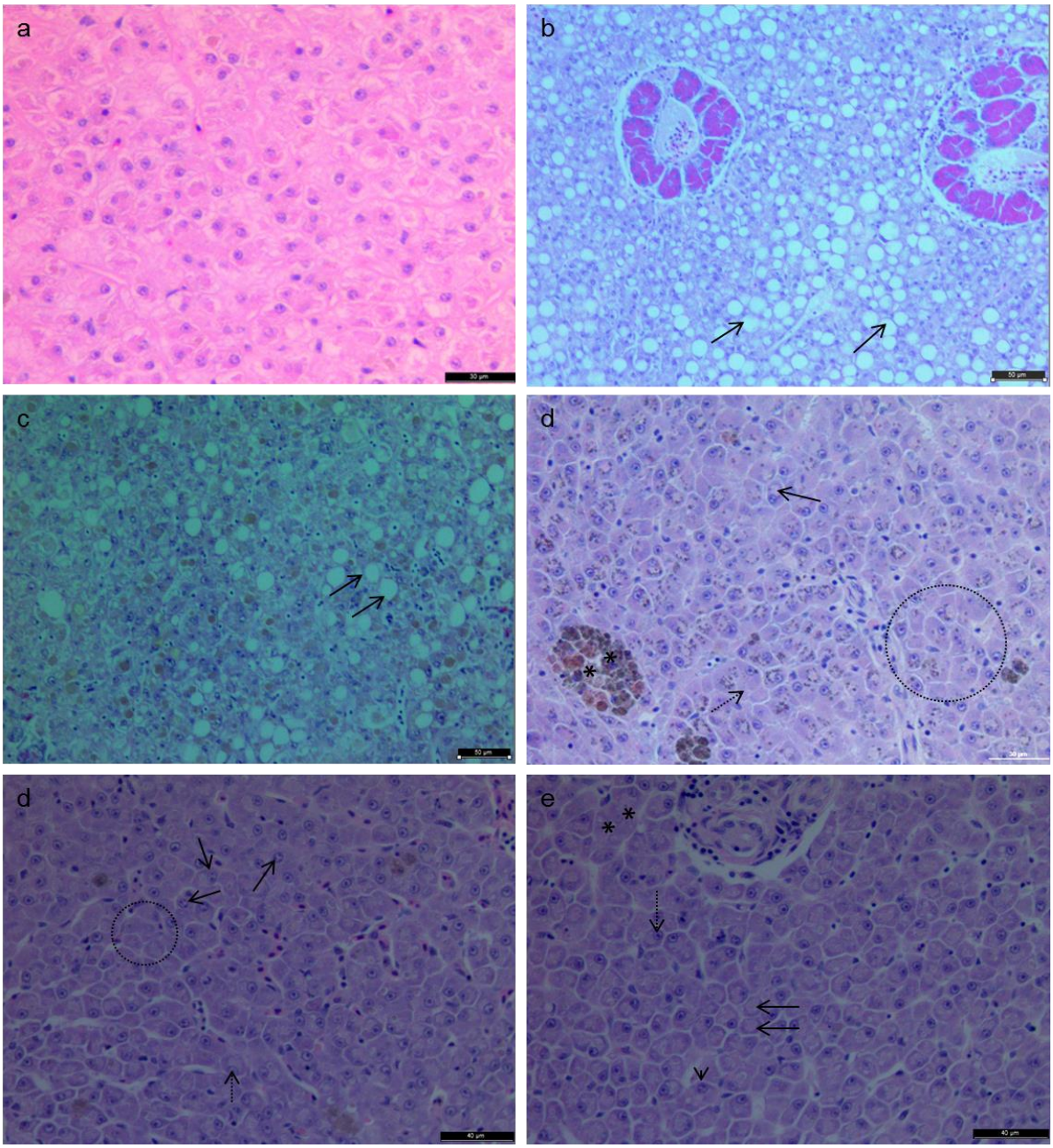

Figure 6.8 a. Normal liver histology; **b.** fatty vacuolisation (arrows); **c.** spread of MMCs, fatty vacuolisation (arrows); **d.** hepatocellular pleomorphism (encircled), hydropic degeneration (solid arrow), necrosis (dotted arrow), MMCs (stars); **e.** hepatocellular degeneration (encircled), peripheral nucleus (dotted arrow), nuclear alteration (solid arrow) **f.** hepatocytes with diffuse vacuolisation in the cytoplasm (solid arrow), necrosis (stars), peripheral nucleus (dotted arrow), nuclear alteration (arrow head).

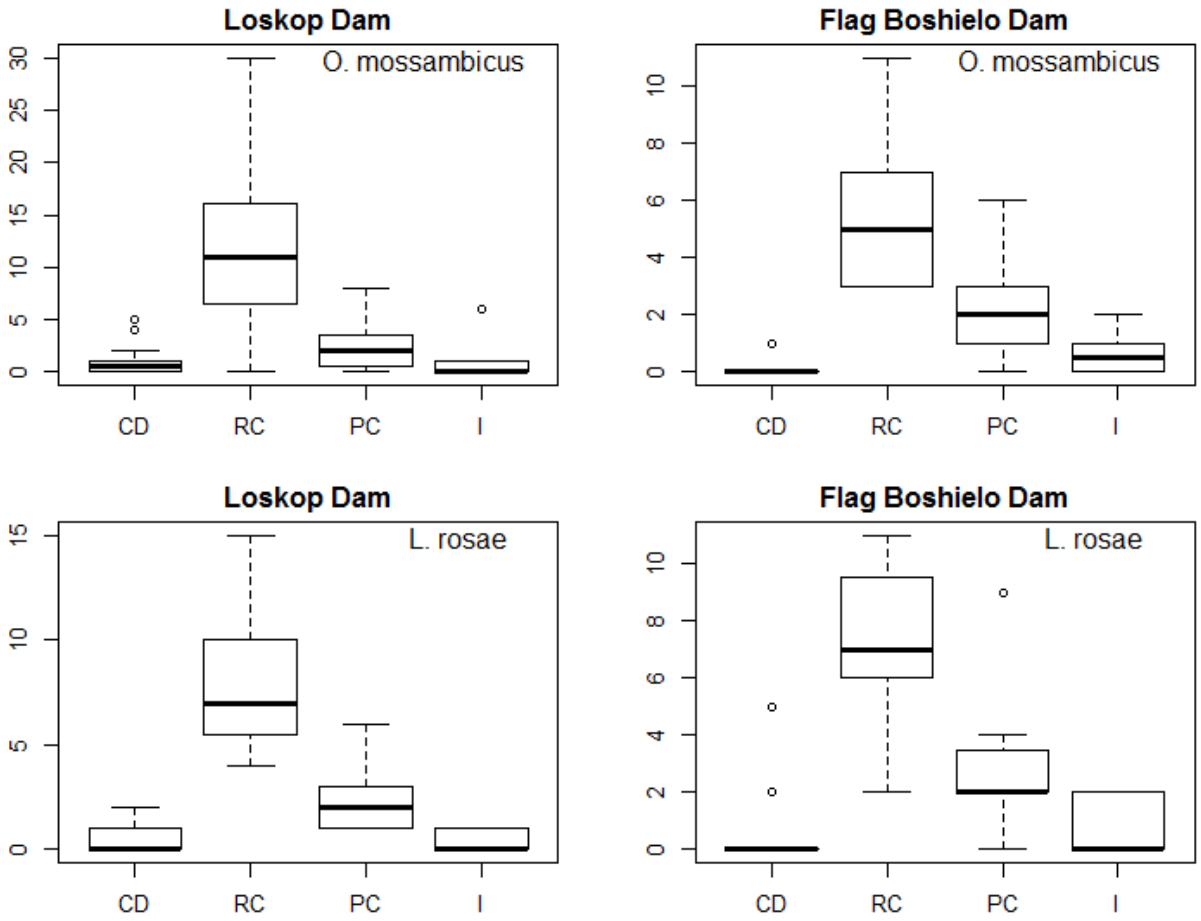

Figure 6.9 Liver reaction patterns recorded for *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams during 2014 surveys.

Table 6.4 Gill and liver indices recorded from *Oreochromis mossambicus* and *Labeo rosae* at Loskop and Flag Boshielo dams during 2014 surveys.

Organ	*O. mossambicus*		*L. rosae*	
	Loskop Dam	Flag Boshielo Dam	Loskop Dam	Flag Boshielo Dam
Gill index	21.60	11.1	11.16	16.40
Liver index	16.75	8.2	10.68	11.05

6.3.4.3 Relationship between metal concentrations in tissues and histopathological condition

In the present study, prevalence and magnitude of alterations in *L. rosae* have shown to increase as metal concentration increase (positive correlation) in both liver and gills. Over 75% of the metals analysed in the liver have shown a higher concentration in *L. rosae* sampled from Flag Boshielo Dam than in those from Loskop Dam (Table 4.1, Chapter 4). A similar trend was observed for about 60% of the metals analysed in the gills of *L. rosae* (Table 4.1, Chapter 4). The metal trend coincided with the liver and gill indices in *L. rosae* which were higher at Flag Boshielo Dam than at Loskop Dam. In *O. mossambicus*, about 80% of the metals analysed in the gills were higher at Flag Boshielo Dam than at Loskop Dam (Table 4.1, Chapter 4). About 90% of the metals analysed in the liver were higher in *O. mossambicus* from Flag Boshielo Dam than in those from Loskop Dam (Table 4.1, Chapter 4). In contrast with the metal trend, higher organ indices were observed in *O. mossambicus* population from Loskop Dam (Table 6.3).

6.4 Discussion

6.4.1 Length-weight relationship

In fish, several ecological and physiological factors are more size-dependent than age-dependent; therefore, size is more biologically relevant than age (Santos *et al.* 2002). The length-weight relationship is important in fishery and fish biology since they allow estimation of average weight of a given length group. Moreover, length-weight relationship analysis may provide information about the health condition and the growth pattern of fish (Santos *et al.* 2002; Mir *et al.* 2012). Despite fish well-being, growth and reproductive state, weight-length relationship may reflect the characteristics of the environment such as habitat quality, water quality and food availability (Liao *et al.* 1995).

In the present study, Loskop Dam exhibited significantly larger fish as compared to Flag Boshielo Dam populations. A negative allometric growth (b<3) was observed for *L. rosae* populations at both dams except for female population at Flag Boshielo Dam, which showed positive allometric growth (b>3). *Oreochromis mossambicus* populations exhibited positive allometric growth (b>3) at Loskop Dam and negative allometric growth (b<3) at Flag Boshielo Dam. There is scant literature on the effect of water quality on the length-weight relationship. Sandhya & Shameem (2003) reported a positive allometric growth for *Liza macrolepis* populations from unpolluted dams and negative allometric growth for those from polluted dams. Similar trend was observed by Stavrescu-Bedivan *et al.* (2015) on *Carassius gibelio* in Romanian aquatic ecosystems which were characterised by different pollution levels.

Although polluted waters exhibited negative allometric growth, positive allometric growth may be expected in meso and eutrophic waters due to food availability (Mir *et al.* 2012). Loskop Dam was characterised by nutrients enrichment (mesotrophic), therefore, the productivity of the dam as one of the factors that have influenced the b parameter for *O. mossambicus* may not be dismissed.

6.4.2 Condition Factor

The condition factor is widely used in fish health studies, fisheries and general fish biology studies. This factor is known as the relationship between the weight of a fish and its length, with the intention of describing the well-being of the fish (Heath *et al.* 2004; Nash *et al.* 2006). The condition factor may differ from species to species, however, the value of 1.60 indicate an excellent condition (trophy class fish) while 0.80 indicate extremely poor fish resembling a barracouta, big head and narrow, thin body (Barnham & Baxter 2003). Jooste *et al.* (2005) emphasised that the condition factor of fish is classified as ideal when a value of one is recorded.

In the present study, *L. rosae* population has shown significantly higher condition factors at Loskop Dam than at Flag Boshielo Dam (Fig. 6.2). For *O. mossambicus,* condition factors were above the normal range as stipulated by Jooste *et al.* (2005) and Barnham & Baxter (2003) at Loskop Dam with some being within the range at Flag Boshielo Dam (Fig. 6.2). Condition factor of 0.27 and 0.37 was recorded for juvenile (fries) *O. mossambicus* at Flag Boshielo Dam and for *L. rosae* at Loskop Dam. The condition factor is greatly influenced by the age of fish, sex, season, stage of maturity, fullness of gut, type of food consumed, amount of fat reserve, degree of muscular development and stage of development of the reproductive organ (Barnham & Baxter 2003; Jenkins 2004). Moreover, elevated condition factors usually reflect food availability and greater feeding activities (Pyle *et al.* 2005). The higher condition factor for fish populations from Loskop Dam might be attributed to the food availability since the dam was mesotrophic (Section 3.2.6, Chapter 3).

6.4.3 Hepatosomatic index

The HSI is associated with the liver energy reserves and metabolic activities and is defined as a ratio of the liver weight to the body weight expressed as a percentage of whole body weight (Nunes *et al.* 2011). Despite being related to the energy reserves, the HSI can provide information on the well-being of fish and is regarded as a useful indicator of water pollution (Di Giulio & Hinton 2008; Lenhardt *et al.* 2009). The index can tell whether the liver has undergone hyperplasia, hypertrophy or atrophy or the

body mass has increased. The HSI varies from species to species and in Osteichthyes it ranges from 1 to 2% (Munshi & Dutta 1996; Marchand 2008).

Significantly higher HSI values were observed for the *O. mossambicus* population from Loskop Dam than those from Flag Boshielo Dam. The increase of HSI values are normally related to the enhanced detoxification capacity in response to the presence of environmental contaminants (Liebel *et al.* 2013). However, *Oreochromis mossambicus* from Loskop Dam were obese with maximum weight of 2.35 kg being observed. An increase in HSI may probably reflects storage of energy reserves presumably because of the increased feeding rates (Pyle *et al.* 2005). Loskop Dam has shown higher productivity as compared to Flag Boshielo Dam; therefore, food availability as the driver for higher HSI values at Loskop Dam may not be dismissed.

The trend observed for the HSI of *O. mossambicus* was not observed for *L. rosae*. *O*ver 75% of *L. rosae* populations from each locality exhibited HSI values below 1 (Fig. 6.3). Therefore, food availability at Loskop Dam has not substantially increase HSI values of *L. rosae*. However, the HSI for *L. rosae* was comparable to the one reported by Lebepe (2012) at Loskop and Flag Boshielo dams. Munshi & Dutta (1996) reported that the HSI may vary from species to species. The current study was not conclusive with regards to HSI, extensive studies are therefore, recommended to determine the ideal HSI value *L. rosae* and other freshwater fishes in South Africa's freshwater ecosystems.

6.4.4 Histopathologic assessment

6.4.4.1 Gills

Gills are in direct contact with the external water environment with the main functions including gaseous exchange, ion regulation, maintenance of acid-base balance, and excretion of nitrogenous wastes (Roberts 2012; Liebel *et al.* 2013). Their vulnerability is considerable because their external location means that they are liable to damage by any irritant material, whether suspended or dissolved in the water (Roberts 2012). Gill alterations reported in this study are comparable to those recorded in other

studies conducted in South Africa and elsewhere (Van Dyk *et al.* 2009a; McHugh *et al.* 2011; Abdel-Moneim *et al.* 2012).

Epithelial lifting, pillar cell rupture, epithelial cells hyperplasia which resulted in secondary lamella fusion, nuclear alterations, necrosis, oedema and haemorrhage were observed for both fish species at Loskop and Flag Boshielo dams. Aneurisms were recorded on *L. rosae* at Flag Boshielo Dam whereas hyperplasia of mucous cells and shortened secondary lamella were observed for *O. mossambicus* at Loskop Dam. *Oreochromis mossambicus* has shown a higher gill index at Loskop Dam with *L. rosae* showing higher gill index at Flag Boshielo Dam. The difference in the trend of histopathological alterations among the two fish populations might infer the difference in sensitivity to pollutants between the two species.

Epithelial lifting and pillar cell rupture dominated histopathological alterations in the gills of *O. mossambicus* and *L. rosae* at both dams. Pillar cells hold epithelia apart to allow blood flow through the secondary lamellae. Epithelial lifting may thus, always accompanied by pillar cell rupture (Evans *et al.* 2005). Moreover, epithelial lifting serve as a defensive mechanism, because it drastically increase the distance across which waterborne irritants must diffuse to reach the bloodstream in the secondary lamellae (Arellano *et al.* 1999; Figueiredo-Fernandes *et al.* 2007). De Oliveira Ribeiro *et al.* (2013) reported that epithelial lifting, fusion of primary and secondary lamella, pillar cell rupture, aneurism and haemorrhage may compromise gill function and consequently result in susceptibility to other pathological conditions, including infection by opportunist organisms.

Arellano *et al.* (1999) and Liebel *et al.* (2013) reported that alterations such as oedema, hyperplasia, swelling and lifting of epithelial cells may represent adaptive strategies to maintain physiological functioning of the organ. However, severe magnitudes of alterations result in lamella fusions which still negatively affect the fish by reducing the surface area of the gill.

6.4.4.2 Liver

Liver is a unique and fragile organ since is composed of a large mass of glandular tissues. However, it plays a vital role in basic metabolism and is also known as a major organ of accumulation, biotransformation and excretion of toxicants in fish (Roberts 2012; Authman *et al.* 2013). The ability of the liver to accumulate toxicants may be overwhelmed by extreme concentrations and result in structural alterations (Ross & Wojciech 2011). Histopathological alterations observed in the present study included cell rupture, nuclear alterations, necrosis, peripheral nucleus, MMCs, fatty vacuolisation, hepatocellular hypertrophy and pleomorphism, hydropic degeneration, neoplasm, haemorrhage and sinusoidal congestion.

These alterations were comparable to those recorded in South Africa and other part of world for *O. mossambicus* and *C. gariepinus* exposed to metals (Van Dyk *et al.* 2007; Marchand *et al.* 2008; Authman *et al.* 2013) and *C. gariepinus* exposed to eutrophic waters (Van Dyk *et al.* 2012). Fatty vacuolisation, steatosis and hydropic degenerations for *L. rosae* were more prevalent at Loskop Dam whereas for *O. mossambicus* they were more prevalent at Flag Boshielo Dam. Although fatty vacuolisation, steatosis and hydropic degenerations are reversible lesions and cells can recover their normal functions (homeostasis) when the stress is removed, the recovery of cells will depend on the severity and duration of exposure to stressors (Guzmán & González 2012). With acid mine drainage from abandoned and operating mines, and untreated and partially treated sewage continuing to flow into the Olifants River system, it is unlikely that these tissues will ultimately recover.

Moreover, hepatocellular hypertrophy, MMCs, haemorrhage, hepatocellular pleomorphism, sinusoidal congestion and neoplasm were more prevalent at Loskop Dam than Flag Boshielo Dam for both species, and so was the magnitude of alterations. Both dams have shown elevated concentrations of metals in the liver with higher concentrations being recorded at Flag Boshielo Dam. Therefore, lesions have shown negative correlation with metal concentrations for both species at Loskop and Flag Boshielo dams. Although the liver of fish populations from Loskop Dam showed lower level of metals compared to those from Flag Boshielo Dam, the lower

concentration at Loskop Dam had more effects on the histopathological condition of fish populations. This might be attributed to the increase in metal toxicity as a result of acid mine drainage emanating from the upper catchment. Similar lesions were also observed in *O. mossambicus* exposed to ^{60}Co radiation in Cauvery River, India (Bukhari *et al.* 2012). Hepatocellular hypertrophy and hyperplasia might be described as the adaptive response to increase detoxification capacity (Salamat & Zarie 2012). Other structural alterations result in necrosis if the concentration exceed the carrying capacity of the liver cells (Ross & Wojciech 2011).

In the present study, the prevalence of necrosis was over 80% for each species at both dams with maximum index value of 30. Necrosis may be described as an irreversible tissue or cell damage (Bernet *et al.* 1999). It was observed in *Astyanax fasciatus* and *Oreochromis niloticus* exposed to industrial and urban sewage (Liebel *et al.* 2013). Ayas *et al.* (2007) described necrosis as one of the most important histopathological responses resulting from exposure to contaminants. Mela *et al.* (2007) and Liebel *et al.* (2013) further elaborated that necrosis in fish liver tissue may be due to the presence of toxicants within the hepatocytes which causes disruption in the biochemical process resulting in enzyme inhibition, failure on protein synthesis, carbohydrate metabolism, reactive oxidative species production, damages in cell membrane and failure of ATP synthesis (Mela *et al.* 2007; Liebel *et al.* 2013). Metals have shown elevated concentrations in the liver tissue of both species at Loskop and Flag Boshielo dams, therefore, the possibility of metals being the driver of necrosis in the fish liver may not be dismissed.

Melanomacrophage centre serve as metabolic dumps for the relocation of debris of damaged cells, including red blood cells and it has shown to increase in size as the fish grows older and tissues degenerate (Agius & Roberts 2003). It has also proved to be one of the robust indicators of fish exposure to contaminants (Fournie *et al.* 2001). The large amount of MMCs recorded in *L. rosae* at Loskop and Flag Boshielo dams might be an evidence of structural and metabolic damage with the little to absence amount in *O. mossambicus* proving the difference in sensitivity to pollutants between the two species.

CHAPTER 7

HUMAN HEALTH RISK ASSOCIATED WITH THE CONSUMPTION OF *Oreochromis mossambicus* AND *Labeo rosae* FROM LOSKOP AND FLAG BOSHIELO DAMS

7.1 Introduction

Inland fisheries are poorly developed in South Africa, mainly because there is limited enormous freshwater bodies and there is no inland fishery policies in place (McCafferty *et al.* 2012). However, recent proposal by African Union pinpointed inland fisheries as a priority investment area for poverty alleviation and regional economic development. It is therefore probable that the efforts to develop inland fisheries may increase (Ellender *et al.* 2009). Eating fish is good for human health and reduces the risk of coronary heart disease, decrease mild hypertension and prevents certain cardiac arrhythmias due to the content of high-quality protein and high content of the two kinds of omega-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid (Castro-González & Méndez-Armenta 2008; Storelli 2008). However, fish are known to accumulate metals within their tissues. Therefore, despite nutritional and health benefits, fish consumption is described as the route of human exposure to a variety of chemical contaminants (Barbour *et al.* 1999; Llobet *et al.* 2003).

The Olifants River is one of the most polluted river systems in South Africa. Its catchment is home to about 10% of South Africa's population dominated by unemployed rural communities (Coetzee *et al.* 2002; Van Vuuren 2010). Due to the serious need for alternative dietary protein supplements, these communities are opting for freshwater fish which are cheap and readily available from the river system. Fish populations in the Olifants River system have been showing a gradual increase of metal concentrations within their tissues over the past few decades (Seymore *et al.* 1995; Coetzee *et al.* 2002; Lebepe 2012). This trend has raised a concern regarding the safety of consuming these contaminated fish.

Most studies conducted in the Olifants River were specifically assessing the trend of metal accumulation across different tissues (Nussey *et al.* 2000; Oberhoster *et al.* 2012). It's only recently where researchers have shifted their focus from evaluating metals concentrations across different tissues to assessment of human health risks which may be associated with the consumption of these contaminated fish. However, very few studies (Addo-Bediako *et al.* 2014b, a; Jooste *et al.* 2014, 2015; Lebepe *et al.* 2016) have been carried out throughout the entire system on target species thus far. Therefore, this section aimed to assess the human health risks associated with the consumption of *Oreochromis mossambicus* and *Labeo rosae* from Loskop and Flag Boshielo dams.

7.2 Materials and methods

7.2.1 Human health risk assessment

A human health risk assessment was conducted using the desktop methodology of the US Environmental Protection Agency (US-EPA 2000). 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:

$$\text{BAF} = \frac{(\text{Concentration in fish})\text{x}(\text{Mass of portion consumed})\text{x}(\text{No. of fish meals per week})}{(\text{Body mass})\text{x}(\text{Average/No. of days in a week})} \ldots$$

……Equation 7.1

where the average fresh weight (fw) metal concentration in mg/kg, mass of portion in kilogram, adult body weight in kilogram and number of days between fish meals in days (US-EPA 2000). In order to calculate the ADD, a number of assumptions were required to characterise the population at risk: 150 g portion of fish muscle once a week; 70 kg adult; and 30 years of exposure (not used in the calculation, but the basis of the risk assessment). The average metal concentration in muscle tissues for each impoundment was used in the risk assessment.

A Hazard Quotient (HQ) 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:

$$\text{HQ} = \frac{\text{ADD}}{\text{RfD}} \quad \ldots\ldots\text{Equation (7.2)}$$

where HQ < 1 suggests adverse health effects are unlikely and HQ > 1 suggests a high probability of adverse health effects (US-EPA 2000). Reference dose levels published by the US-EPA were used (US-EPA 2013). The Pb RfD value from Ashraf *et al.* (2012), who referenced the US-EPA as their source, was used for this study. Box and whisker plots were prepared for the HQ values derived from the human health risk assessment for both dams using R-3.1.1 (R Development Core Team 2014).

7.3 Results

7.3.1 Metal concentration in the muscle tissue

Notable concentrations of all selected metals were recorded in the muscle of both species at both Loskop and Flag Boshielo dams (Table 7.1). Arsenic, barium, cobalt, iron, antimony, selenium and vanadium have shown relatively higher concentrations at Loskop Dam as compared to Flag Boshielo Dam for both species (Table 7.1).

Table 7.1 Mean concentration of metals (mg/kg dry weight) recorded in different tissues of *Labeo rosae* and *Oreochromis mossambicus* at Loskop and Flag Boshielo dam.

Metals	***Labeo rosae***		***Oreochromis mossambicus***	
	Loskop Dam (N=20)	Flag Boshielo Dam (N=20)	Loskop Dam (N=23)	Flag Boshielo Dam (N=20)
Ag	0.88 ± 1.01	3.70 ± 3.30	1.06 ± 1.43	0.83 ± 0.73
Al	26.60 ± 9.97	34.11 ± 43.17	13.01 ± 5.21	16.33 ± 7.75
As	1.16 ± 1.25	0.73 ± 0.86	2.63 ± 1.91	0.62 ± 0.98
B	0.17 ± 0.21	0.38 ± 0.21	0.43 ± 0.37	0.52 ± 0.49
Ba	1.25 ± 1.35	1.16 ± 0.47	0.26 ± 0.37	0.23 ± 0.27
Cd	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01
Co	0.03 ± 0.02	0.02 ± 0.02	0.13 ± 0.05	0.06 ± 0.05
Cr	0.87 ± 2.34	0.42 ± 0.71	0.21 ± 0.19	0.29 ± 0.29
Cu	0.49 ± 0.37	0.73 ± 0.43	0.75 ± 0.34	0.90 ± 0.43
Fe	20.97 ± 7.72	18.11 ± 11.23	21.64 ± 14.35	11.20 ± 4.84
Mn	2.18 ± 1.34	2.42 ± 1.08	1.37 ± 0.99	5.41 ± 6.40
Ni	0.34 ± 0.27	0.58 ± 1.10	0.32 ± 0.24	0.34 ± 0.21
Pb	0.07 ± 0.04	0.17 ± 0.32	0.02 ± 0.01	0.04 ± 0.04
Sb	0.15 ± 0.20	0.13 ± 0.13	0.17 ± 0.22	0.11 ± 0.07
Se	3.33 ± 4.64	3.26 ± 1.56	3.89 ± 3.37	2.02 ± 2.37
Sn	0.03 ± 0.02	0.06 ± 0.01	0.01 ± 0.01	0.04 ± 0.01
Sr	8.72 ± 4.42	10.36 ± 2.66	1.33 ± 3.00	1.09 ± 1.30
V	0.26 ± 0.11	0.14 ± 0.05	0.09 ± 0.08	0.07 ± 0.07
Zn	10.84 ± 3.77	24.79 ± 6.69	18.04 ± 3.13	20.42 ± 3.50

Mean ± standard deviation

7.3.2 Human health risk assessment

7.3.2.1 *Labeo rosae*

Hazard quotient values for *L. rosae* populations are presented in Figures 7.1, 7.2 & 7.3. Both dams exhibited the mean HQ values exceeding the international recommended value of 1 for arsenic and lead (Fig. 7.1). However, arsenic HQ value greater than 1 was recorded from 85% of the sampled population with 70% of the population showing lead HQ values above 1 at Loskop Dam (Fig. 7.2). At Flag Boshielo Dam, 5% of the sampled *L. rosae* population showed HQ values less than 1 for lead and 21% for arsenic. A HQ value greater than 1 was observed for antimony on 21% of the Flag Boshielo Dam population and 20% of Loskop Dam population exhibited HQ value of 0.6 (Fig 7.2). For selenium, 32% of the sampled *L. rosae* population has shown a HQ value of 1 with 26% showing a value above 0.6 (Fig. 7.2).

7.3.2.2 *Oreochromis mossambicus*

Oreochromis mossambicus population has shown a mean HQ value of greater than 1 for arsenic at both Loskop and Flag Boshielo dams (Fig. 7.1). Only 25% of the population sampled from Flag Boshielo Dam showed HQ value greater than 1 for arsenic (Fig. 7.3). At Loskop Dam, arsenic HQ value of greater than 1 was observed from 96% of the sampled *O. mossambicus* population (Fig. 7.3). Although lead was found to be problematic in *L. rosae* at both dams; only 4% of sampled *O. mossambicus* population has shown HQ value of greater than 1 at Loskop Dam. At Flag Boshielo Dam, 25% of the population exhibited lead HQ value of greater than 1. Antimony and selenium exhibited the HQ value of greater than 0.5 for about 10% of the sampled *O. mossambicus* at Flag Boshielo Dam (Fig. 7.3). At Loskop Dam, silver, cobalt and lead have shown the HQ value of greater than 0.5 from about 10% of the sampled population. Furthermore, antimony exhibited the HQ value of greater than 0.6 from about 40% of the sampled population (Fig. 7.3).

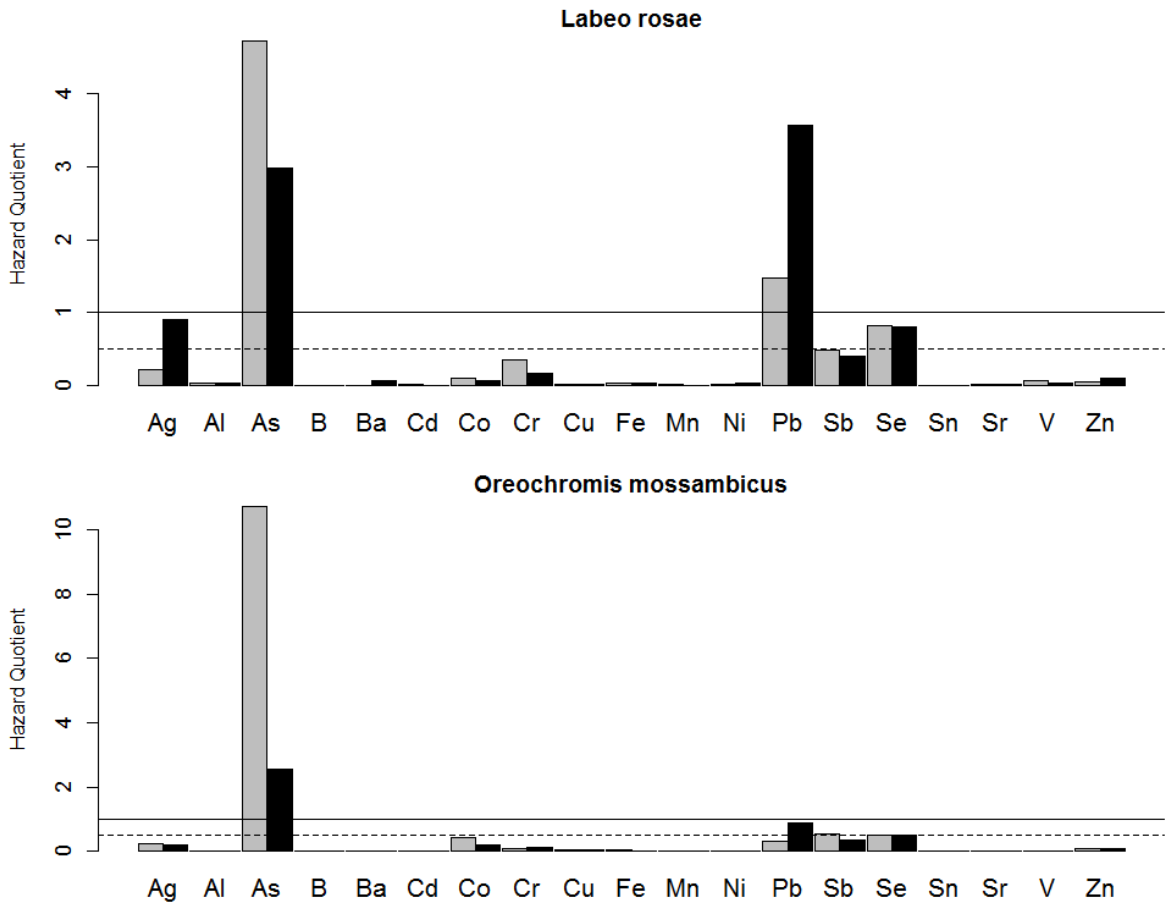

Figure 7.1 Hazard quotient values recorded for *Oreochromis mossambicus* and *Labeo rosae* sampled from Loskop (grey) and Flag Boshielo dams (black) during 2014 surveys. Abline drawn denote value above which the human adverse effects are probable.

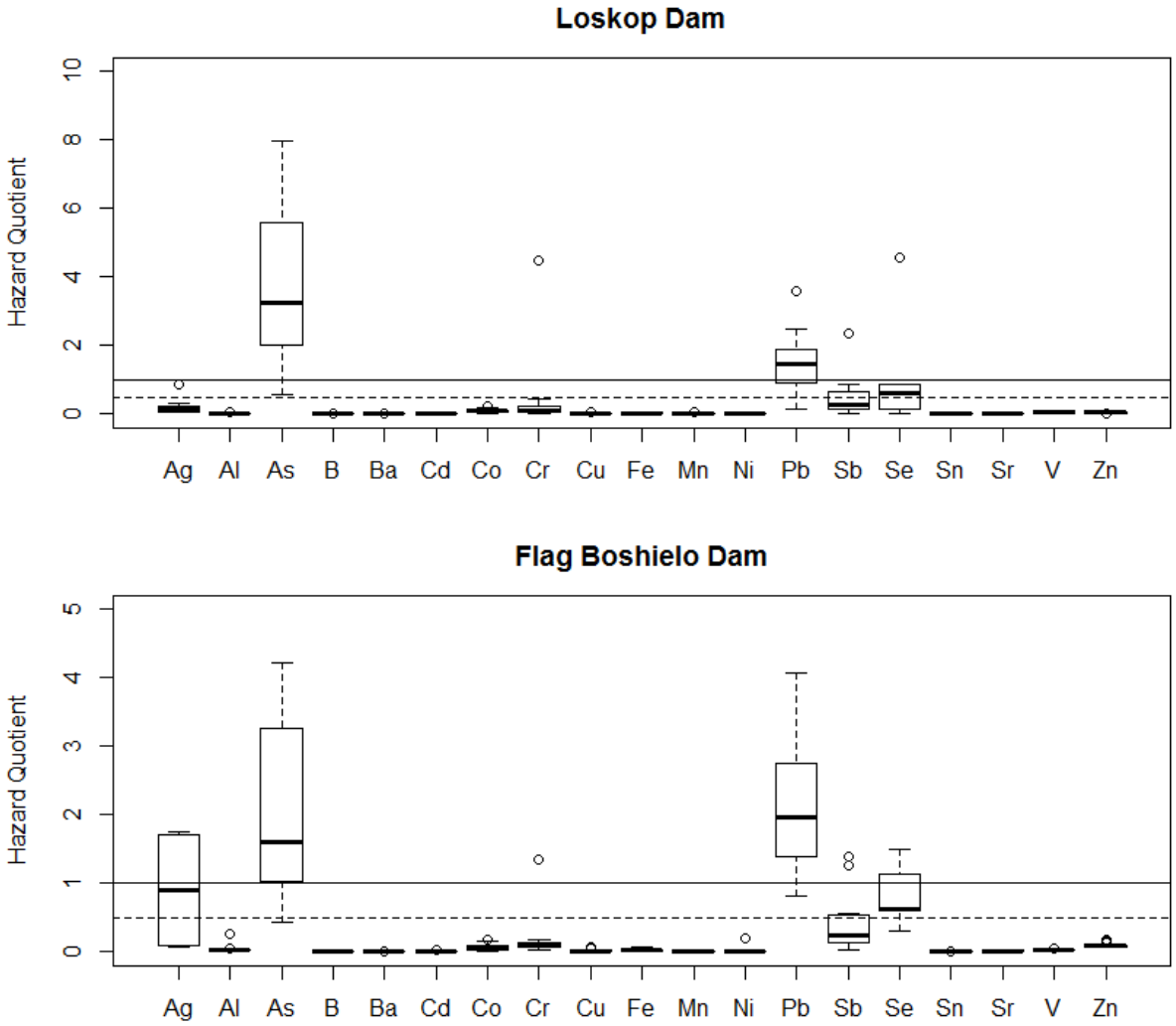

Figure 7.2 Box and whisker plots for hazard quotient values recorded for *Labeo rosae* from Loskop and Flag Boshielo dams during 2014 surveys.

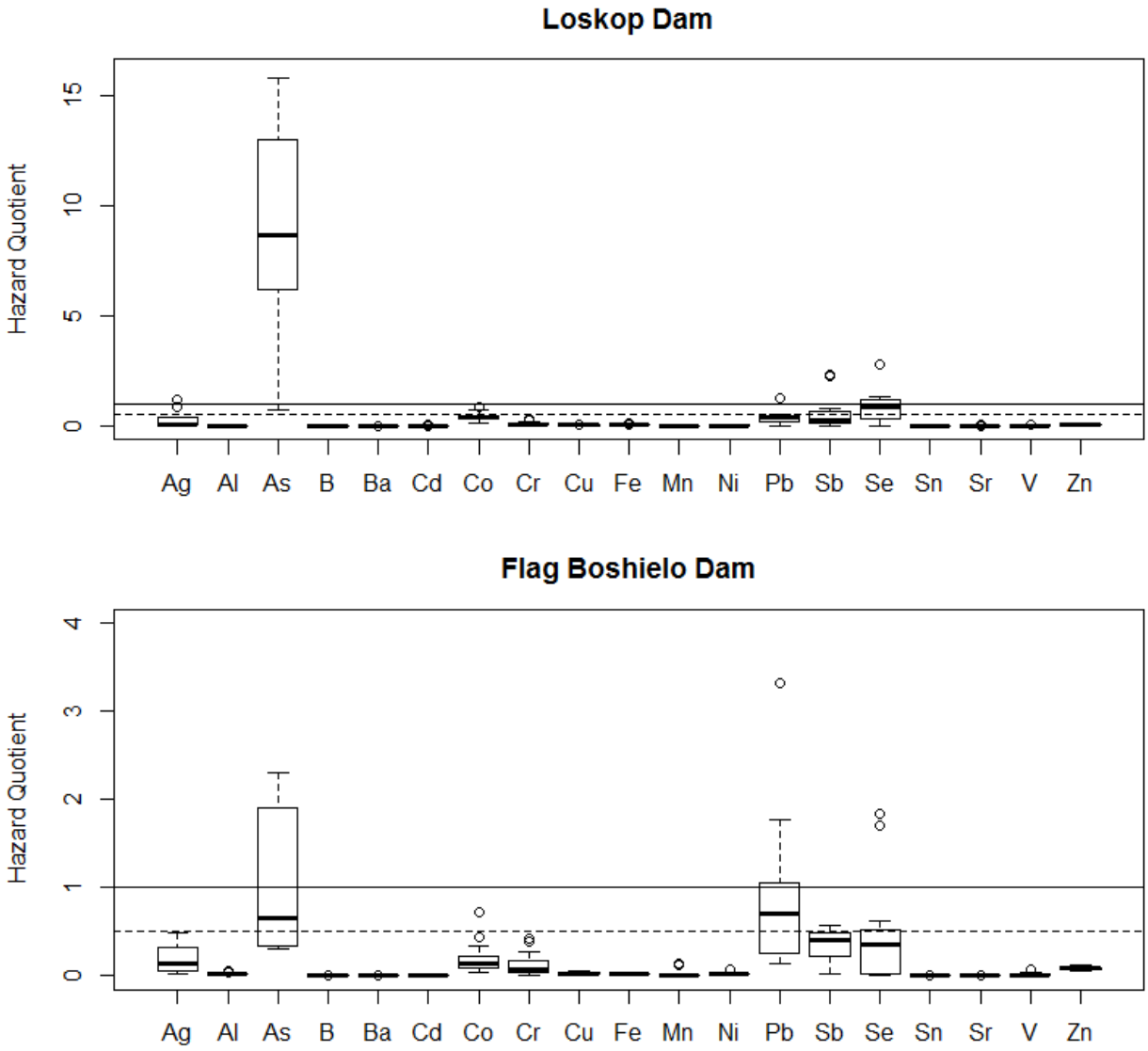

Figure 7.3 Box and whisker plots for hazard quotient values recorded for *Oreochromis mossambicus* from Loskop and Flag Boshielo dams during 2014 surveys.

7.4 Discussion

Recent studies reported that metals in fish from Loskop and Flag Boshielo dams have increased beyond international acceptable levels for human consumption (Addo-Bediako *et al.* 2014b; Lebepe *et al.* 2016). Antimony, chromium and lead concentrations exceeding international level for safe consumption were recorded in the muscle tissue of *O. mossambicus* (Addo-Bediako *et al.* 2014b), *L. rosae* (Jooste *et al.* 2014; Lebepe *et al.* 2016), *Clarias gariepinus* (Jooste *et al.* 2015) and *Schilbe intermedius* (Addo-Bediako *et al.* 2014a) sampled from Flag Boshielo Dam.

In the present study, more metals i.e. antimony, lead, silver, arsenic, and selenium showed to have exceeded international threshold for safe consumption at both Loskop and Flag Boshielo dams for both species as compared to previous studies. Although chromium and cobalt concentrations were within the acceptable range, they were on the verge of exceeding threshold levels for safe consumption since the HQ values greater than 0.6 but less than 1. Moreover, this assessment was carried out based on the assumptions that 150g portion is being consumed by 70kg adult once per week. Therefore, reported chromium, cobalt and zinc concentrations may result in adverse human health effects if the consumption rate increases or if the fish is consumed by an individual less than 70 kg. The effects of these metals in human are ranging from poor development in foetus to neurotoxicity and carcinogenicity in adults (Becker *et al.* 2007).

Arsenic is of particular concern since its known to be carcinogenic and several human systems and/or organs may be affected by even low concentrations (Heath *et al.* 2004). Developmental effects such as incidents of infants with congenital malformations and decreased birth weight were reported in arsenic exposed woman (ATSDR 2007b). Acute oral exposure to lower levels of arsenic has resulted in effects on the gastrointestinal system (nausea, vomiting, diarrhoea); central nervous system (headaches, weakness, lethargy, delirium); cardiovascular system (sinus tachycardia, hypotension, shock); and the liver, kidney, and blood (anaemia, leukopenia). The primary effects reported in humans from chronic exposure to arsenic are effects on the skin (US-EPA 2000; ATSDR 2007b).

Lead concentration has been a cause for concern in the Olifants River system. Several studies has found it to be exceeding international threshold for safe consumption in various fish species (Addo-Bediako *et al.* 2014a, b; Jooste *et al.* 2014, 2015; Lebepe *et al.* 2016). Lead may pose serious health effects in various organs even at low concentration. The nervous system of children is known to be the primary target for lead toxicity. Early symptoms that may develop within weeks of initial exposure include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory, and hallucinations (ATSDR 2007a).

Oral exposure of antimony may result in gastrointestinal complications such as stomach pains, colic, nausea and vomiting (ATSDR 1992; Sundar & Chakravarty 2010). Human health effects as a result of selenium exposure include aches and pains, irritability, chills and tremors, dizziness, pulmonary oedema and lesions of the lung as well as other respiratory, neurological and reproductive complications (Vinceti *et al.* 2001; ATSDR 2003). Silver is not considered to be carcinogenic and no toxic effects have been documented on the immune, cardiovascular, nervous, or reproductive by any form of silver (Drake & Hazelwood 2005). Only neurological complications have been reported from silver exposure (ATSDR 1990).

The increase of metal concentration in fish tissues in the Olifants River system has been reported to be driven by acid mine drainage decanting from derelict and abandoned mines (Hodgson & Krantz 1998; Dabrowski & de Klerk 2013). Measures were put in place to address the acid mine drainage problem in the catchment which include building of the two water treatment plants, Brugspruit Water Pollution Control Works and Emalahleni Water Reclamation Plant (Hobbs *et al.* 2008; Hutton *et al.* 2009). However, the efficiency of these plants towards addressing acid mine drainage problem in the upper Olifants River system is yet to be seen. There has been a significant increase of metal concentrations during their operational years. Coal mining in the upper Olifants River catchment commenced in 1895 and is still ongoing (Hobbs *et al.* 2008). Given that the coalfield has not reached its production peak in the catchment yet (Hobbs *et al.* 2008), and inability of the responsible authorities to employ effective measures to reduce the acid mine drainage seeping

from operating and abandoned mines, it is thus, reasonable to predict that human health risks upon consuming fish from the Olifants River system would increase.

The present study has found that consumption of *O. mossambicus* and *L. rosae* from Loskop and Flag Boshielo dams such that 70 kg adults consume 150 g portion once per week, may be exposing themselves to adverse health effects. More metals are showing to exceed acceptable levels for safe consumption as compared to studies conducted few years ago in this river system. Although water exhibited low metal concentrations, significantly high concentrations recorded in sediment remain a cause for concern. Primarily because these fixed metals will at some point remobilise back into the water column and be taken up by fish through muscle and gills. Moreover, metals fixed in sediment may get their way into the detritus and sediment feeding fish through diet. These dams are being considered for the development of inland fisheries in South Africa (Lebepe *et al.* 2016). Therefore, regular monitoring of metal concentrations in the muscle of the target fish species need to be conducted so that any drastic increase can be detected. Moreover, awareness and health advisories are recommended to acquaint communities about the safe consumption of fish from the Olifants River system.

CHAPTER 8

CONCLUDING REMARKS AND RECOMMENDATIONS

The aim of this study was to assess the effects of environmental contaminants on the health of *Oreochromis mossambicus* and *Labeo rosae* using enzymatic and histopathologic biomarkers at Loskop and Flag Boshielo dams. *Oreochromis mossambicus* and *L. rosae* are abundant at both dams and were among the species recorded during the mass fish mortalities in the Olifants River system. Both species exhibit the same mode of feeding and recent studies have reported elevated concentrations of metals in fish from Loskop and Flag Boshielo dams (Addo-Bediako *et al.* 2014b; Lebepe *et al.* 2016). Considering that local communities are consuming *O. mossambicus* and *L. rosae* from both dams, the present study further conducted the human health risk assessment to determine if it is safe to consume these fish species from the two dams.

Most water constituents showed to be within the guidelines for aquatic ecosystems at both dams. However, sulphate exhibited significantly high concentrations at both dams. The high sulphate concentration confirmed that although the water showed alkaline pH, the acid mine drainage problem is still a matter of concern. This was further supported by escalating metal concentrations in sediment and fish tissues at both Loskop and Flag Boshielo dams. Other constituents which showed significant concentrations were anions and cations; however, they are of least concern since they are not toxic to aquatic biota. Extensive agricultural activities in the middle Olifants River catchment have not increased the nitrogen input at Flag Boshielo Dam; hence, the dam was oligotrophic. Although previous studies have reported eutrophication at Loskop Dam (Oberholster *et al.* 2010; Nchabeleng *et al.* 2014), nitrogen concentration was mesotrophic during the present study. Extensive agricultural activities and sewage pollution from the upper catchment have not significantly increased the nitrogen concentration at Loskop Dam.

Few metals were detected in the water column; however, most analysed metals have shown significant concentrations in sediment at both dams. Change in physico-chemical properties of the water and biological processes in aquatic ecosystems may

remobilised metals back into the water column (Coetzee *et al.* 2002). Therefore, metal concentrations in the sediment remain a cause for concern. The water at Loskop and Flag Boshielo dams are at risk of experiencing metal pollution without inputs from external sources. Moreover, organisms feeding on detritus, benthos and sediment may be exposed to these increased metal concentrations.

Fish at both dams exhibited elevated metal concentrations across their tissues. Popular metal concentration trend of liver>gills>muscle was observed for *O. mossambicus* and *L. rosae* at both dams. However, lead, manganese and strontium exhibited higher concentrations in the gills for both species at both dams. The present study demonstrated that liver and gills may be used as indicative organs for the estimation of metal pollution in aquatic ecosystems. Muscle is not an active site for metal biotransformation and accumulation (El-Moselhy *et al.* 2014), and this fact was also supported in the current study. No relationship was observed between fish size and metal concentration on both species at Loskop and Flag Boshielo dams. Fish populations at Loskop Dam were extremely large but showed relatively lower metal concentrations as compared to those from Flag Boshielo Dam. The increased metal concentrations in the water and sediment at Flag Boshielo Dam may be the explanation for the higher levels in Flag Boshielo Dam populations.

Both *O. mossambicus* and *L. rosae* populations exhibited elevated concentrations of metals at both dams. These increased metal concentrations in the liver were accompanied by irregular activities of LDH, G6PDH and GST. Activities of the three enzymes have shown relationship with metal concentrations for both fish populations. Metabolic enzymes, LDH and G6PDH have shown different trend when exposed to increased metal concentrations. Glucose-6-phosphate dehydrogenase was induced whereas LDH was inhibited. Therefore, both enzymes proved to be useful biomarkers of contaminant exposure and reliable when used together.

Metal concentrations were higher in Flag Boshielo Dam populations as compared to those from Loskop Dam whereas the GST activity was lower in Flag Boshielo Dam populations. According to Kopecka *et al.* (2006), GST is one of the main enzymes in detoxification pathway and its activity may increase with increasing concentrations of

contaminants; however, this hypothesis was not supported during the present study. From these results, it is evident that elevated metal concentrations are capable of inhibiting GST.

Inhibition of AChE was observed from Flag Boshielo Dam populations for both species. Van der Oost *et al.* (2003) reported that Acetylcholinesterase is highly sensitive to carbamates and organophosphorus, and it was found responding to even low concentrations. Results observed in the present study suggest that pesticides applied at the Groblersdal and Marble Hall areas are inhibiting the AChE at Flag Boshielo Dam.

Despite biomarker responses to elevated metal concentrations, condition factor has not deviated from the normal range. Hepatosomatic index has been used in ecological studies to discriminate populations from different environmental quality; however, it was not conclusive in the present study. The overall conditions of both species were good at both Loskop and Flag Boshielo dams.

Histopathologic alterations were similar for both species at both dams but differed in severity. Prevalence and severity of alterations were relatively high in the gills as compared to the liver for both species at both dams. Gills exhibited moderate modifications for *O. mossambicus* at Loskop Dam and slight modification at Flag Boshielo Dam. Slight modifications were observed in the gill of *L. rosae* populations at both dams. Liver exhibited slight modifications for *O. mossambicus* and *L. rosae* populations at Loskop and Flag Boshielo dams. Slight modification in the liver coincide with condition factor results which classified the fish overall conditions as good. The different trend of histopathological alterations between the two species may suggest that these species differ with regard to sensitivity to contaminants.

Histopathologic and enzymatic biomarkers have proven to be reliable tools for detecting early warning signs of the effect of pollution on the health of fish. Despite condition factor falling within the normal range, histopathologic and enzymatic biomarkers have shown that fish at Loskop and Flag Boshielo dams are living under stressful conditions. Given that responsible authorities are not doing enough to address the acid mine drainage problem in the Olifants River system, it is predicted

that the health of fish will deteriorate. Therefore, regular studies of this kind are recommended to keep a close eye on the health of fish in this river system.

Both species have proven to accumulate metals with muscle exhibiting relatively low concentrations. Considering that muscle is the tissue consumed by human, low concentrations is enough to cause a concern. Comparing to concentrations reported in previous studies, metal concentrations in muscle of fish in the Olifants River system have substantially increased over the past two decades. Lead, chromium, cobalt and antimony concentrations have been the only metals of concern over the past few years, nevertheless, the present study has shown that arsenic, silver and selenium have also exceeded international levels for safe consumption.

The mining of coal in the upper Olifants River catchment commenced in 1895 and the coalfield has not reached its production peak yet. Acid mine drainage from operating and abandoned coal mines remain a threat since the operating water treatment plants in the catchment are not doing enough to ensure that the water reaching the system are good for aquatic ecosystem (Hobbs *et al.* 2008). If another intervention is not made to reduce pollution in the Olifants River system, the health risks of consuming fish from this system would increase and more metals will ultimately exceed the international acceptable level for safe consumption in *O. mossambicus* and *L. rosae.*

Given that both Loskop and Flag Boshielo dams are being considered for inland fisheries (Lebepe *et al.* 2016), it is therefore, recommended that the implementation should be put to a halt until the acid mine drainage problem is addressed. However, there are local communities fishing for subsistence, therefore, regular monitoring of metal concentrations in fish tissues is recommended so that any drastic increase may be detected and communicated in time. Moreover, health advisory should be established to acquaint these communities with knowledge about the safe consumption of fish from the Olifants River system. More studies on metal variations with species, fish age and sizes along the longitudinal gradient of the Olifants River system are encouraged to determine the fish group which may be safe for human consumption.

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APPENDIX

Table 1: Biometric data of *Labeo rosae* sampled from Loskop Dam during winter and summer 2014.

Fish #	Sex	Mass (g)	Total length (cm)	Standard length (cm)	Liver weight (g)
28 July 2014					
1	M	1500	45.4	37	9.28
2	M	1100	43.9	36.5	8.84
3	F	1550	46.1	38.5	10.98
4	M	1200	43.8	36.2	7.87
5	F	1530	49.8	41.1	20.6
6	M	1300	44.8	37.1	9.6
7	F	1300	46.4	38.7	12.1
8	M	1200	45.6	37.1	9.8
9	M	1100	43.1	35.4	9.4
10	F	720	38.2	31.1	5.8
19 November 2014					
1	F	1066.99	42.5	34.9	8.74
2	M	498.7	32.1	26.5	4.04
3	F	784.2	37.1	30.9	7.22
4	M	1354.52	36.66	45.3	7.19
5	F	1877.26	41.9	50.3	19.21
6	F	1510.11	37.8	45.9	11.01
7	F	1759.44	39.9	48.9	12.2
8	F	1570.08	40.9	49.9	15.9
9	M	1447.47	46.3	38.4	11.08
10	F	1787.87	39.5	48	15.35
11	F	1914.83	41.7	49.6	18.21
12	M	1935.4	42.1	50.3	16.41

Table 2: Biometric data of *Labeo rosae* sampled from Flag Boshielo Dam during winter and summer 2014.

28 July 2014					
Fish #	Sex	Mass (g)	Total length (cm)	Standard length (cm)	Liver weight (g)
1	F	721.35	38.5	31.9	10.57
2	M	219.31	26.9	21.4	2.83
3	F	586.61	36.1	29.1	7.25
4	M	255.32	30	24.5	2.21
5	M	337.02	24.3	31.5	2.64
6	M	311.03	24.9	30.5	2.5
7	M	254.94	29.6	23.4	1.89
8	M	279.94	30.3	25.2	2.36
9	F	374.8	33.5	26	6.28
10	F	382	35.2	26.9	5.2
18 November 2014					
1	F	403.3	31.12	25.91	3.08
2	F	391.82	32.85	26.01	3.65
3	M	489.72	35.67	28.51	3.06
4	F	362.05	31.3	25.72	3.18
5	M	301.59	30.3	24.1	1.8
6	M	477.9	34.6	27.9	3.58
7	F	483.72	33.6	27.9	3.46
8	M	370.39	35.5	31.8	3.24
9	M	340.03	30.7	24.7	2.57
10	M	400.01	34.1	26.4	2.72
11	M	350.62	30.5	25.5	2.06
12	M	294.41	29.5	23	1.71
13	M	262.56	29.1	23.5	1.5
14	F	72.72	19.7	15.5	0.71

Table 3: Biometric data of *Oreochromis mossambicus* sampled from Loskop Dam during winter and summer 2014.

28 July 2014					
Fish #	Sex	Mass (g)	Total length (cm)	Standard length (cm)	Liver weight (g)
1	M	2100	44.7	39.1	21.6
2	F	1650	41	35.9	22
3	M	2350	46	39.6	25.3
4	M	1200	37.5	32.8	15.4
5	M	1800	43.7	38.1	23.7
6	M	2250	46.1	40.9	23.3
7	F	1350	40.1	31.9	18.4
8	M	1750	42.9	36.9	17.2
9	M	1950	43.1	38.9	27.5
10	M	1450	40.3	34.5	22.5
11	M	1650	44	37	30.5
12	F	1500	39.2	31.9	15.2
19 November 2014					
1	F	1253.16	38.6	32.1	19.95
2	M	1561.75	42.7	35.6	16.36
3	F	363.69	25.3	21.3	3.97
4	M	1807.48	44.9	37.7	19.58
5	F	1491.01	39.2	32.9	18.28
6	M	1489.16	42.5	34.7	14.09
7	F	1249.16	38.8	32.9	11.24
8	F	154.19	20.4	17.1	1.1
9	M	222.91	22.3	18.1	1.93
10	F	1186.3	40.7	34.9	12.93
11	M	2094.2	46.6	39.1	73.88

Table 4: Biometric data of *Oreochromis mossambicus* sampled from Flag Boshielo Dam during winter and summer 2014.

19 August 2014					
Fish #	Sex	Mass (g)	Total length (cm)	Standard length (cm)	Liver weight (g)
1	M	899.6	37.2	31	6.78
2	F	502.25	29	24.1	3.24
3	F	413.84	27.1	22.9	3.07
4	F	313.79	25.4	21.1	2.79
5	F	349.7	26.1	21.9	2.69
6	M	295.28	24.9	20.9	2.18
7	M	280.14	24.5	20.1	1.76
8	F	285.4	24.5	20.1	2.31
9	M	386.73	27.6	22.3	2.9
10	M	264.72	24.3	19.9	1.46
18 November 2014					
1	M	149.57	20.3	16.9	1.14
2	F	119.85	18.1	14.5	0.72
3	M	139.33	19.9	16.4	0.9
4	F	749.73	35.5	30	5.83
5	M	648.8	33.5	28.5	5.33
6	M	159.02	20.51	16.02	0.95
7	F	159.02	20.31	16.91	1.24
8	F	153.5	24.5	20.5	2.11
9	M	158.43	20.1	16.5	0.87
10	F	145.67	19.5	15.9	0.83
11	M	139.85	19.5	16.3	1.04
12	M	114.93	18.3	15.1	0.43
13	M	48.02	14	11.5	-
14	F	44.77	13.5	11.2	-