



# Assessing the effects of multiple stressors on the Incomati River Catchment

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## ABSTRACT

Freshwater ecosystems, such as rivers, lakes, and wetlands, are among the most diverse and productive ecosystems on the planet. They support a wide variety of species and provide important ecosystem services, including water for drinking, agriculture, and industrial activities. It also provides habitat for aquatic organisms. These freshwater resources are under immense pressure due to a growing population, urbanisation, industrialisation, agricultural expansion, and climate change. Anthropogenic activities such as agriculture, forestation, mining, as well as wastewater pollution contribute to the decline in the quality of these ecosystems. Although trace elements are naturally occurring, an increase in these anthropogenic activities close to river catchments has led to an increase in the concentrations of trace elements in water and sediment of freshwater systems. Some trace elements (zinc, copper, and iron) are essential for the metabolic processes of aquatic organisms, but at high concentrations, they pose a threat to the health of these organisms. Elements such as cadmium, mercury and lead, are non-essential and highly toxic even at low concentrations. The Limpopo River and Incomati River catchments are important rivers that provide water to millions of people that live in and around these catchments. Both of these rivers flow through the Kruger National Park (KNP) and where there are few anthropogenic activities that could affect the health of the ecosystem, whereas just outside of the KNP, both of these catchments are affected by many anthropogenic activities such as mining, agriculture, as well as industrial activities.

Therefore, the aims of the present study were to determine the water and sediment quality of both the Limpopo River and the Incomati River catchments, determine the accumulation of trace elements in *Oreochromis mossambicus* (Mozambique tilapia) from selected sites in the Limpopo River and Incomati River catchments, and to determine whether there is a relationship between environmental element exposure (as reflected in the bioaccumulation in *O. mossambicus*) and ecosystem health (as reflected by the water, sediment and fish index scores). Firstly, the physico-chemical variables of the water and sediment of these systems were assessed. Secondly, the bioaccumulation of trace elements in the muscle tissue of fish was assessed to determine the bioavailability of trace elements in the environment. Thirdly, the Aquatic Toxicity Index (ATI), Sediment Quality Index (SeQI) and Fish Response Index (FRAI) were assessed in relation to the accumulated trace element concentrations in the muscle tissue of fish.

From the results, it was evident that trace element concentrations in the water and sediment of the Limpopo River and Incomati River catchments are influenced by the unique anthropogenic activities in their respective tributaries. It was also determined that trace element concentrations in the water and sediment of the Limpopo River catchment were higher than the trace element concentrations in the water and sediment of the Incomati River catchment. *Oreochromis mossambicus* was an

effective bioindicator for trace elements in both of these systems. The fish from the Limpopo River had higher trace element concentrations compared to fish from the Incomati River catchment. It was also evident that there was a relationship between environmental element exposure, indicated by element bioaccumulation in *O. mossambicus*, and the ecological status as indicated by water, and biotic (fish) indices.

The present study concluded that the Limpopo River and the Incomati River catchments are subjected to various stressors that contribute to increased levels of nutrients, as well as the trace element concentrations in these systems. The accumulation of trace elements in *O. mossambicus* from the Limpopo River and Incomati River catchments serves as a valuable indicator of environmental quality, pointing to significant pollution risks from industrial, agricultural, and mining activities. The stressors affecting these catchments need to be addressed, to help preserve both the aquatic organisms that inhabit these catchments, as well as the communities that are dependent on these water resources.

**Keywords:** Bioaccumulation, biomonitoring, trace elements, water quality, sediment quality, *Oreochromis mossambicus* (Mozambique tilapia)

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# Chapter 1: General Introduction

## 1.1 Background:

The Limpopo River catchment is one of the largest catchments in South Africa. It covers approximately 416,300 km<sup>2</sup> (Kapangaziwiri *et al.*, 2021) and flows through four neighbouring countries, South Africa (45%), Botswana (19%), Mozambique (21%), and Zimbabwe (15%) (Kahinda *et al.*, 2016). The upper reaches of the Limpopo River form the border between Botswana and South Africa, the middle reaches form the border between South Africa and Zimbabwe, whereas the lower reaches of the river are located entirely in Mozambique where it discharges into the Indian Ocean (Kahinda *et al.*, 2016). Some of the main tributaries of the upper reaches of the Limpopo River catchment in South Africa are the Crocodile, Matlabas, Lephale, and Mogolakwa Rivers, the middle reaches include the Sand, and Luvuvhu Rivers, and the lower reaches include the Olifants River (Kapangaziwiri *et al.*, 2021).

The Crocodile River (West) catchment is the second largest tributary of the Limpopo River and has a catchment area of 29,572 km<sup>2</sup> (Dickens *et al.*, 2021). Some of the pressures that affect the water quality of the catchment are urbanisation, industrial activities, mining activities, and large-scale irrigation schemes (Walsh & Wepener, 2009; Du Preez *et al.*, 2018). The Hex River, a large tributary of the lower reaches of the Crocodile River, is impacted by large platinum and chromium smelter activities that affect the water and sediment quality (Dickens *et al.*, 2021; Erasmus *et al.*, 2022; Stevens *et al.*, 2023). The Matlabas River covers an area of 3,448 km<sup>2</sup> and is one of the smallest tributaries of the Limpopo River catchment (Dickens *et al.*, 2021). According to Lombaard *et al.* (2015), the irrigation sector is the major water user of the Matlabas River catchment. The Lephale River is a small tributary of the Limpopo River catchment and covers an area of 4,868 km<sup>2</sup> with less influence from anthropogenic activities, although extractive mining is permitted along the catchment with agricultural and domestic usage also putting pressure on the water quality of this catchment (Dickens *et al.*, 2021).

The Luvuvhu River covers an area of 5,941 km<sup>2</sup> and rises from the Soutpansberg Mountains (Edokpayi *et al.*, 2022). According to Dickens *et al.* (2021), land-use activities include commercial forestry, agriculture, conservation areas and urban. Trace element contamination in the Luvuvhu River is due to mining in the tributaries of the Luvuvhu River (Gerber *et al.*, 2015a). Some of the tributaries of the Limpopo River catchment are non-perennial rivers. The Shingwedzi River is one of these rivers and is influenced by rural settlements along the catchment, as well as subsistence farming activities (Fouche & Vlok, 2010). According to

Dickens *et al.* (2021), the Letaba River covers an area of 13,400 km<sup>2</sup> and is subjected to numerous anthropogenic activities such as water abstraction, flow regulation, commercial agriculture and forestry, which affect the water quantity, as well as the quality. The Olifants River is the largest tributary of the Limpopo River catchment and covers an area of 54,475 km<sup>2</sup> (McCartney *et al.*, 2004). It is regarded by many as one of South Africa's most polluted river systems due to anthropogenic activities such as mining, agriculture, forestation, and domestic and industrial activities (Kotze *et al.*, 1999; Wepener *et al.*, 1999; Heath *et al.*, 2010; Ashton & Dabrowski, 2011; Gerber *et al.*, 2015a; Malakane *et al.*, 2020;). According to Nhiwatiwa *et al.* (2017), acid mine drainage from abandoned mines in the upper catchment results in the acidification of river water and the mobilisation of metals from the bedrock and sediment. The Olifants River is also one of the biggest rivers flowing through the Kruger National Park (KNP). The development of mining activities has affected the water quality of the Olifants River and is introducing trace elements, as well as salts into this system (Riddell *et al.*, 2019).

The Incomati River catchment is an international catchment that flows through three countries namely South Africa (Mpumalanga), Eswatini (Swaziland), and Mozambique, where it is discharged into the Indian Ocean (Kleinhans *et al.*, 2013). The catchment covers about 46,800 km<sup>2</sup>, most of which is located in South Africa (63%), whereas only 32% of the river is located in Mozambique and 5% in Swaziland. This catchment is very important in South Africa and includes sub-catchments such as the Komati, Crocodile, Elands, Sabie and Kaap Rivers (Kleinhans *et al.*, 2013). The Crocodile River covers an area of 10,440 km<sup>2</sup> (Kleinhans *et al.*, 2013) and the water quality is influenced by agricultural runoff, as well as mining activities (Madonsela *et al.*, 2024). According to Riddell *et al.* (2019), the Sabie River is considered one of the most pristine and biodiverse rivers in the whole of South Africa, however, up until the mid-1940s when livestock farmers successfully lobbied for the South African government to prevent upstream gold mine tailings along the Drakensberg Escarpment, the effects of cyanide leachate were noted all the way to the KNP.

Both the Limpopo River and the Incomati River catchments flow through the KNP before flowing across the border into Mozambique. The KNP is the biggest conservation area in South Africa, covers an area of two million hectares and is situated in the savanna biome (Riddell *et al.*, 2019). Despite its important conservation status, the rivers of the KNP have been subjected to metal pollution ever since the first mining activities were initiated in the Sabie River catchment in the 1800's (Riddell *et al.*, 2019). The challenge in managing the water quality of

these large perennial rivers that flow through the KNP is that each river has different anthropogenic activities in the upper catchments. According to Riddell *et al.* (2019) there is a need to study the diffuse common impacts (e.g. metal elements) on an inter-catchment basis for effective engagement with upstream water users and to contribute to effective sustainable catchment management of these rivers.

*Oreochromis mossambicus* (Mozambique tilapia) is native to the eastward-flowing rivers of central and southern Africa (Russell *et al.*, 2011). Maddern *et al.* (2007) stated that *O. mossambicus* is indigenous to fresh and brackish waters of southeastern Africa and has been cultured in many countries, most notably in Africa and Asia, for human consumption. They are very tolerable fish and can survive in a wide range of temperatures and salinity, different flow velocities, as well as tolerate high concentrations of ammonia and nitrate (Maddern *et al.*, 2007). The feeding habits of *O. mossambicus* depend on the season and life stage of the species but they generally feed on debris in reed beds, as well as algae (Mannzhi *et al.*, 2021). Trace elements bioaccumulate in the tissue of aquatic organisms, such as fish, through food, water, as well as sediments (Lebepe *et al.*, 2020). Thus, the Mozambique tilapia has commonly been used as an indicator species for element contamination of freshwater systems of the north-eastern regions of South Africa (Chatterjee *et al.*, 2006; Arain *et al.*, 2007; Addo-Bediako *et al.* 2014; Yap *et al.*, 2015; Plessl *et al.*, 2017; Lebepe *et al.*, 2020; Mannzhi *et al.*, 2021; Erasmus *et al.*, 2022; Lebepe *et al.*, 2024).

## **1.2 Assessing the ecosystem health of rivers**

The water quality of the rivers in South Africa can be attributed to an array of anthropogenic activities. According to Madonsela *et al.* (2024), the major contributors of the deterioration of South Africa's water quality include agricultural runoff, extensive coal mining activities, industrial activities, and a general decline in the operation and management of wastewater treatment infrastructure, especially sewage treatment. Water from the Limpopo River catchment is used for irrigation that accounts for about 50%, while urban use is only 30% and other uses like mining, power generation and rural water use each account for about 6% (Munnik, 2020). The surrounding areas of the catchment are mainly used for commercial forestry, grazing, and irrigation (Saraiva-Okello *et al.*, 2015). According to Kleinhans *et al.* (2013), the intensive use of water in the Incomati River system for irrigation purposes has impacted the ecosystem's health. In the eastern regions of South Africa, the production of sugarcane uses water from the Crocodile, Lomati, as well as the Komati Rivers as their main source for irrigation (Van der Laan *et al.*, 2012). As stated by Nhiwatiwa *et al.* (2017), the development of intensive agricultural areas can have a significant impact on the integrity of

river ecosystems in terms of ecological health and water quality. There are multiple sugarcane plantations next to these catchments that are affecting the water quality of these systems through ions that are leached from these activities into these systems when making use of flood irrigation (Nhiwatiwa *et al.*, 2017).

The health of ecosystems around the world has been affected by anthropogenic activities (O'Brien *et al.*, 2016), thus affecting the ecosystem services that they provide. It is, therefore, necessary to assess the health of ecosystems such as rivers, to prevent further deterioration. There are different approaches that can be used to assess the health of river ecosystems. Some of the approaches include assessing the physical, and chemical variables of water and sediment, as well as biological indicators. Different indices are also used to assess the health of ecosystems. In this study, an ecotoxicological approach is followed to assess the health of these ecosystems.

### **1.2.1 Water and Sediment Quality assessment**

“Quality” of water is a human construct that is used to describe the usefulness of water, which is also dependent on the way it is going to be used (Dallas & Day, 2004). Differences in climate, geomorphology, geology, soils and biotic composition contribute to the differences in the water quality of different regions, as well as continents (Dickens *et al.*, 2021). The water and sediment quality of a river system is dependent on whether it should be assessed according to whether it will be related to ecosystem health or human consumption. The physical properties of rivers can vary a lot, depending on the flow, temperature, type of sediment, as well as the geology. It is important to monitor the health of a river system seasonally due to the fluctuations in the hydrology. Water quality is only an indication of the health of the system at that point in time since it is influenced by different variables such as *in situ* parameters (*i.e.* pH, electrical conductivity (EC), total dissolved solids (TDS), dissolved oxygen (DO), and temperature), anions, cations, as well as trace elements, whereas sediment quality variables (trace elements, particle size and organic carbon content) could be an indication of the long term effects of contaminants on a system (Erasmus, 2020). Contaminants that are bioavailable in the water and sediment of river systems can also accumulate in aquatic biota. It is thus also important to determine the trace element concentrations in aquatic biota to provide a better overview of the health of the aquatic ecosystem. According to O'Brien *et al.* (2016), multi-metric indices are useful in that they can distil a lot of information and parameters onto a uniform scale that can be used to provide an indication the health of an ecosystem. The Aquatic Toxicity Index (ATI) can be used to assess the health of an ecosystem by integrating the water quality data and providing an index score that can be used to determine the ability

of a system to sustain aquatic life (Gerber *et al.*, 2015a). The Sediment Quality Index (SeQI) is also used to determine the health of an ecosystem by integrating sediment quality variables and providing an index score that provides insight into the effects that anthropogenic activities have on sediment quality (Gerber *et al.*, 2015b).

### **1.2.2 Fish assessment**

Aquatic biota can be used as indicators for contamination to determine the health of an aquatic ecosystem. Fish live longer than macroinvertebrates and are exposed to contaminants for longer periods. According to Authman *et al.* (2015), due to feeding and living in aquatic environments, fish are particularly vulnerable and heavily exposed to pollution because they cannot escape from the detrimental effects of pollutants. This makes fish good indicators of environmental health by determining the concentration of trace elements in the fish inhabiting these aquatic systems. Trace element concentrations in fish depend on a number of factors *i.e.* pH, EC, temperature, salinity, fish age, size, sex and the presence of other elements (Lebepe *et al.*, 2024). Different biological indices have been developed to assess the ecosystem health of aquatic ecosystems. According to Mangadze *et al.* (2018), the fish response assessment index (FRAI) is an assessment index based on the environmental intolerances and preferences of the reference fish assemblage and the response of the constituent species of the assemblage to particular groups of environmental determinants or drivers. The FRAI measures how fish assemblages respond to different environmental drivers such as hydrology, geomorphology as well as the physico-chemical water quality properties (Levin *et al.*, 2019).

### **1.3 Problem statement, hypotheses, and aims.**

Trace elements occur naturally in the crust of the earth and leach into the environment from the geology, and are present in low concentrations (Edokpayi *et al.*, 2022). Different guidelines are set to determine if the water and sediment quality of a system is good or bad for specific uses. According to Edokpayi *et al.* (2022), several water quality indices have been developed to aid in presenting levels of risk associated with metals to water managers. Trace elements such as iron, copper, manganese, magnesium and zinc are essential in the metabolism of organisms, but only at optimal concentrations, whereas non-essential elements such as mercury, cadmium, silver and lead are toxic to aquatic organisms even at low concentrations (Vasanthi *et al.*, 2019). There is a multitude of research done on water pollution of South African freshwater systems, as well as the Limpopo River and what pollution does to the water quality as well as the effects it has on biota (*e.g.*, Ashton *et al.*, 2001; Dallas & Day, 2004;

Petrie *et al.*, 2015, Kahinda *et al.*, 2016; De Necker *et al.*, 2020; Erasmus *et al.*, 2020a; 2020b; Munnik, 2020; Dickens *et al.*, 2021). These studies indicated that the Limpopo River catchment is affected by contamination of trace elements, however, the Incomati River catchment only has limited information regarding the water and sediment quality in Mozambique and South Africa, which is concerning since there are multiple communities that make use of these rivers as a source of water and other ecosystem services. It is necessary to understand the distribution of trace elements, water quality trends, and ecosystem health of these systems to help preserve these rivers and ensure the continued availability of the vital resources that these systems provide. This study assessed the water and sediment quality of the Incomati River catchment, as well as the accumulation patterns of trace elements in *O. mossambicus* from both the Limpopo River and Incomati River catchments. Measurements of element concentrations in water and sediment only reflect the momentary pollution status and do not account for other factors such as the temperature or pH of the water, thus it is necessary to determine the accumulation of elements in biota (Bervoets *et al.*, 2020). Relating the accumulation of elements in biota such as fish, to different ecosystem health indices can potentially be used to predict the ecological effects of contamination on aquatic communities (Bervoets *et al.*, 2020). The association of accumulation patterns with ecosystem health indices has not been assessed in South Africa, whereas it has been proven to work in European countries. The present study also assessed the association of different ecosystem health indices to the accumulation patterns of trace elements in *O. mossambicus* from the Limpopo River and the Incomati River catchments.

### **1.3.1 General hypotheses**

#### **The hypotheses tested in the present study:**

- The trace element concentrations in the water and sediment of the Limpopo River and Incomati River catchments are influenced by the unique anthropogenic activities in their respective tributaries.
- The degree of element pollution at the different sites will be reflected in the water and sediment quality indices.
- Bioaccumulation of elements in fish will reflect the degree of element contamination of water and sediments of the different tributaries of the two catchments.
- Environmental element exposure, indicated by element bioaccumulation in *O. mossambicus*, will reflect the ecological status as indicated by water, sediment and biotic (fish) indices.

### 1.3.2 Aims and Objectives

This study mainly focussed on selected tributaries of the Limpopo River and Incomati River catchments. The aims of this study were to 1) determine the water and sediment quality of both catchments, 2) determine the accumulation of trace elements in *O. mossambicus* from selected sites in the Limpopo River and Incomati River catchments, and 3) to determine whether there is a relationship between environmental element exposure (as reflected in the bioaccumulation in *O. mossambicus*) and ecosystem health (as reflected by the water, sediment and fish index scores).

The following objectives were set to be able to achieve the aims set out in this study:

- Conduct surveys at selected sites in the Limpopo River and Incomati River catchment to collect water, sediment and *O. mossambicus* muscle tissue samples.
- Determine the water and sediment physico-chemical parameters (including element concentrations) at selected sites in both catchments and assess the spatial relationships of these parameters.
- Determine the element concentrations in muscle tissue of *O. mossambicus* from selected sites in both catchments and assess the relationship with element concentrations in water and sediments from the sites.
- Determine if there are any relationships between element exposure (bioaccumulation in muscle tissue of *O. mossambicus*) and ecosystem health (the different indices that were assessed at each of the sites).

### 1.4 Chapter layout:

The present study has been divided into four chapters:

**Chapter 1: General Introduction:** This chapter provides background information on the Limpopo River and the Incomati River catchments, as well as some of their main tributaries. Background on why fish make a good bioindicator of trace elements in an aquatic environment, as well as on the specific species used in this study.

**Chapter 2: Assessing the water and sediment quality of two catchments in southern Africa:** In this chapter, the water and sediment quality at 13 sites on the Limpopo River and seven sites on the Incomati River were determined. The water and sediment quality of both catchments were also compared to each other. The Aquatic Toxicity Index (ATI) and Sediment Quality Index (SeQI) were assessed for each site.

**Chapter 3: Element bioaccumulation in *Oreochromis mossambicus* from the Limpopo and Incomati River catchments:** This chapter presents the results of trace element

concentrations in *O. mossambicus* muscle tissue from six sites in the Limpopo River and Incomati River catchments. Correlations between different indices and trace element concentrations were assessed.

**Chapter 4: General conclusion and recommendations:** Gives an overall conclusion based on the results of the water and sediment quality, as well as the trace element concentrations that accumulate in *O. mossambicus* from selected sites. The chapter also gives recommendations for possible future studies.

**References:** A list of all the references used in this study.

## **Chapter 2: Assessing the water and sediment quality of two catchments in southern Africa.**

### **2.1 Introduction**

Natural resources all over the world are under huge pressure due to several anthropogenic stressors such as climate change, industrial development, increased agricultural activities, as well as population growth (Saraiva-Okello *et al.*, 2015). Freshwater is one of these natural resources that have been put under immense pressure. The increase in agricultural activities, as well as industrial development, influences the water quality of water bodies through the introduction of different pollutants. Chetty and Pillay (2019) stated that nitrates, sulphates, phosphates, copper (Cu) and iron (Fe) are often associated with agricultural-based activities, whereas other trace elements (cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and Zinc (Zn)) are one of the most common pollutant by-products of industrial processes. The introduction of these pollutants is also affecting the aquatic ecosystems that provide essential resources for the well-being of humans, as well as providing habitat for the aquatic organisms (Van Rooyen *et al.*, 2022).

The Limpopo River and the Incomati River catchments are both very important to South Africa. The Limpopo River catchment is a transboundary catchment that is shared by more than 14 million people spread out across four countries, South Africa (45%), Botswana (19%), Mozambique (21%), and Zimbabwe (15%), with most of the water users situated at the upper Limpopo River catchment (Kahinda *et al.*, 2016). Most of the water from the catchment is used for agricultural purposes with domestic water supply also being one of the major water uses. The Olifants River is one of the tributaries of the Limpopo River catchment and is regarded as one of the most polluted river systems in South Africa (Verhaert *et al.*, 2019). Stressors affecting the water quality of the Olifants River are mainly agriculture, as well as mining, industrial activities and tourism (Mudaly & Van Der Laan, 2020). These are just some of the sources of water quality stressors affecting the Limpopo River catchment. In South Africa, there are around 760 rural communities around the catchment area (Kahinda *et al.*, 2016). These communities rely on the ecosystem services that are provided by the catchment for their livelihoods.

The Incomati River catchment is also a transboundary catchment that flows through three countries, South Africa (63%), Eswatini (5%), and Mozambique (32%), in Southern Africa and was used by 2.2 million people in 2007 in South Africa (Riddell *et al.*, 2020). Some of the stressors on the Incomati River catchment include agricultural and forestry activities, urban

development, coal combustion power stations, as well as coal and gold mining activities (Erasmus *et al.*, 2023). According to Kleynhans *et al.* (2013), the intensive use of water in the Incomati system for irrigation purposes has impacted the ecosystem's health. In the eastern regions of South Africa, the production of sugarcane uses water from the Crocodile, Lomati, as well as the Komati Rivers as their main source for irrigation (Van der Laan *et al.*, 2012). An increase in urbanisation has led to considerable changes in the natural flow patterns of the river due to an increase in water abstraction (Saraiva-Okello *et al.*, 2015). An additional threat to the system is the extensive coal and gold mining in the catchments of major tributaries, which can lead to acidic water and metals entering the system (Darwall *et al.*, 2009).

Both river systems are mostly situated in arid and semi-arid areas. Rainfall across the Limpopo River catchment is also only seasonal with as little as 400 mm per annum near the middle reaches of the Limpopo River catchment and over 1 000 mm per annum on the Drakensberg Mountains (Ashton *et al.*, 2001). The river also has high and variable evaporation rates and mostly the evaporation loss exceeds the rainfall (Ashton *et al.*, 2001). The catchment is also comprised of several alluvial aquifers, as well as deep river pools that need to fill up before there is any surface water flow (Kahinda *et al.*, 2016). The mean annual precipitation for the Incomati River catchment is around 740 mm, which is primarily only during the summer months (Saraiva-Okello *et al.*, 2015).

Both catchments not only support a large population of rural communities but also supply water to most of the rivers in the KNP. Thus, not only supporting human populations but biodiversity in South Africa's largest conservation area. Therefore, the water quality of the Limpopo River catchment, as well as the Incomati River catchment is very important for the well-being of these communities, as well as for the ecosystem. The Aquatic Toxicity Index (ATI) is a calculation that was developed by Wepener *et al.* (1992) to determine if an aquatic ecosystem is suitable for maintaining aquatic biota (Van Rooyen *et al.*, 2022). It is a reflection of the effect of different water quality variables on fish, as representatives of aquatic ecosystem health (Van Rooyen *et al.*, 2022). The Sediment Quality Index (SeQI) is an effective tool that assesses the degree to which anthropogenic activities affect the sediment quality, as well as with respect to its relative desired state (Van Rooyen *et al.*, 2022).

During the present study, the water quality of both systems was assessed based on different water quality variables and classified these systems according to the ATI. Sediment quality will also be classified according to the SeQI and assessed according to particle size and total organic carbon (TOC). The aim of this chapter is to determine the water quality status of both

catchments, relative to each other, as well as within a catchment. Supplementary objectives were to interpret water quality in terms of its suitability for use by fish populations, achieved by assessing the various factors affecting water quality and applying an ATI to the data.

## **2.2 Materials and methods**

### **2.2.1 Historical water quality data**

Where possible, historical water quality data were obtained from the Department of Water and Sanitation (DWS) Water Management System (WMS) ([https://www.dws.gov.za/iwqs/wms/data/WMS\\_pri\\_txt.asp](https://www.dws.gov.za/iwqs/wms/data/WMS_pri_txt.asp)). Summary statistics of the data were generated using the online WMS statistical tool. Current sites are comparable to historical data since the same sites were sampled.

### **2.2.2 Field surveys**

Water and sediment samples were collected in triplicate in pre-cleaned 250 mL poly-ethylene containers at pre-selected sites in the Limpopo River catchment during May – July of 2021, as well as from the Incomati River catchment during May – July of 2022. A total of 13 sites were sampled in triplicate from the Limpopo River catchment (**Figure 2.1**) and seven sites were sampled from the Incomati River catchment (**Figure 2.2**). The Limpopo River catchment includes one site on each of the following tributaries: Crocodile River (CROC), Matlabas River (MAT), Lephalala River (LEPH), Mogalakwena River (MOG), Sand River (SAND), Luvuvhu River (LUV), Shingwidzi River (SHIN), two sites on the Letaba (GLET and LET) and Olifants (MAM and BAL), as well as two sites on the mainstem Limpopo River (LR1 and LR2) (**Figure 2.3**). The Incomati River catchment sites are on the following tributaries: Sabie River (SR KNP, LSR, and SR Moz), Crocodile River (VG), and three sites on the mainstem Incomati River (IR1, IR2 and IR3) (**Figure 2.4**).

Extech instruments were used to measure the *in-situ* variables including pH, electrical conductivity (EC;  $\mu\text{S}/\text{cm}$ ), total dissolved solids (TDS;  $\text{mg}/\text{L}$ ) and temperature ( $^{\circ}\text{C}$ ) (ExStik EC500), as well as dissolved oxygen (DO;  $\text{mg}/\text{l}$ ) and oxygen saturation (%) (ExStik DO600). All samples were frozen, to prevent organic decomposition, until further analysis could be done in the laboratory.

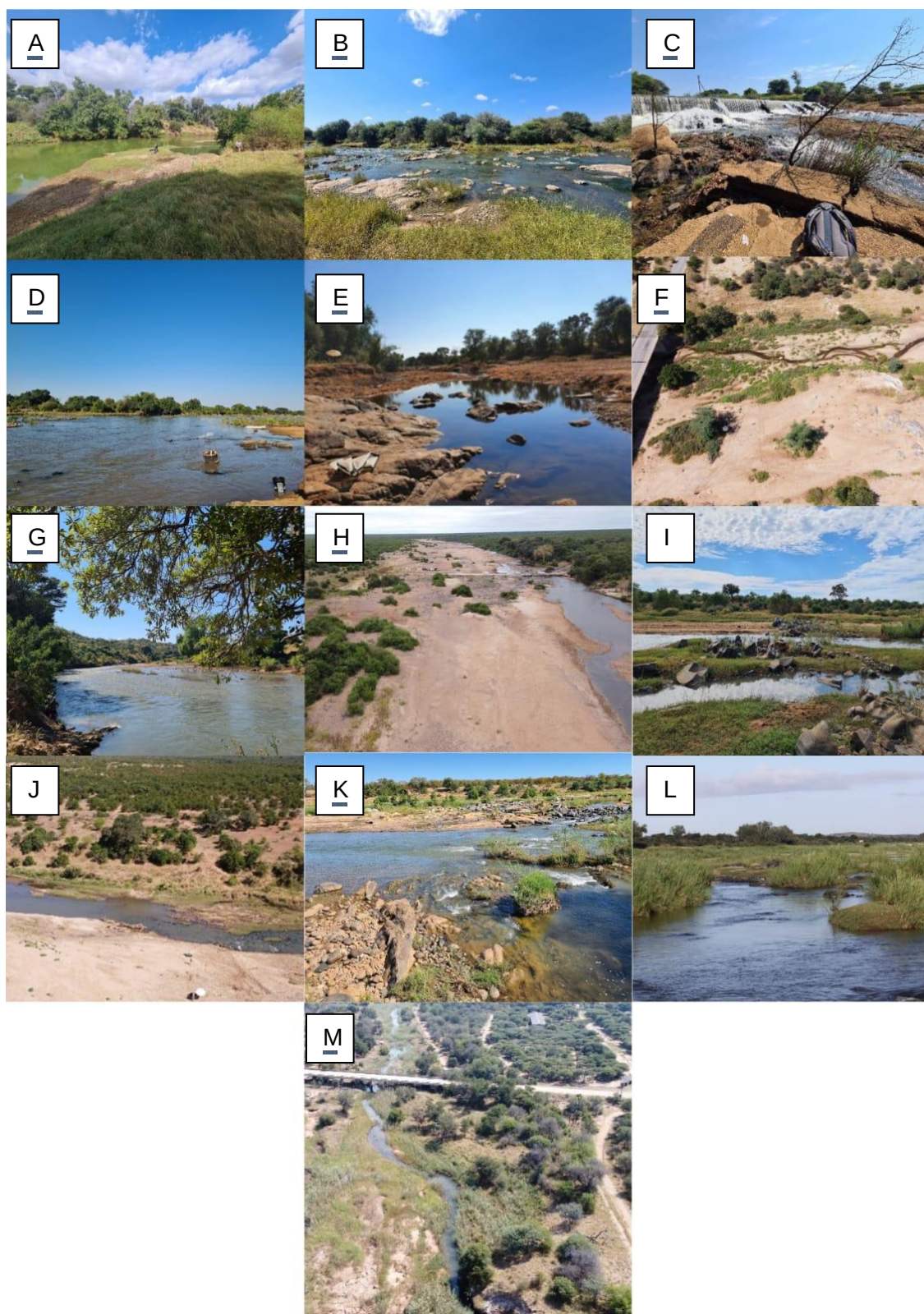


**Figure 2.1:** A) Crocodile River (CROC), B) mainstem Limpopo River (LR1), C) Matlabas River (MAT), D) Lephalala River (LEPH), E) mainstem Limpopo River (LR2), F) Mogalakwena River (MOG), G) Sand River (SAND), H) Luvuvhu River (LUV), I) Shingwidzi River (SHIN), J) Groot Letaba River (GLET) and K) Letaba River (LET), L) Olifants Mamba (MAM) and M) Olifants Balule (BAL).



**Figure 2.2:** A) Sabie River KNP (SR KNP), B) Lower Sabie River (LSR), C) Sabie River Moz (SR Moz), D) Crocodile River (VG), and three sites on the E) Incomati River (IR1), F) Incomati River (IR2) and G) Incomati River (IR3)

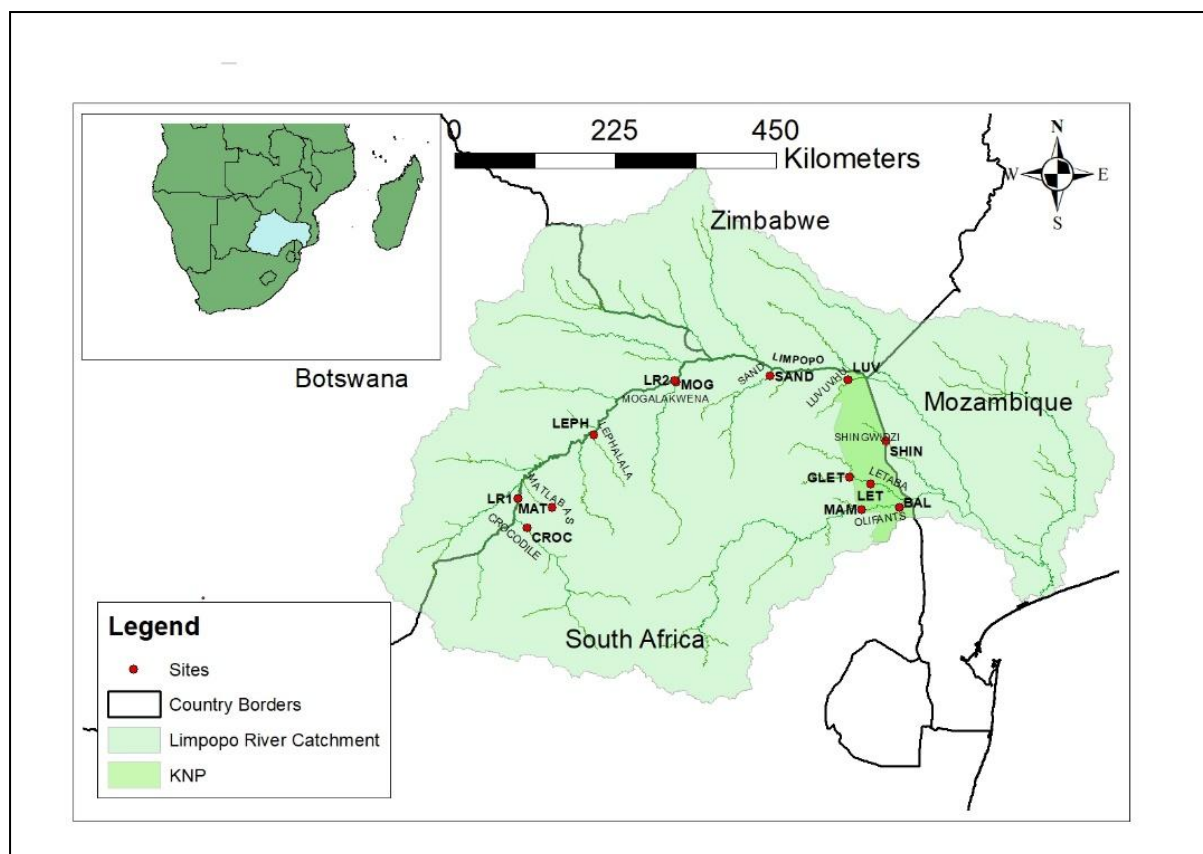


**Figure 2.3:** Sampling sites on the Limpopo River catchment.

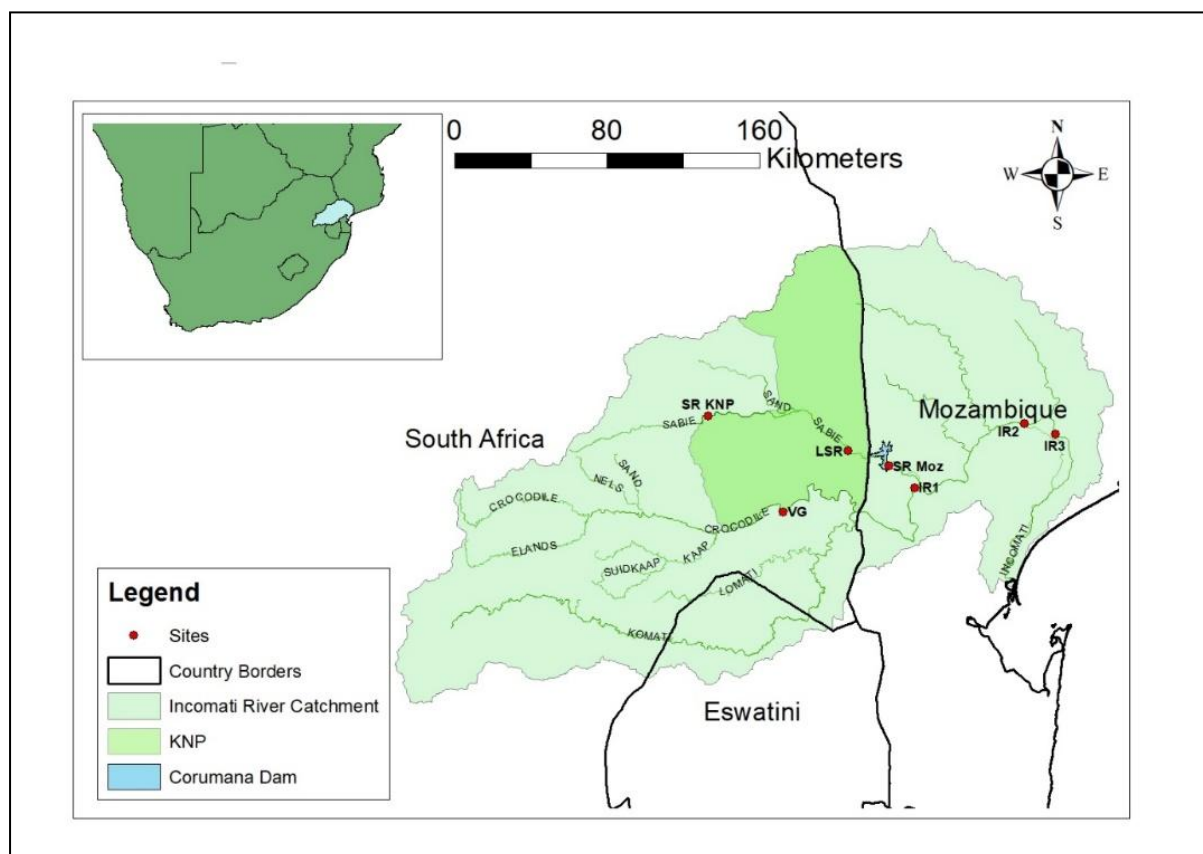


**Figure 2.4:** Sampling sites on the Incomati River catchment.

### 2.2.3 Water Quality Analysis

Water samples were left to defrost to room temperature after which the unfiltered samples were analysed for turbidity, nutrients (ammonium, nitrates, nitrites, ortho-phosphates), and anions (alkalinity, chloride and sulphate). All physico-chemical analyses were done using Merck photometric test kits and a Merck Pharo 300 Spectroquant. Samples were prepared for element analysis by filtering them through a cellulose nitrate filter (0.45 µm, Sartorius Stedim Biotech) and acidified with nitric acid (HNO<sub>3</sub>) (65% supra pure quality, Merck) to an acid concentration of 1% (Erasmus *et al.*, 2020). Samples were analysed for elements using a quadrupole inductively coupled plasma mass spectrometry (ICP-MS) (PerkinElmer, Elan 6000) equipped with an autosampler system (PerkinElmer, AS-90), with chromium being analysed on an atomic absorption spectrometer (AAS) (PerkinElmer, PinAAcle 900T) equipped with Zeeman-effect background correction and mercury on a flow injection mercury system (FIMS 400) equipped with an autosampler system (PerkinElmer, S10).

### 2.2.4 Sediment Quality Analysis

Sediment samples were oven-dried at 60°C for approximately 72 hours. The particle size distribution was determined using methods described by Wepener and Vermeulen (2005). Particle size categories that were applied include very coarse sand (4000 - 2000 µm), coarse sand (2000 - 500 µm), medium sand (500 - 212 µm), fine sand (212 - 65 µm) and mud (< 65 µm) (Erasmus *et al.*, 2020). Crucibles were pre-weighed after which dried sediment samples were added and weighed again to determine the mass of the sediment. Samples were then transferred into an incinerator (L40/11, Nabertherm) at 600°C for six hours, allowed to cool, and re-weighed. Weight was used to calculate the total organic carbon (TOC) using the following equation:

$$\% \text{ Organic Carbon} = \left[ \frac{(Mb - Ma)}{Mb} \right] \times 100$$

Where, Mb = mass of sediment before incineration (g) and Ma = mass of sediment after incineration (g). Classes for TOC were classified according to USEPA (2001), as high (> 4%), medium (2 - 4%), moderately low (1 – 2%), low (0.05 – 1%) and very low (< 0.05%) (Van Rooyen *et al.*, 2022).

For trace element analysis, approximately 0.2 g of dried sediment sample was weighed and placed in Teflon digestion tubes with a mixture of 7.5 mL HCl (37%, suprapure quality, Merck) and 2.5 mL HNO<sub>3</sub> (65%, suprapure quality, Merck) and digested using a Milestone Ethos Easy

Microwave digestion system. After the samples were digested and decanted into a 50 mL volumetric flask, diluted with 1% HNO<sub>3</sub>, the samples were transferred into 50 mL falcon tubes.

Element analysis was performed on an ICP-MS equipped with an autosampler, whereas chromium was analysed on an AAS equipped with Zeeman-effect background correction and mercury on a FIMS equipped with an autosampler, due to several interferences on the ICP-MS. The analytical quality assurance was carried out using certified reference material (CRM) (NCS DC 73310 Stream Sediment, China National Analysis Centre for Iron and Steel) for sediments. The percentage recoveries of the CRM are reported in **Table 2.1**. The percentage recoveries for all the elements analysed were within the 20% deviation range (80 - 120%). For this study, no correction factor was applied. For each round of digestion, triplicate blank samples were analysed to determine if the acid was pure and if the digestion process did not contaminate the samples. The detection limits and limits of quantification for sediment samples were determined as three times and nine times the standard deviation of the blank measurements, respectively and divided by the mean sample weight of 194.6 mg.

**Table 2.1:** Limit of Detection (LOD) and Limit of Quantification (LOQ) reported in mg/kg dry weight (DW) in sediment samples, as well as the percentage recoveries of the elements of interest from the sediment CRM.

| Element | Sediment |        | % recovery   |
|---------|----------|--------|--------------|
|         | LOD      | LOQ    | NCS DC 73310 |
| As      | 0.017    | 0.052  | 117          |
| Cd      | 0.0002   | 0.0006 | 91           |
| Cr      | 0.017    | 0.052  | 82           |
| Cu      | 0.003    | 0.008  | 91           |
| Hg      | 0.010    | 0.031  | 88           |
| Ni      | 0.053    | 0.159  | 84           |
| Pb      | 0.035    | 0.011  | 81           |
| Zn      | 0.063    | 0.019  | 91           |

### 2.2.5 Statistical analysis

Data was tested for normality and homogeneity of variance using D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test, respectively, using GraphPad Prism 10 (GraphPad Software, Inc.) (Erasmus, 2020). Differences in spatial and temporal water and sediment data were assessed using analysis of variance (ANOVA). A one-way ANOVA was performed for metal and water quality variables using GraphPad 10 (GraphPad Software, Inc.). The significant differences between groups were determined using Tukey–Kramer post-hoc analyses (Erasmus, 2020). The statistical significance was set at  $p < 0.05$ .

Maucha diagrams were used to provide a visual representation of the ionic (milli-equivalents per litre) composition at each site with the star symbol showing anions and cations in different colours (Maucha, 1932). Aquatic Toxicity Index (ATI) developed by Wepener *et al.* (1992) was applied to the water quality data. Scores were calculated for each of the sampling sites for both catchments. The water quality variables used to calculate the index scores were as follows: pH, TDS, turbidity (log NTU), DO (mg/l), PO<sub>4</sub>-P, NH<sub>4</sub>-N; Zn, Mn, Pb, Ni, Cu, and K. Scores were calculated using a computer-based program, Water 2. Wepener *et al.* (1992) used the Solway Modified Unweighted Additive Aggregation Function to calculate the final index scores:

$$ATI = \frac{1}{100} \left( \frac{1}{n} \sum_{i=1}^n q_i \right)^2$$

Where, ATI = final score; n = number of determinants in the indexing system, and  $q_i$  = the quality of each parameter (value between 0 and 100). Final scores represent a value between 0 and 100 and indicate whether the water quality is suitable for aquatic biodiversity. Wepener *et al.* (1992) defined the generic interpretation of the ATI scale as follows: a score of 60 and lower is unsuitable for sustaining aquatic biodiversity. A score between 60 and 100 is suitable for sustaining aquatic biodiversity and a score of 100 indicates pristine conditions.

The Sediment Quality Index provides three components: (1) scope – referring to the number of variables that did not meet the guidelines, (2) frequency – the frequency of which the guidelines were not met, and (3) amplitude – the extent of which the guidelines were not met. By combining these three components, a single value between 0 (worst sediment quality) and 100 (pristine sediment quality) is produced that describes the sediment quality in relation to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (ISQG) (CCME, 2007). The following sediment variables were used to derive the index values and quality

scores: As, Cd, Cr, Cu, Pb, Ni, Zn and TOC. For Ni, the sediment guidelines of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000) were selected since no ISQG guidelines are available from the CCME database (Van Rooyen *et al.*, 2022). Aluminium, Mn, Co and Fe were excluded from the calculation as there are no ISQGs for these metals. The international guidelines were used as South Africa does not currently have targeted guidelines for sediment quality. The SeQI scores were calculated as follows:

$$SeQI = 100 - \left( \frac{\sqrt{F_1^2 + F_2^2 + F_3^2}}{1.732} \right)$$

Where, F1 = Scope; F2 = Frequency; F3 = Amplitude. The value, 1.732, was used as a divisor as it normalises the values to a range between 0 and 100. For a detailed description of the calculation of F1-3, refer to CCME (2007).

Additionally, a principal component analysis (PCA) was performed to interpret the general temporal and spatial water and sediment quality patterns. When the data were parametric the data was log-transformed to ensure normality and the PCA biplots were constructed using the Canoco 5 for Windows (v. 5.15) statistical package (Erasmus, 2020).

## 2.3 Results

### 2.3.1 Water Quality

The historical and current water quality for sites sampled in the Limpopo River catchment is provided in **Table 2.2** and **Table 2.3**, respectively. Most of the variables at **CROC**, **LR1**, **LUV**, **SHIN** and **LET** were higher than historically. The **MAT** site recorded elevated nitrate and nitrite levels with very high EC. The EC at site **LEPH** was also elevated, but all other water quality variables were similar or lower than historically. Most of the sites on the Limpopo River catchment indicated similar water quality levels. Concentrations of the current water quality variables ranged between 7.1 – 9.2 for pH, 381 – 2415 µS/cm for EC, 9 – 16.2 mg/L for DO, 20.3 – 27.7°C for temperature, 10.4 – 603 mg/L Cl, 1.33 – 217 mg/L for SO<sub>4</sub>, 0.01 – 0.46 mg/L for PO<sub>4</sub>, 0.01 – 0.18 mg/L for NH<sub>4</sub>, 0.21 – 2.41 mg/L for NO<sub>3</sub> + NO<sub>2</sub>, 34.6 – 473 mg/L HCO<sub>3</sub> for alkalinity and 4.67 – 35 NTU for turbidity.

**Table 2.2:** Historical water quality represented as the mean and range, where the range is indicated in brackets, for the period of the Limpopo River catchment.

| Element                                  | CROC                  | LR1                  | MAT                  | LEPH                | LUV                  | SHIN                  | LET                   |
|------------------------------------------|-----------------------|----------------------|----------------------|---------------------|----------------------|-----------------------|-----------------------|
| Sampling period                          | 2004 – 2018           | 1980 - 2018          | 1971 – 2018          | 1995 – 2018         | 1983 – 2017          | 1983 – 2018           | 1983 – 2018           |
| pH                                       | 8.40 (6.58 – 9.04)    | 7.71 (6.15 – 8.71)   | 7.13 (4.43 – 8.48)   | 7.7 (7.24 – 9.17)   | 7.84 (5.9 – 9.09)    | 8.23 (6.29 – 8.8)     | 8.26 (6.08 – 8.91)    |
| EC (µS/cm)                               | 740 (439 – 975)       | 258 (73 -955)        | 540 (150 – 586)      | 112 (27 – 545)      | 148 (76 – 975)       | 268 (54 – 1510)       | 412 (136 – 1300)      |
| Cl (mg/L)                                | 87 (40 – 144.6)       | N/A                  | 5 (1.5 – 87.9)       | 12.1 (3.2 – 230)    | 13.6 (4.8 – 148)     | 16.7 (3.6 – 580)      | 34.1 (9.54 – 188)     |
| SO <sub>4</sub> (mg/L)                   | 73.1 (41.7 – 167.7)   | 16.6 (2 – 89.7)      | 2 (0.6 – 13)         | 6 (0.44 – 450)      | 4.2 (0.375 – 29.9)   | 7.3 (1 – 92.1)        | 9.25 (1.6 – 41.9)     |
| PO <sub>4</sub> (mg/L)                   | 0.105 (0.005 – 0.442) | 0.015 (0.003 – 0.96) | 0.01 (0.003 – 0.181) | 0.02 (0.005 – 6.6)  | 0.017 (0.003 – 7.27) | 0.025 (0.003 – 0.489) | 0.019 (0.003 – 0.445) |
| NH <sub>4</sub> (mg/L)                   | 0.053 (0.015 – 0.295) | 0.0253 (0.02 – 0.4)  | 0.0253 (0.02 – 0.4)  | 0.02 (0.015 – 3)    | 0.04 (0.015 – 1.4)   | 0.06 (0.015 – 19.8)   | 0.072 (0.015 – 6.53)  |
| NO <sub>2</sub> + NO <sub>3</sub> (mg/L) | 0.94 (0.025 – 3.87)   | 0.082 (0.005 – 1.72) | 0.02 (0.02 – 1.4)    | 0.218 (0.02 – 6.01) | 0.062 (0.005 – 1.83) | 0.097 (0.005 – 8.04)  | 0.094 (0.005 – 7.05)  |

N/A – no available data

**Table 2.3:** Water quality variables analysed for samples collected in the Limpopo River catchment during the 2021 survey. Data presented as mean  $\pm$  standard error.

| Element                                     | CROC               | LR1                | MAT                | LEPH                | LR2                | MOG                | SAND               | LUV                | SHIN                                | GLET               | LET                 | MAM                  | BAL                 |
|---------------------------------------------|--------------------|--------------------|--------------------|---------------------|--------------------|--------------------|--------------------|--------------------|-------------------------------------|--------------------|---------------------|----------------------|---------------------|
| pH                                          | 9.2 $\pm$<br>0.03  | 8.7 $\pm$<br>0.01  | 7.1                | 7.6 $\pm$<br>0.04   | 8.5 $\pm$<br>0.14  | 7.5 $\pm$<br>0.27  | 8.6 $\pm$<br>0.07  | 8.3 $\pm$<br>0.04  | 8.2 $\pm$<br>0.06                   | 8.3 $\pm$<br>0.09  | 8.4 $\pm$<br>0.44   | 8.8 $\pm$<br>0.06    | 8.6 $\pm$<br>0.02   |
| EC ( $\mu$ S/cm)                            | 782 $\pm$<br>11    | 837 $\pm$<br>58    | 6855 $\pm$<br>515  | 857 $\pm$<br>2.0    | 936 $\pm$<br>159   | 2415 $\pm$<br>255  | 2580               | 381                | 900 $\pm$<br>31                     | 1007               | 805 $\pm$<br>427    | 1285.5 $\pm$<br>7.5  | 547 $\pm$<br>4.5    |
| DO (mg/L)                                   | 10.8 $\pm$<br>1.8  | 11.3 $\pm$<br>0.6  | 9.3                | 9.4 $\pm$<br>0.4    | 10.4 $\pm$<br>0.1  | 12.8 $\pm$<br>4.6  | 16.2 $\pm$<br>0.4  | 10 $\pm$<br>0.2    | 9 $\pm$<br>0.7                      | 10.2 $\pm$<br>0.6  | 9.7 $\pm$<br>0.4    | 11 $\pm$<br>0.4      | 10.2                |
| Temperature<br>(°C)                         | 22.4 $\pm$<br>0.8  | 22.1 $\pm$<br>0.8  | 22.3               | 21.6 $\pm$<br>0.1   | 22.5 $\pm$<br>0.3  | 23.6 $\pm$<br>1.2  | 27.7 $\pm$<br>1.3  | 24.9 $\pm$<br>0.5  | 21.5 $\pm$<br>0.6                   | 22.3 $\pm$<br>0.2  | 22.7 $\pm$<br>1.5   | 22.7 $\pm$<br>0.1    | 20.3 $\pm$<br>0.5   |
| Cl (mg/L)                                   | 92.7 $\pm$<br>2.1  | 81.3 $\pm$<br>9.1  | 10.4 $\pm$<br>1.1  | 10.7 $\pm$<br>3.8   | 53 $\pm$<br>8.7    | 603 $\pm$<br>90.2  | 444 $\pm$<br>49.6  | 13.4 $\pm$<br>0.31 | 87.3 $\pm$<br>0.58                  | 19.3 $\pm$<br>2.52 | 28.3 $\pm$<br>8.39  | 26 $\pm$<br>2        | 32.7 $\pm$<br>0.58  |
| SO <sub>4</sub> (mg/L)                      | 61 $\pm$<br>1.0    | 58                 | 1.3 $\pm$<br>0.58  | 4.7 $\pm$<br>1.2    | 14 $\pm$<br>1.7    | 217 $\pm$<br>65.3  | 133 $\pm$<br>3.5   | 4 $\pm$<br>1       | 12 $\pm$<br>1                       | 14.7 $\pm$<br>3.79 | 13 $\pm$<br>1       | 69.7 $\pm$<br>0.58   | 62.67 $\pm$<br>0.58 |
| PO <sub>4</sub> (mg/L)                      | 0.15 $\pm$<br>0.01 | 0.04 $\pm$<br>0.02 | 0.05 $\pm$<br>0.04 | 0.46 $\pm$<br>0.32  | 0.24 $\pm$<br>0.28 | 0.16 $\pm$<br>0.01 | 0.23 $\pm$<br>0.3  | 0.1 $\pm$<br>0.06  | 0.05 $\pm$<br>0.04                  | 0.03 $\pm$<br>0.01 | 0.02 $\pm$<br>0.01  | 0.01 $\pm$<br>0.01   | 0.11 $\pm$<br>0.08  |
| NH <sub>4</sub> (mg/L)                      | 0.08 $\pm$<br>0.01 | 0.04 $\pm$<br>0.01 | 0.07 $\pm$<br>0.01 | 0.01 $\pm$<br>0.001 | 0.06 $\pm$<br>0.01 | 0.14 $\pm$<br>0.13 | 0.13 $\pm$<br>0.06 | 0.1 $\pm$<br>0.001 | 0.18 $\pm$<br>$3.4 \times 10^{-15}$ | 0.05 $\pm$<br>0.02 | 0.02 $\pm$<br>0.001 | 0.01 $\pm$<br>0.0006 | 0.12 $\pm$<br>0.002 |
| NO <sub>2</sub> + NO <sub>3</sub><br>(mg/L) | 1.31 $\pm$<br>0.78 | 1.4 $\pm$<br>0.67  | 2.4 $\pm$<br>0.35  | 0.91 $\pm$<br>0.35  | 1.9 $\pm$<br>0.33  | 0.94 $\pm$<br>0.12 | 1.66 $\pm$<br>0.24 | 0.41 $\pm$<br>0.1  | 0.87 $\pm$<br>0.23                  | 0.21 $\pm$<br>0.15 | 0.28 $\pm$<br>0.29  | 1.01 $\pm$<br>1.48   | 1.97 $\pm$<br>1.21  |
| Alkalinity                                  | 218 $\pm$<br>28    | 206 $\pm$<br>7.1   | 34.6 $\pm$<br>7.7  | 38.7 $\pm$<br>1.8   | 91.5 $\pm$<br>8.1  | 328 $\pm$<br>94    | 473 $\pm$<br>8.1   | 71.2 $\pm$<br>1.8  | 350 $\pm$<br>7.7                    | 95.6 $\pm$<br>4.7  | 146 $\pm$<br>50.4   | 165 $\pm$<br>8.07    | 169 $\pm$<br>13.76  |
| Turbidity                                   | 35 $\pm$<br>3.6    | 20 $\pm$<br>1.0    | 4.67 $\pm$<br>2.3  | 7.3 $\pm$<br>0.58   | 9.7 $\pm$<br>7.1   | 8.0 $\pm$<br>3.0   | 13.7 $\pm$<br>2.9  | 8 $\pm$<br>1.7     | 17.7 $\pm$<br>2.08                  | 11.7 $\pm$<br>1.53 | 12.7 $\pm$<br>2.89  | 13 $\pm$<br>3        | 12 $\pm$<br>1.73    |

\*Samples with no standard error did not have any deviation between the replicate samples.

The historical and current water quality of the Incomati River catchment is provided in **Table 2.4** and **Table 2.5**, respectively. There are no historical water quality data for the site in Mozambique whereas the sites in the Kruger National Park had historical data. The sites in the KNP indicated similar water quality compared to the historical data. The current water quality variable concentrations ranged between 7.43 – 8.72 for pH, 137 – 1080  $\mu\text{S}/\text{cm}$  for EC, 7.41 – 11.5 mg/L for DO, 18.1 – 23.13°C for temperature, 8.67 – 64 mg/L for Cl, 4 – 55.2 mg/L for  $\text{SO}_4$ , 0.03 – 0.1 mg/L for  $\text{PO}_4$ , 0.02 – 0.07 for  $\text{NH}_4$ , 0.01 and 0.62 mg/L for  $\text{NO}_3 + \text{NO}_2$ , 70.1 – 172  $\text{HCO}_3$  for alkalinity, and 3.67 and 13 for turbidity.

**Table 2.4:** Historical water quality represented as the mean and range, where the range is indicated in brackets, for the period of the Incomati River catchment.

| Element                            | SR KNP                | VG                   | LSR                   | SR Moz | IR1 | IR2 | IR3 |
|------------------------------------|-----------------------|----------------------|-----------------------|--------|-----|-----|-----|
| Sampling period                    | 2006 – 2019           | 1983 – 2019          | 1983 - 2019           | N/A    | N/A | N/A | N/A |
| pH                                 | 7.74 (6.27 – 9.2)     | 8.01 (5.4 – 8.98)    | 7.76 (5.21 – 9.03)    | N/A    | N/A | N/A | N/A |
| EC ( $\mu\text{S}/\text{cm}$ )     | 129 (47 – 80.9)       | 326 (95.4 – 1320)    | 145 (68 – 828)        | N/A    | N/A | N/A | N/A |
| Cl (mg/L)                          | 8 (2.5 – 82)          | 17.4 (5.1 -114)      | 9.6 (1.5 – 80.7)      | N/A    | N/A | N/A | N/A |
| $\text{SO}_4$ (mg/L)               | 2.5 (1.5 – 17)        | 20.5 (1 – 102)       | 6.36 (0.6 – 50.5)     | N/A    | N/A | N/A | N/A |
| $\text{PO}_4$ (mg/L)               | 0.05 (0.005 – 8.3)    | 0.027 (0.003 – 5.4)  | 0.015 (0.003 – 0.232) | N/A    | N/A | N/A | N/A |
| $\text{NH}_4$ (mg/L)               | 0.1 (0.025 – 27.5)    | 0.05 (0.015 – 7.1)   | 0.05 (0.015 – 0.65)   | N/A    | N/A | N/A | N/A |
| $\text{NO}_2 + \text{NO}_3$ (mg/L) | 0.236 – (0.025 – 4.4) | 0.586 (0.005 - 11.3) | 0.084 (0.005 – 4.33)  | N/A    | N/A | N/A | N/A |

N/A – no available data

**Table 2.5:** Water quality variables analysed for samples collected in the Incomati River catchment during the 2022 survey. Data presented as mean  $\pm$  standard error.

| Element                                  | SR KNP             | VG                 | LSR                | SR Moz              | IR1                | IR2                 | IR3                 |
|------------------------------------------|--------------------|--------------------|--------------------|---------------------|--------------------|---------------------|---------------------|
| pH                                       | 7.43 $\pm$<br>0.1  | 8.12 $\pm$<br>0.17 | 7.45 $\pm$<br>0.09 | 7.56 $\pm$<br>0.2   | 7.94 $\pm$<br>0.12 | 8.21 $\pm$<br>0.29  | 8.72                |
| EC ( $\mu$ S/cm)                         | 137 $\pm$<br>1.07  | 412 $\pm$<br>95.9  | 161 $\pm$<br>1.17  | 204 $\pm$<br>4.62   | 596 $\pm$<br>3.46  | 640 $\pm$<br>348    | 1080                |
| DO (mg/L)                                | 7.41 $\pm$<br>0.21 | 6.87 $\pm$<br>0.73 | 7.42 $\pm$<br>1.29 | 9.24 $\pm$<br>0.38  | 10.5 $\pm$<br>0.19 | 11.5 $\pm$<br>0.41  | -                   |
| Temperature ( $^{\circ}$ C)              | 18.6 $\pm$<br>0.06 | 20.4 $\pm$<br>1.64 | 20.7 $\pm$<br>0.1  | 23.13 $\pm$<br>0.32 | 21.1 $\pm$<br>0.12 | 22.7 $\pm$<br>0.68  | 18.1                |
| Cl (mg/L)                                | 19.7 $\pm$<br>5.34 | 39.8 $\pm$<br>7.65 | 26.7 $\pm$<br>2.51 | 8.67 $\pm$<br>1.53  | 64 $\pm$<br>8.15   | 41.5 $\pm$<br>7.31  | 21.3 $\pm$<br>4.51  |
| SO <sub>4</sub> (mg/L)                   | 4 $\pm$<br>1.41    | 34.2 $\pm$<br>2.32 | 15.7 $\pm$<br>8.15 | 52.7 $\pm$<br>37.8  | 44 $\pm$<br>21.2   | 55.2 $\pm$<br>36.16 | 7.67 $\pm$<br>3.79  |
| PO <sub>4</sub> (mg/L)                   | 0.05 $\pm$<br>0.02 | 0.1 $\pm$<br>0.02  | 0.08 $\pm$<br>0.01 | 0.03                | 0.05 $\pm$<br>0.02 | 0.09 $\pm$<br>0.08  | 0.08 $\pm$<br>0.03  |
| NH <sub>4</sub> (mg/L)                   | 0.03 $\pm$<br>0.01 | 0.07 $\pm$<br>0.02 | 0.02               | 0.02                | 0.03 $\pm$<br>0.01 | 0.03 $\pm$<br>0.004 | 0.03 $\pm$<br>0.01  |
| NO <sub>2</sub> + NO <sub>3</sub> (mg/L) | 0.11 $\pm$<br>0.18 | 0.62 $\pm$<br>0.14 | -                  | 0.01 $\pm$<br>0.001 | 0.2 $\pm$<br>0.21  | 0.45 $\pm$<br>0.24  | 0.01 $\pm$<br>0.002 |
| Alkalinity                               | 70.1 $\pm$<br>5.28 | 172 $\pm$<br>27.6  | 86.5 $\pm$<br>1.76 | 91.5 $\pm$<br>3.05  | 160 $\pm$<br>35.7  | 140 $\pm$<br>10.3   | 78.3 $\pm$<br>12.3  |
| Turbidity                                | 3.67 $\pm$<br>0.58 | 10.3 $\pm$<br>4.08 | 4                  | 5 $\pm$<br>1.73     | 9.83 $\pm$<br>6.68 | 12.2 $\pm$<br>8.3   | 13 $\pm$<br>10.4    |

\*Samples with no standard error did not have any deviation between the replicate samples.

Maucha diagrams for the Limpopo River catchment are displayed in **Figures 2.5, 2.6 and 2.7**. Most of the rivers in the Limpopo River catchment were dominated by TAL, as well as Na, while Mg also contributed to the general ionic composition of the downstream sites. Increased Cl was also evident at most of the sites and dominated at **MOG** and **SAND**.

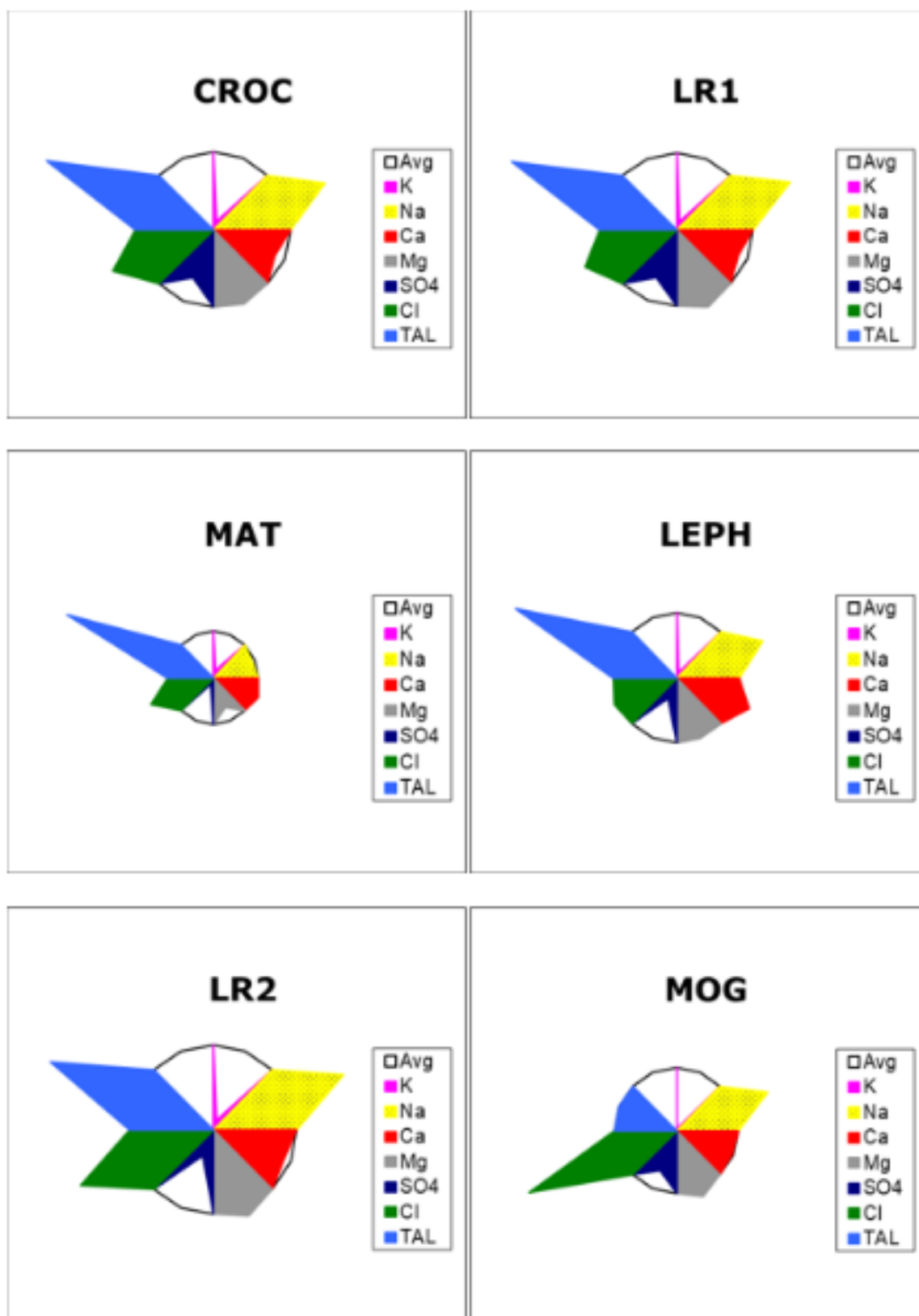


**Figure 2.5:** Maucha diagrams representing the Crocodile (CROC), Limpopo River (LR1 and LR2), Matlabas (MAT), Mogolokwena (MOG) and Lephalale (LEPH) rivers in the Limpopo River catchment starting with the most upstream site and moving downstream (May – July 2021).

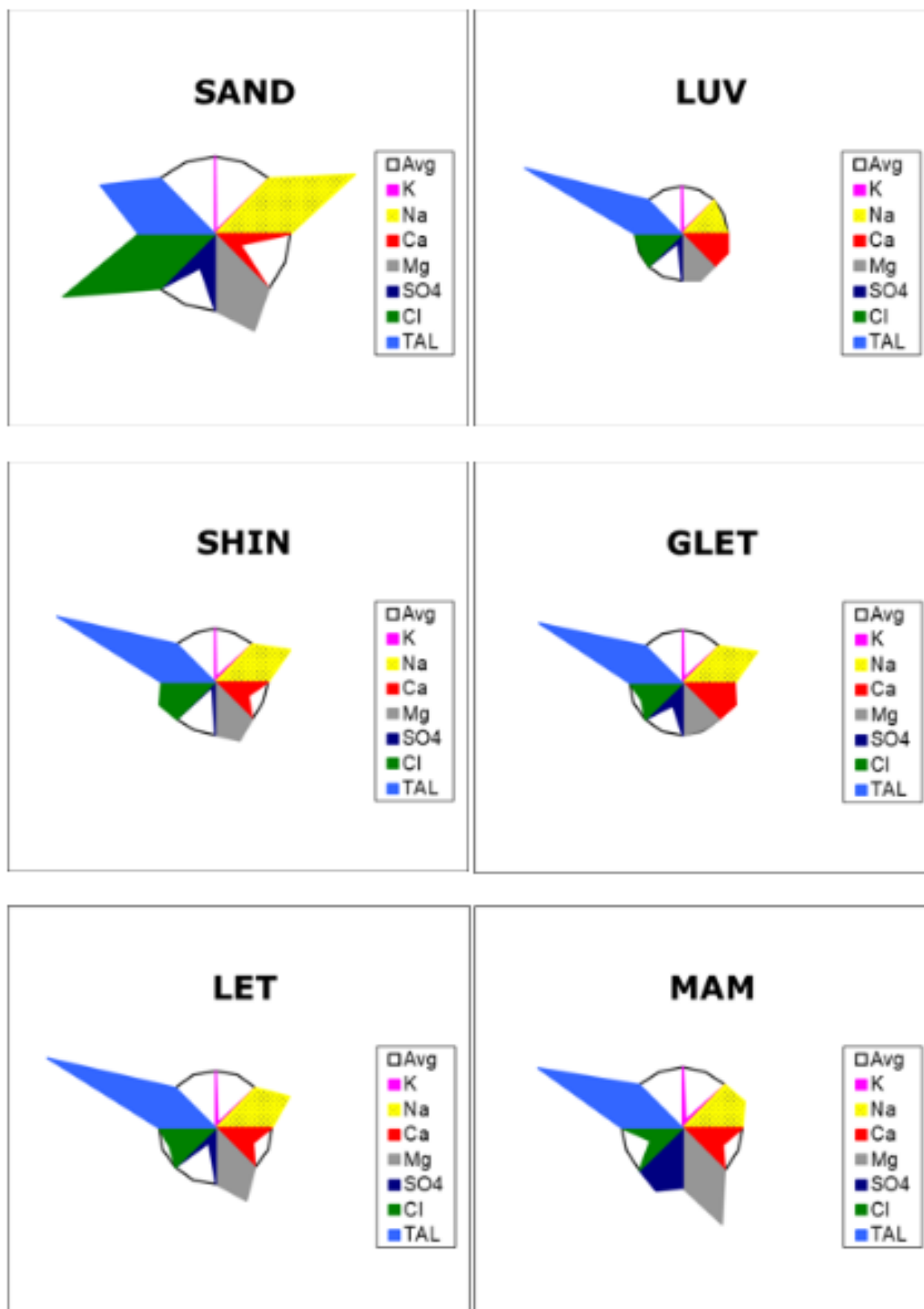


**Figure 2.6:** Maucha diagrams representing the Sand (SAND), Luvuvhu (LUV), Shingwidzi (SHIN), Groot Letaba (GLET), Letaba (LET) and Mamba (MAM) rivers in the Limpopo River catchment starting with the most upstream site and moving downstream (May – July 2021).

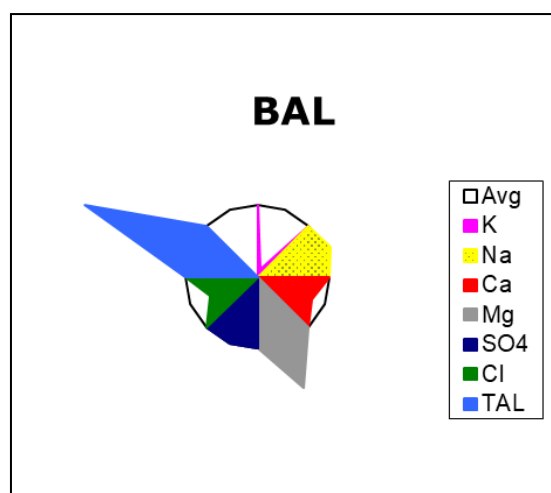


**Figure 2.7:** Maucha diagrams representing Balule River (BAL) in the Limpopo River catchment (May – July 2021).

The ionic chemistry is displayed as Maucha diagrams and used to determine the different ions that contributed to the water chemistry during the period in which the survey was conducted. All the rivers in the Incomati River catchment (**Figures 2.8, & 2.9**) were dominated by total alkalinity (TAL), with most of the sites indicating elevated levels of chloride (Cl). Some sites also had elevated levels of magnesium (Mg) (**SR KNP, VG, IR2**), sulphate (SO<sub>4</sub>) (**SR Moz**), calcium (Ca) (**SR KNP, SR Moz, IR3**) and sodium (Na) (**IR2**).

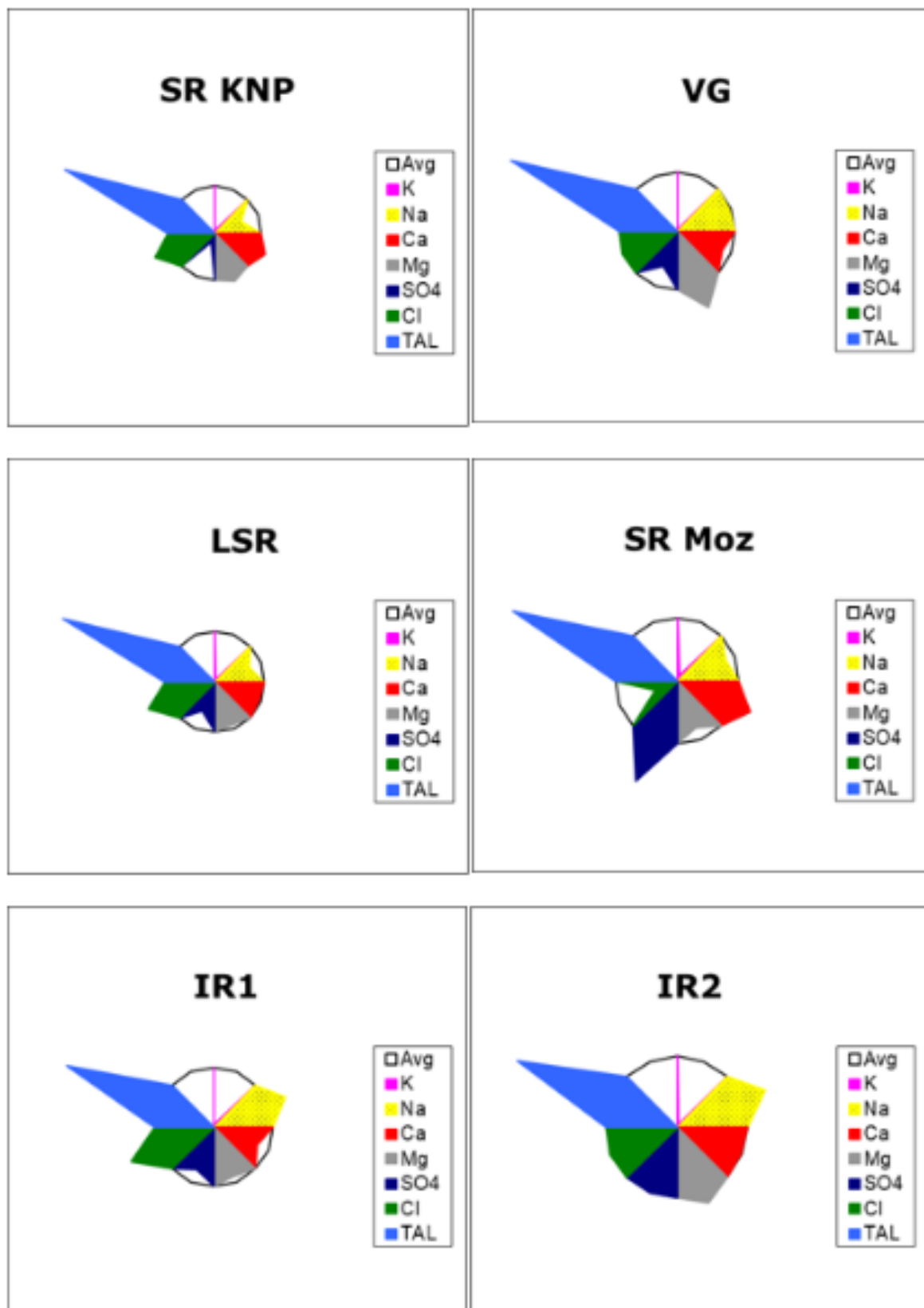


**Figure 2.8:** Maucha diagrams representing the Sabie (SR KNP, LSR, SR Moz), Crocodile (VG) and Incomati (IR1 and IR2) rivers in the Incomati River catchment starting at the most upstream site and moving downstream (May – July 2022).

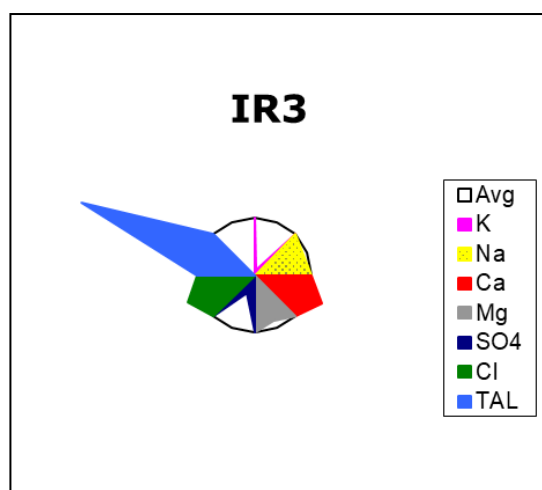


**Figure 2.9:** Maucha diagrams representing IR3 in the Incomati River catchment (May – July 2022).

Concentrations of trace elements (**Table 2.6**) in the Limpopo River catchment ranged between 0.08 – 1.66 µg/L for As, 0.01 – 0.04 µg/L for Cd, 0.08 - 5.39 µg/L for Cr, 0.76 – 5.61 µg/L for Cu, 0.02 – 38.54 µg/L for Hg, 1.15 – 26.14 µg/L for Ni, 0.01 – 0.21 µg/L for Pb, 1.99 – 17.35 for Zn. **CROC** had the highest concentrations for As, whereas **LUV** had the highest concentrations for Cr, Hg and Ni. Site **LR2** had the highest concentrations of Zn and Cu. The highest concentrations of Cd were evident at **SAND** and the highest concentrations of Pb were evident at **GLET**. There were no significant differences between any of the sites for Cr, Cd, Ni, Hg or Pb. Copper concentrations at **LR2** differed significantly from all of the sites. The Zn concentrations at **LR2** differed from most of the sites, except for **MAT**. The As concentrations at most of the sites indicated a significant difference between most of the sites on the Limpopo River catchment, except between **SHIN** and **LR2**, **SHIN** and **MOG**, or between **LR2** and **MOG**.

**Table 2.6:** The mean  $\pm$  standard error of the trace element concentrations ( $\mu\text{g/L}$ ) in water samples collected from the Limpopo River catchment.

| Element | CROC                       | LR1                       | MAT                       | LEPH                      | LR2                   | MOG                  | SAND                      | LUV                       | SHIN                 | GLET                      | LET                       | MAM                      | BAL                       |
|---------|----------------------------|---------------------------|---------------------------|---------------------------|-----------------------|----------------------|---------------------------|---------------------------|----------------------|---------------------------|---------------------------|--------------------------|---------------------------|
| As      | 1.66 $\pm$<br>0.003*#\$\$@ | 1.56 $\pm$<br>0.01*#\$\$@ | 0.27 $\pm$<br>0.02*#\$\$@ | 0.14 $\pm$<br>0.01*#\$\$@ | 0.5 $\pm$<br>0.04#    | 0.51 $\pm$<br>0.01\$ | 0.88 $\pm$<br>0.02*#\$\$@ | 0.08 $\pm$<br>0.01*#\$\$@ | 0.55 $\pm$<br>0.02@  | 0.29 $\pm$<br>0.01*#\$\$@ | 0.37 $\pm$<br>0.03*#\$\$@ | 1.09 $\pm$<br>0.02*#\$\$ | 0.94 $\pm$<br>0.01*#\$\$@ |
| Cd      | 0.02 $\pm$<br>0.01         | 0.01 $\pm$<br>0.01        | 0.03 $\pm$<br>0.01        | 0.01 $\pm$<br>0.01        | 0.03 $\pm$<br>0.01    | 0.02 $\pm$<br>0.003  | 0.04 $\pm$<br>0.02        | 0.02 $\pm$<br>0.01        | 0.02 $\pm$<br>0.002  | 0.01 $\pm$<br>0.004       | 0.01 $\pm$<br>0.003       | 0.01 $\pm$<br>0.01       | 0.02 $\pm$<br>0.01        |
| Cr      | 0.2 $\pm$<br>0.04          | 0.15 $\pm$<br>0.06        | 0.29 $\pm$<br>0.12        | 0.24 $\pm$<br>0.04        | 0.41 $\pm$<br>0.16    | 0.08 $\pm$<br>0.02   | 0.16 $\pm$<br>0.02        | 5.39 $\pm$<br>8.84        | 0.26 $\pm$<br>0.09   | 0.18 $\pm$<br>0.02        | 0.39 $\pm$<br>0.42        | 0.31 $\pm$<br>0.18       | 0.26 $\pm$<br>0.07        |
| Cu      | 1.98 $\pm$<br>0.14*#       | 1.54 $\pm$<br>0.3*#       | 1.62 $\pm$<br>0.11*       | 0.76 $\pm$<br>0.08*#      | 5.61 $\pm$<br>2.13*#  | 1.49 $\pm$<br>0.4*#  | 1.91 $\pm$<br>0.11*#      | 0.97 $\pm$<br>0.11*#      | 1.75 $\pm$<br>0.33*# | 1.51 $\pm$<br>0.16*#      | 1.75 $\pm$<br>0.51*#      | 1.33 $\pm$<br>0.32*#     | 1.48 $\pm$<br>0.18*#      |
| Hg      | <LOD                       | <LOD                      | <LOD                      | <LOD                      | 0.02                  | <LOD                 | <LOD                      | 38.54 $\pm$<br>65.18      | <LOD                 | 0.08 $\pm$<br>0.09        | <LOD                      | <LOD                     | <LOD                      |
| Ni      | 4.17 $\pm$<br>0.21         | 3.93 $\pm$<br>0.1         | 1.68 $\pm$<br>0.18        | 1.16 $\pm$<br>0.06        | 3.21 $\pm$<br>0.19    | 3.21 $\pm$<br>0.2    | 2.59 $\pm$<br>0.16        | 26.14 $\pm$<br>42.36      | 2.88 $\pm$<br>0.04   | 1.15 $\pm$<br>0.21        | 1.31 $\pm$<br>0.02        | 1.23 $\pm$<br>0.11       | 1.96 $\pm$<br>0.07        |
| Pb      | 0.06 $\pm$<br>0.02         | 0.04 $\pm$<br>0.01        | 0.08 $\pm$<br>0.05        | 0.01 $\pm$<br>0.02        | 0.11 $\pm$<br>0.04    | 0.05 $\pm$<br>0.004  | 0.16 $\pm$<br>0.12        | 0.03                      | 0.2 $\pm$<br>0.09    | 0.21 $\pm$<br>0.25        | 0.19                      | <LOD                     | 0.06 $\pm$<br>0.01        |
| Zn      | 4.12 $\pm$<br>1.32*        | 2.68 $\pm$<br>0.49*       | 8.78 $\pm$<br>1.85        | 5.35 $\pm$<br>0.83*       | 17.35 $\pm$<br>11.05* | 4.89 $\pm$<br>3.23*  | 3.48 $\pm$<br>0.98*       | 5.84 $\pm$<br>3.3*        | 5.28 $\pm$<br>0.49*  | 2.95 $\pm$<br>1.19*       | 1.99 $\pm$<br>0.57*       | 2.55 $\pm$<br>0.55*      | 3.06 $\pm$<br>1.14*       |

<LOD – Below the limit of detection, significant differences per element are indicated with common superscripts.

Concentrations of trace elements (**Table 2.7**) in the Incomati River catchment ranged between 2.01 – 6.84 µg/L for As, 0.02 – 0.04 µg/L for Cd, 0.05 – 0.23 µg/L for Cr, 0.01 – 0.03 µg/L for Hg, 0.03 – 0.55 µg/L for Ni, and 0.53 – 2.07 µg/L for Zn. Copper was below the detection limit at all the sites, whereas Pb concentrations above the limit of detection were only evident at **IR3**. The sites **SR KNP** had the highest concentrations for As and Hg, whereas **IR2** had the highest concentrations for Cd. Site **IR3** had the highest concentrations of Cr. The highest concentrations of Ni were evident at **VG** and the highest concentrations of Zn was evident at **SR Moz**. There were no significant differences between any of the sites for Cd, Cu, Pb and Zn. Arsenic concentrations at **IR3**, and **VG** differed significantly from most of the sites, except for **SR Moz**. The As concentrations at **SR Moz** differed significantly from the As concentrations at **IR2** ( $p < 0.0001$ ), **LSR** ( $p < 0.0001$ ), **SR KNP** ( $p < 0.0001$ ). Chromium concentrations differed significantly between **SR Moz** and **IR2**, as well as **LSR** and **IR2** ( $p = 0.0425$ ). The Ni concentrations at **LSR** differed from **IR1** ( $p = 0.0145$ ), **IR2** ( $p = 0.0374$ ) and **VG** ( $p = 0.0044$ ), as well as between the **SR KNP** and **IR1** ( $p = 0.0052$ ), **IR2** ( $p = 0.0138$ ) and **VG** ( $p = 0.0016$ ). The Hg concentrations at **IR3** indicated a significant difference from the Hg concentrations at **VG** ( $p = 0.0457$ ), **SR Moz** ( $p = 0.0193$ ) and **IR2** ( $p = 0.0391$ ).

**Table 2.7:** The mean  $\pm$  standard error of the trace element concentrations (µg/L) in water samples collected from the Incomati River catchment.

| Element   | LSR                  | VG                   | IR1                  | SR Moz               | IR2                  | IR3                  | SR KNP              |
|-----------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|---------------------|
| <b>As</b> | 2.36 $\pm$<br>0.2*#@ | 5.19 $\pm$<br>1*#    | 2.77 $\pm$<br>0.1*#  | 6.69 $\pm$<br>0.2@   | 2.13 $\pm$<br>0.5*#@ | 2.01 $\pm$<br>0.3*#  | 6.84 $\pm$<br>1*#@  |
| <b>Cd</b> | 0.03 $\pm$<br>0.008  | 0.03 $\pm$<br>0.005  | 0.02 $\pm$<br>0.001  | 0.02 $\pm$<br>0.01   | 0.04 $\pm$<br>0.01   | 0.03 $\pm$<br>0.01   | 0.03 $\pm$<br>0.004 |
| <b>Cr</b> | 0.08 $\pm$<br>0.04#  | 0.16 $\pm$<br>0.08   | 0.05 $\pm$<br>0.03   | <LOD*                | 0.12 $\pm$<br>0.1*#  | 0.23 $\pm$<br>0.07   | 0.17 $\pm$<br>0.06  |
| <b>Cu</b> | <LOD                 | <LOD                 | <LOD                 | <LOD                 | <LOD                 | <LOD                 | <LOD                |
| <b>Hg</b> | 0.01 $\pm$<br>0.002  | 0.01 $\pm$<br>0.004* | 0.01 $\pm$<br>0.0005 | 0.01 $\pm$<br>0.003* | 0.02 $\pm$<br>0.01*  | 0.01 $\pm$<br>0.009* | 0.03 $\pm$<br>0.001 |
| <b>Ni</b> | 0.03 $\pm$<br>0.03*  | 0.55 $\pm$<br>0.1*#  | 0.08 $\pm$<br>0.04*# | 0.38 $\pm$<br>0.05   | 0.48 $\pm$<br>0.3*#  | 0.44 $\pm$<br>0.09   | 0.31 $\pm$<br>0.09# |
| <b>Pb</b> | <LOD                 | <LOD                 | <LOD                 | <LOD                 | <LOD                 | 0.02                 | <LOD                |
| <b>Zn</b> | 1.61 $\pm$<br>0.9    | 0.64 $\pm$<br>0.3    | 1.13 $\pm$<br>0.5    | 2.07 $\pm$<br>1      | 0.97 $\pm$<br>0.5    | 1.25 $\pm$<br>0.8    | 0.53 $\pm$<br>0.4   |

<LOD – Below the limit of detection, significant differences per element are indicated with common superscripts.

The individual ATI scores calculated for each site in the Limpopo River catchment are presented in **Table 2.8**. The variables contributing to the lowest scores are included. Between the Limpopo River and its tributaries, the ATI scores indicated spatial differences as the Letaba River (**LET**; 76.8) scored higher than the Sand River (**SAND**; 61.13). The ATI scores for all the rivers were above the acceptable level (60). Different variables (e.g., NH<sub>4</sub>-N, PO<sub>4</sub>-P, TDS, turbidity, pH, Cr, Cu, and Mn) contributed to the lower scores at the different sites, with the lowest rating score of 3.83 for TDS concentration at the **MAT** site, whereas the lowest rating score of 16 for Zn concentrations was evident at the rest of the sites.

**Table 2.8:** ATI scores for each of the sites on the Limpopo River catchment with its contributing chemical variables, as well as the variable with the lowest rating.

| Sites       | ATI Score | Contributing Chemical Variables                                                           | Lowest Rating |
|-------------|-----------|-------------------------------------------------------------------------------------------|---------------|
| <b>CROC</b> | 64.83     | Ortho-phosphate (69.27) / TDS (73.93) / Ammonium (74.45)                                  | Zinc (16)     |
| <b>LR1</b>  | 72.76     | TDS (73.25) / Ammonium (88.33) / Ortho-phosphate (90.85)                                  | Zinc (16)     |
| <b>MAT</b>  | 61.26     | TDS (3.83) / Ammonium (76.52) / Ortho-phosphate (87.85)                                   | TDS (3.83)    |
| <b>LEPH</b> | 67.97     | Ortho-phosphate (33.07) / TDS (71.25)                                                     | Zinc (16)     |
| <b>LR2</b>  | 67.66     | TDS (68.05) / Ammonium (80) / Ortho-phosphates (56.62) / Chromium (97.03), Copper (97.80) | Zinc (16)     |
| <b>MOG</b>  | 61.93     | TDS (30.97) / Ammonium (64.58) / Ortho-phosphates (67.95) / Manganese (98.04)             | Zinc (16)     |
| <b>SAND</b> | 61.13     | TDS (27.99) / Ammonium (66.25) / Ortho-phosphates (57.17)                                 | Zinc (16)     |
| <b>LUV</b>  | 73.71     | TDS (90.91) / Ammonium (71.88) / Ortho-phosphates (77.91)                                 | Zinc (16)     |
| <b>SHIN</b> | 69.84     | Ammonium (58.06) / TDS (69.67) / Ortho-phosphate (88.69)                                  | Zinc (16)     |
| <b>GLET</b> | 74.06     | TDS (49.71) / Ammonium (71.88) / Ortho-phosphates (77.91)                                 | Zinc (16)     |
| <b>LET</b>  | 76.8      | TDS (73.029) / Ortho-phosphate (96.23)                                                    | Zinc (16)     |
| <b>MAM</b>  | 73.20     | TDS (56.47) / Ortho-phosphates (98.10)                                                    | Zinc (16)     |
| <b>BAL</b>  | 68.86     | TDS (68.61) / Ammonium (67.82) / Ortho-phosphates (77.35)                                 | Zinc (16)     |

The individual ATI scores (a value between 0 and 100) calculated for each site in the Incomati River catchment are presented in **Table 2.9**. The variables contributing to the lowest scores are included. Between the Incomati River and its tributaries, the ATI scores indicated spatial differences as the upstream site in the Incomati River (**IR2**; 75.52) scored higher than the downstream site in the Incomati River (**IR3**; 67.31). The ATI scores for all the rivers in the Incomati River catchment were above the acceptable level (60). Different variables (e.g., NH<sub>4</sub>-N, PO<sub>4</sub>-P, TDS, turbidity and Fluoride) contributed to the lower scores at the different sites, with the lowest rating score of 16 for Zn concentrations across all the sites.

**Table 2.9:** ATI scores for the different sites on the Incomati River catchment with its contributing chemical variables, as well as the variable with the lowest rating.

| Sites         | ATI Score | Contributing chemical variables                                             | Lowest rating |
|---------------|-----------|-----------------------------------------------------------------------------|---------------|
| <b>SR KNP</b> | 74.26     | Fluoride (88.96) / Ammonium (93.5)                                          | Zinc (16)     |
| <b>VG</b>     | 67.82     | Ammonium (73.64) / Ortho-phosphate (82.53) / TDS (87.14) / Fluoride (88.47) | Zinc (16)     |
| <b>LSR</b>    | 72.72     | Fluoride (89.69)/ Ortho-phosphate (81.94)                                   | Zinc (16)     |
| <b>SR Moz</b> | 75.39     | Fluoride (87.85) / TDS (99.351)                                             | Zinc (16)     |
| <b>IR1</b>    | 73.52     | TDS (81.45)                                                                 | Zinc (16)     |
| <b>IR2</b>    | 75.52     | TDS (87.06) / Fluoride (93.89) / Ammonium (95)                              | Zinc (16)     |
| <b>IR3</b>    | 67.31     | Ortho-phosphate (83.93) / Fluoride (88.96) / Ammonium (93.5)                | Zinc (16)     |

There was clear spatial variation in the water quality variables of the sites on the Limpopo River catchment (**Figure 2.10**). Some of the sites (**LUV**, **GLET**, **LET**, **LEPH** and **MAT**) were characterised by higher concentrations of trace elements (Cr, Hg, Ni and Zn) with **LUV**, **GLET** and **LET** associated with a higher ATI score. Most of the sites (**CROC**, **LR2**, **SHIN**, **MAM** and **BAL**) were associated with a higher pH, turbidity and alkalinity, as well as higher concentrations of As and SO<sub>4</sub>. Higher concentrations of trace elements (Cd, Cu), nutrients (Tot N, PO<sub>4</sub>), as well as high electrical conductivity and dissolved oxygen were evident at **LR1**, **MOG** and **SAND**.

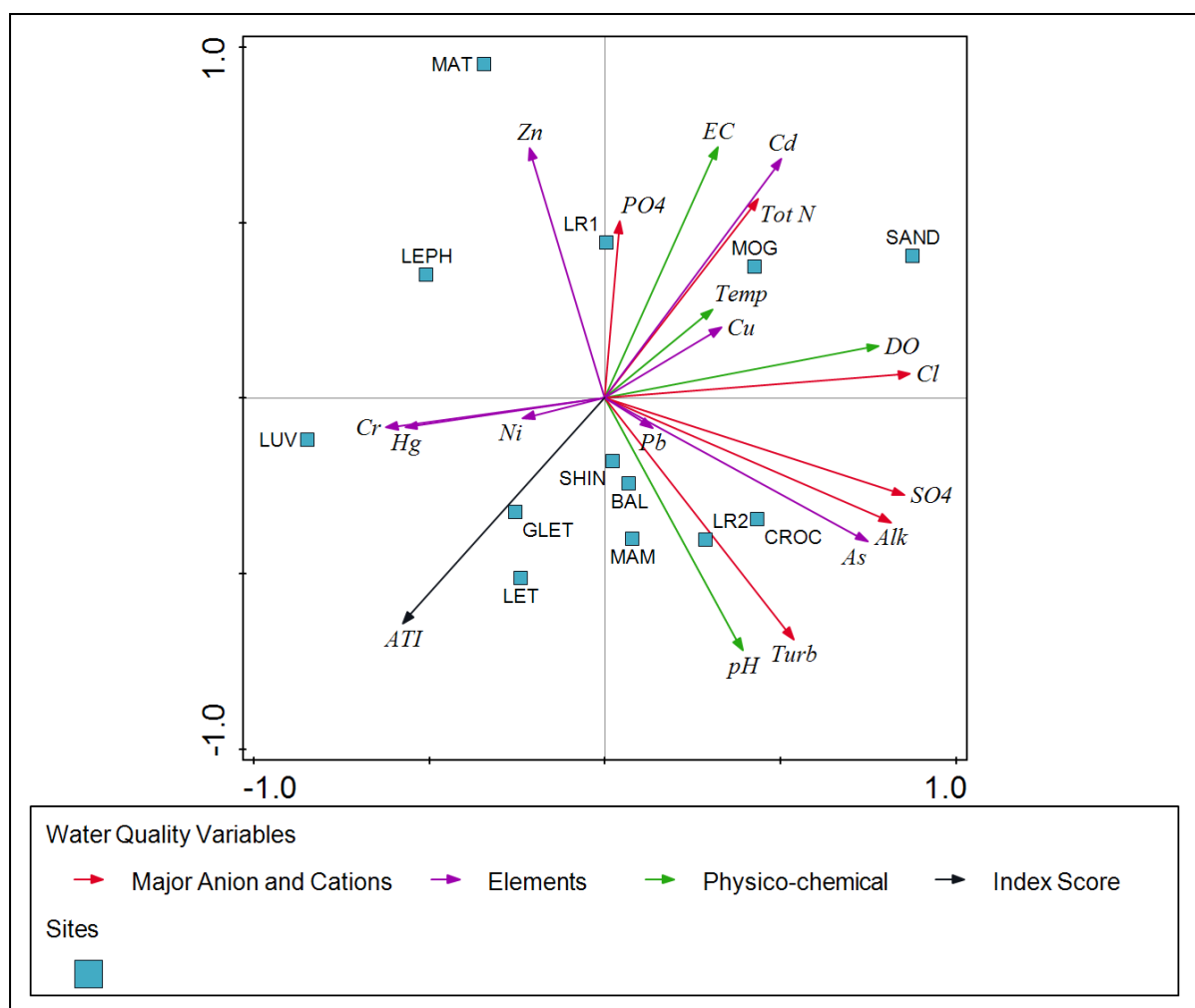


**Figure 2.10:** PCA biplot for the Limpopo River catchment indicates differences in element concentrations, major cation and anion concentrations, and the physico-chemical concentrations at sample sites in 2021. This biplot describes 50.82% of the variation in the data, where 29.86% is displayed on the first axis, while 20.96% is displayed on the second axis.

There was clear spatial variance in the water quality variables of the sites on the Incomati River catchment (**Figure 2.11**). Most of the sites that were situated inside the Kruger National Park (KNP) (**SR KNP** and **LSR**) were not characterised by any specific variables. **SR Moz** was characterised by high concentrations of Zn and a high ATI score. Sites **VG**, **IR1** and **IR2** were characterised by high dissolved oxygen, turbidity, nutrients (Tot N), trace elements (Cr, Ni), as well as salts (Cl and  $SO_4$ ). Site **IR3** was associated with a higher pH, electrical conductivity, nutrients ( $PO_4$ ), as well as trace elements (As, Cd, and Hg).

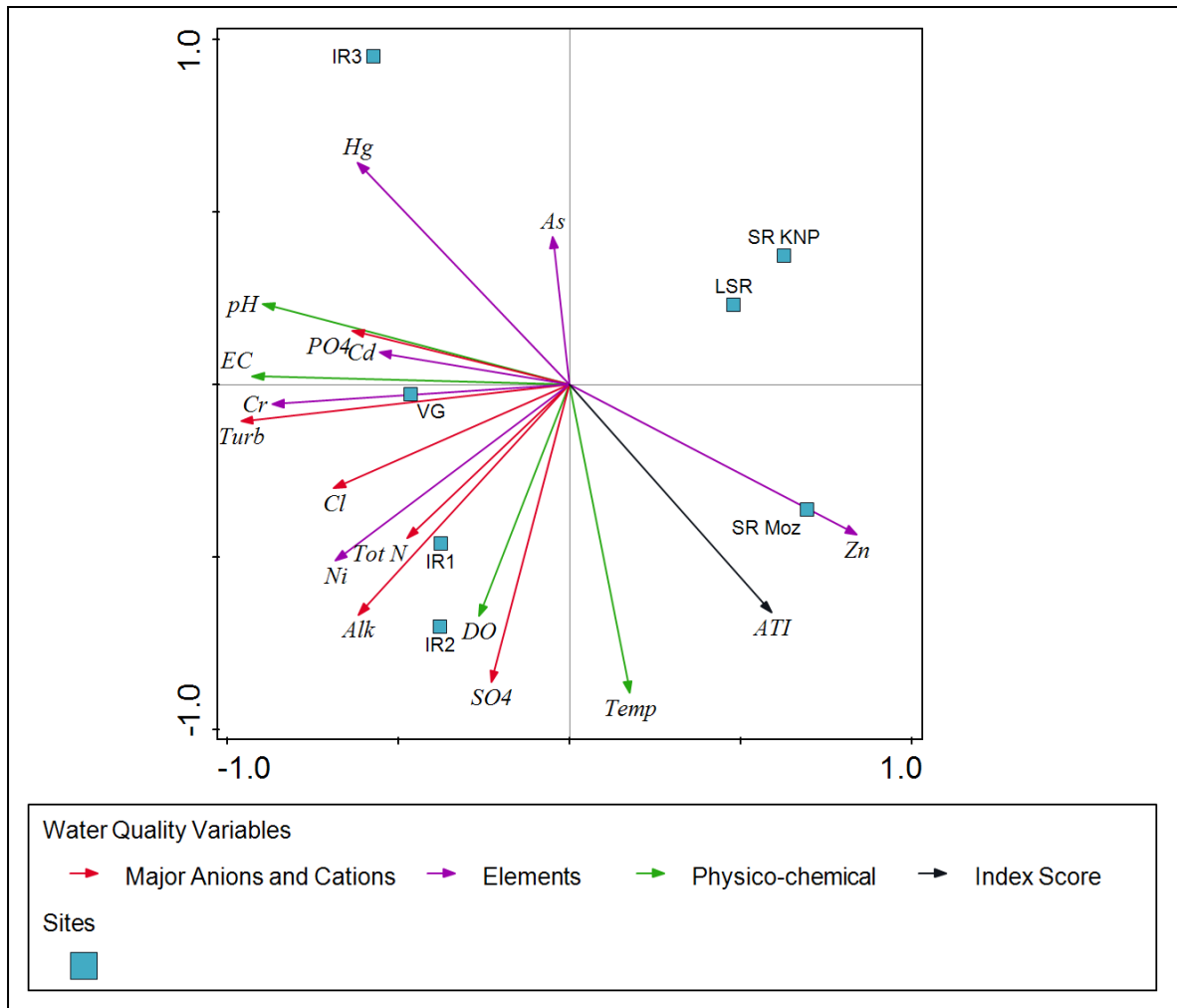


**Figure 2.11:** PCA biplot for the Incomati River catchment indicating differences in element concentrations, major cation and anion concentrations, as well as the physico-chemical concentrations at sample sites in 2022. This biplot describes 67.76% of the variation in the data, where 42.32% is displayed on the first axis, while 25.44% is displayed on the second axis.

The PCA biplot representing the water quality comparison between the Limpopo River catchment and the Incomati River catchment (**Figure 2.12**) indicates a separation between the sites on the Limpopo River catchment and the sites on the Incomati River catchment. Along the first component (explaining 33.69% of the variation) the individual ions, trace elements and physico-chemical variables differentiate between the two systems. Along the second component (explaining 20.18% of the variation) the variables differentiate between sites within a system. All of the sites on the Incomati River catchment were characterised by As concentrations, whereas most of the sites (**CROC**, **LR2**, **MOG**, **SAND**, **SHIN**, **MAM** and **BAL**) on the Limpopo River catchment were characterised by high salts (Cl and SO<sub>4</sub>), electrical conductivity and dissolved oxygen. Some sites (**LR1**, **MAT**, **LUV**, **GLET** and **LET**)

were characterised by high trace element concentrations (Cr, Cu, Hg, Ni, Zn), as well as high concentrations of nutrients (Tot N, PO<sub>4</sub>). Sites that were not characterised by any of the water quality variables were characterised by a high ATI score.

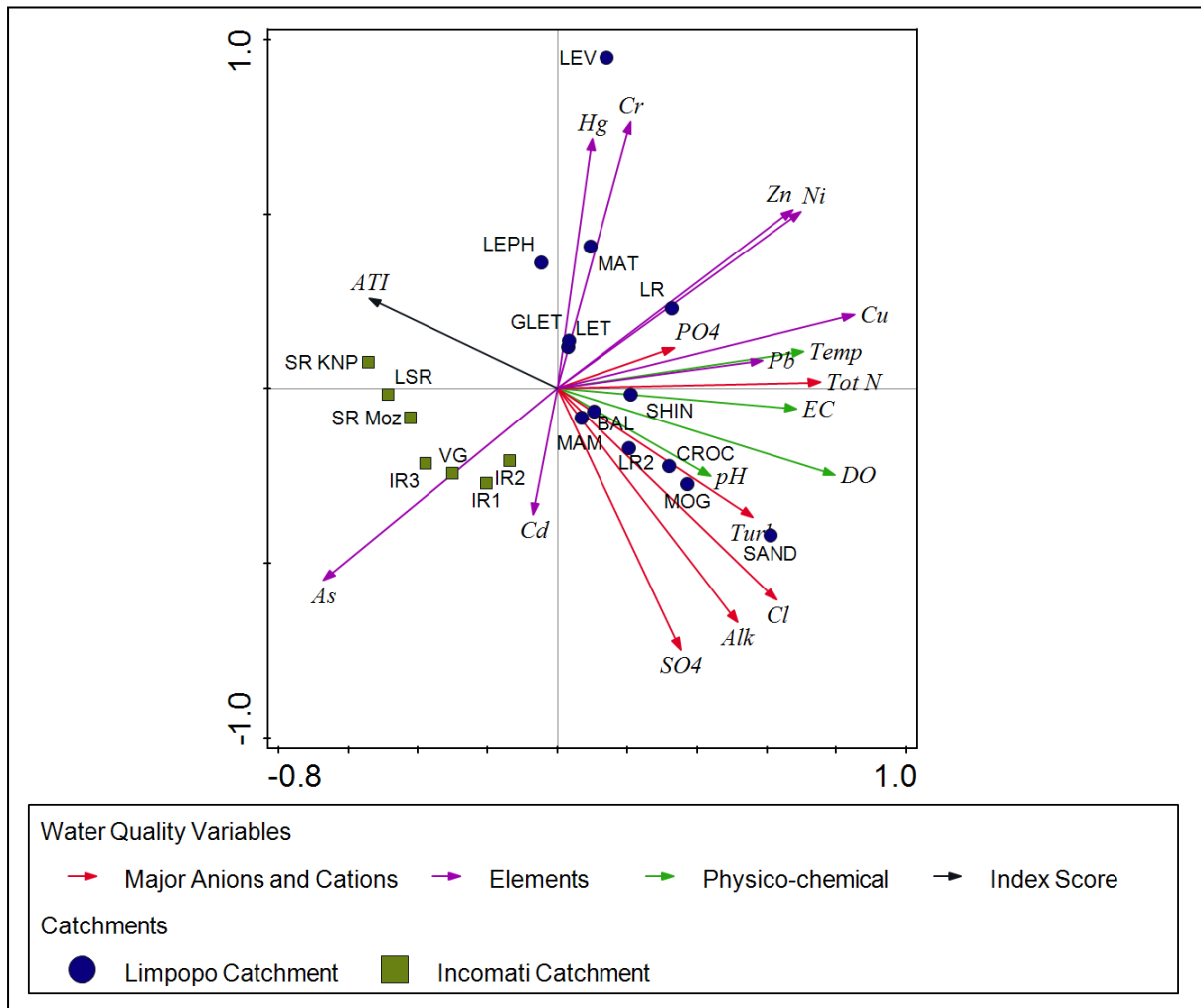


**Figure 2.12:** PCA biplot for the Limpopo River catchment and Incomati River catchment indicating differences in element concentrations, major cation, anion concentrations and the physico-chemical concentrations at sample sites in 2021 and 2022. This biplot describes 53.88% of the variation in the data, where 33.69% is displayed on the first axis, while 20.18% is displayed on the second axis.

### 2.3.2 Sediment Quality

Sediment sampled from the **MAT**, **GLET**, **LET**, and **BAL** sites had predominantly small grain sizes < 500 µm, whereas the rest of the samples all had predominantly larger grain sizes, i.e., > 500 µm. Sediment samples were analysed according to particle size, as well as the total organic carbon. Sediment samples from the **LSR**, **SR Moz** and **IR1** had a predominantly larger grain size, i.e., > 500 µm, whereas **SR KNP**, **VG**, **IR2**, and **IR3** had a predominantly small grain size, i.e., < 500 µm (**Figure 2.13**). The total organic carbon found in the sediment samples of all the rivers ranged from 0.31% to 8.4%. According to the classification system, as set out by the USEPA (2001), the organic carbon content of the **LSR** and the **IR1** is low. Sites **IR2** and **VG** have moderately low and medium organic carbon content, while site **IR3** has a high organic carbon content.

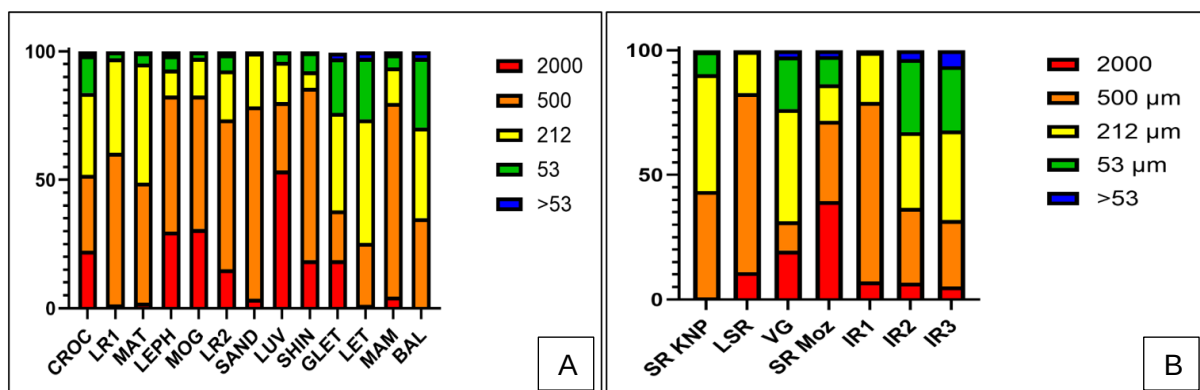


**Figure 2.13:** Sediment Particle size at each of the sites A.) Limpopo River catchment and B.) Incomati River catchment.

The SeQI scores were calculated using the Canadian Council of Ministers of the Environment (CCME) sediment quality guidelines (SQGs). In **Table 2.10** below, the number of variables that were used to calculate the scores, as well as the variables that failed to meet the SQGs set out by the CCME for the Limpopo River catchment are indicated. The SeQI scores ranged between 43 and 100 for the sites on the Limpopo River catchment. Sites with a score of 100 include **MAT** and **GLET**, and did not have any variables that failed. Most of the other sites had two or more variables (As, Cr, Cu, Ni and Zn) that failed.

**Table 2.10:** SeQI score for the Limpopo River catchment with the number of variables taken into account and the variables that failed.

| Site        | Index score | Scope (F <sub>1</sub> ) | Amplitude (F <sub>3</sub> ) | Variables | Variables failed |
|-------------|-------------|-------------------------|-----------------------------|-----------|------------------|
| <b>CROC</b> | 57          | 38                      | 48                          | 8 (3)     | As, Cr, Ni       |
| <b>LR1</b>  | 57          | 38                      | 48                          | 8 (3)     | As, Cr, Ni       |
| <b>MAT</b>  | 100         | 0                       | 0                           | 8 (0)     | -                |
| <b>LEPH</b> | 77          | 25                      | 21                          | 8 (2)     | Cr, Ni           |
| <b>LR2</b>  | 81          | 25                      | 10                          | 8 (2)     | Cr, Ni           |
| <b>MOG</b>  | 56          | 25                      | 57                          | 8 (2)     | Cr, Ni           |
| <b>SAND</b> | 78          | 29                      | 14                          | 7 (2)     | Cr, Ni           |
| <b>LUV</b>  | 55          | 43                      | 47                          | 7 (3)     | Cr, Cu, Ni       |
| <b>SHIN</b> | 55          | 43                      | 48                          | 7 (3)     | Cr, Cu, Ni       |
| <b>GLET</b> | 100         | 0                       | 0                           | 8 (0)     | -                |
| <b>LET</b>  | 69          | 29                      | 33                          | 7 (2)     | Cr, Ni           |
| <b>MAM</b>  | 43          | 57                      | 57                          | 7 (4)     | Cr, Cu, Zn Ni    |
| <b>BAL</b>  | 53          | 43                      | 51                          | 7 (3)     | Cr, Cu, Ni       |

In **Table 2.11** are the SeQI scores and the variables that affected the scores. All the sites on the Incomati River catchment had a SeQI score of 70 and above, with the **LSR**, **VG**, and **IR1** sites having a score of 100 and did not have any variables that failed. Other sites that had a lower score had two or more variables (As, Cr, Ni) that failed, except for **SR KNP**, which only had one variable (As) that failed.

**Table 2.11:** SeQI score for the Incomati River catchment with the number of variables taken into account and variables that failed.

| Site          | Index score | Scope (F <sub>1</sub> ) | Amplitude (F <sub>3</sub> ) | Variables | Variables failed |
|---------------|-------------|-------------------------|-----------------------------|-----------|------------------|
| <b>SR KNP</b> | 83          | 13                      | 21                          | 8 (1)     | As               |
| <b>LSR</b>    | 100         | 0                       | 0                           | 8 (0)     | -                |
| <b>VG</b>     | 100         | 0                       | 0                           | 8 (0)     | -                |
| <b>SR Moz</b> | 78          | 25                      | 17                          | 8 (2)     | As, Cr           |
| <b>IR1</b>    | 100         | 0                       | 0                           | 8 (0)     | -                |
| <b>IR2</b>    | 70          | 38                      | 18                          | 8 (3)     | As, Cr, Ni       |
| <b>IR3</b>    | 79          | 25                      | 16                          | 8 (2)     | Cr, Ni           |

Concentrations of trace elements (**Table 2.12**) in the Limpopo River catchment ranged between 0.69 – 10.07 µg/g for As, 0.022 – 0.431 µg/g for Cd, 22.58 – 362.5 µg/g for Cr, 3.93 – 56.1 µg/g for Cu, <LOD – 0.024 µg/g for Hg, 8.47 – 107.7 µg/g for Ni, 3 – 31.32 µg/g for Pb, 19.42 – 125.4 µg/g for Zn. Site **LR1** had the highest concentrations for As, whereas **LUV** had the highest concentrations for Cd and Cu. Site **MOG** had the highest concentrations of Cr and Pb. The highest concentrations of Hg were evident at **GLET** and the highest concentrations of Ni was evident at **SHIN**. Site **MAM** had the highest concentrations of Zn. There were no significant differences between any of the sites for Cr. As concentrations at **LR1** differed significantly from most of the sites, except when compared to As concentrations at **LR2**, **CROC** and **SHIN** ( $p = 0.0379$ ), **CROC** and **LET** ( $p = 0.0334$ ), **CROC** and **LR2** ( $p = 0.0169$ ), **CROC** and **SAND** ( $p = 0.004$ ) and **CROC** and **GLET** ( $p = 0.0008$ ) also differed significantly. The Cd concentrations at **LR2** differed from most of the sites, except for **MAT**. The Cd concentrations at **LUV** indicated a significant difference between all of the sites on the Limpopo River catchment. Significant differences between Ni concentrations were evident between **MAT** and **SHIN** ( $p = 0.001$ ), **MAT** and **LUV** ( $p = 0.0193$ ), **MAT** and **MAM** ( $p = 0.0038$ ), **MAT** and **BAL** ( $p = 0.0207$ ), **SHIN** and **LR2** ( $p = 0.0054$ ), **SHIN** and **SAND** ( $p = 0.0336$ ), **SHIN** and **GLET** ( $p = 0.0008$ ), **LR2** and **MAM** ( $p = 0.02$ ), **LUV** and **GLET** ( $p = 0.0159$ ), **MAM** and **GLET** ( $p = 0.0031$ ), as well as **BAL** and **GLET** ( $p = 0.0171$ ). Significant differences between Cu concentrations were evident between **MAT** and **SHIN** ( $p = 0.0002$ ), **MAT** and **LUV** ( $p = 0.0002$ ), **MAT** and **MAM** ( $p = 0.0082$ ), **MAT** and **BAL** ( $p = 0.0129$ ), **CROC** and **SHIN** ( $p = 0.0477$ ), **CROC** and **LUV** ( $p = 0.0381$ ), **LR1** and **SHIN** ( $p = 0.0453$ ), **LR1** and **LUV** ( $p = 0.0361$ ), **SHIN** and **LR2** ( $p = 0.0002$ ), **SHIN** and **SAND** ( $p = 0.0192$ ), **SHIN** and **GLET** ( $p = 0.0008$ ), **LR2** and **LUV** ( $p = 0.0002$ ), **LR2** and **MAM** ( $p = 0.0069$ ), **LR2** and **BAL** ( $p = 0.0107$ ), **SAND** and **LUV** ( $p = 0.0151$ ), **LUV** and **GLET** ( $p = 0.0006$ ), **MAM** and **GLET** ( $p = 0.0263$ ), as well as **BAL** and **GLET** ( $p = 0.04$ ). Zinc concentrations at **MAM** differed significantly from most of the sites, except between **MAM** and **LUV**, and **MAM** and **BAL**. The Zn concentrations at **LUV** differed significantly from **MAT** ( $p = 0.0258$ ), **LR2** ( $p = 0.0137$ ), **SAND** ( $p = 0.045$ ), and **GLET** ( $p = 0.0012$ ), and significant differences were also observed between **LR2** and **BAL** ( $p = 0.0444$ ), as well as **BAL** and **GLET** ( $p = 0.0041$ ). The Hg concentrations at **GLET** differed significantly from all the sites, whereas the Hg concentrations at **MAT**, **LEPH**, **CROC**, **LR1** and **LR2** differed significantly from **SHIN**, **LET**, **MOG**, **SAND**, **LUV**, **MAM** and **BAL** ( $p < 0.0001$ ). The Pb concentrations at **MOG** differed significantly from **GLET** ( $p = 0.0337$ ).

**Table 2.12:** The mean  $\pm$  standard error of the trace element concentrations ( $\mu\text{g/g}$ ) of sediment samples collected from the Limpopo River catchment.

| Element | CROC                                      | LR1                                | MAT                                     | LEPH                               | LR2                                    | MOG                                | SAND                                | LUV                                    | SHIN                                   | GLET                               | LET                                | MAM                                              | BAL                                     |
|---------|-------------------------------------------|------------------------------------|-----------------------------------------|------------------------------------|----------------------------------------|------------------------------------|-------------------------------------|----------------------------------------|----------------------------------------|------------------------------------|------------------------------------|--------------------------------------------------|-----------------------------------------|
| As      | 7.52 $\pm$<br>1.01* <sup>#@&amp;\$a</sup> | 10.07 $\pm$<br>2.96*               | 3.85 $\pm$<br>1.26*                     | 3.55 $\pm$<br>0.67*                | 2.26 $\pm$<br>0.24 <sup>\$</sup>       | 4.26 $\pm$<br>4.07*                | 1.5 $\pm$<br>0.27* <sup>&amp;</sup> | 3.66 $\pm$<br>0.51*                    | 2.7 $\pm$<br>0.67* <sup>#</sup>        | 0.69 $\pm$<br>0.21* <sup>a</sup>   | 2.63 $\pm$<br>0.71* <sup>@</sup>   | 4.74 $\pm$<br>0.74*                              | 4.21 $\pm$<br>1.29*                     |
| Cd      | 0.151 $\pm$<br>0.078* <sup>#</sup>        | 0.127 $\pm$<br>0.039* <sup>#</sup> | 0.094 $\pm$<br>0.004 <sup>#</sup>       | 0.172 $\pm$<br>0.066* <sup>#</sup> | 0.170 $\pm$<br>0.066* <sup>#</sup>     | 0.155 $\pm$<br>0.081* <sup>#</sup> | 0.131 $\pm$<br>0.061* <sup>#</sup>  | 0.431 $\pm$<br>0.188* <sup>#</sup>     | 0.146 $\pm$<br>0.074* <sup>#</sup>     | 0.022 $\pm$<br>0.005* <sup>#</sup> | 0.153 $\pm$<br>0.056* <sup>#</sup> | 0.166 $\pm$<br>0.065* <sup>#</sup>               | 0.144 $\pm$<br>0.048* <sup>#</sup>      |
| Cr      | 224.3 $\pm$<br>50.27                      | 204.6 $\pm$<br>63                  | 30.18 $\pm$<br>6.03                     | 80.01 $\pm$<br>31.28               | 68.58 $\pm$<br>14.15                   | 362.5 $\pm$<br>484                 | 50.53 $\pm$<br>8.07                 | 129.3 $\pm$<br>17.83                   | 99.41 $\pm$<br>16.62                   | 22.58 $\pm$<br>8.47                | 120.4 $\pm$<br>22.54               | 242.4 $\pm$<br>85.81                             | 188.6 $\pm$<br>74.11                    |
| Cu      | 23.42 $\pm$<br>9.59 <sup>ab</sup>         | 23.22 $\pm$<br>15.77 <sup>cd</sup> | 4.59 $\pm$<br>1.88* <sup>#&amp;\$</sup> | 26.84 $\pm$<br>11.46               | 3.93 $\pm$<br>1.09 <sup>ehij</sup>     | 27.23 $\pm$<br>13.65               | 19.96 $\pm$<br>1.03 <sup>fk</sup>   | 56.10 $\pm$<br>11.12 <sup>#bdhkl</sup> | 55.22 $\pm$<br>21.67* <sup>acefg</sup> | 8.82 $\pm$<br>2.7 <sup>gilmn</sup> | 28.02 $\pm$<br>1.73                | 42.91 $\pm$<br>3.66 <sup>sin</sup>               | 41.31 $\pm$<br>13.98 <sup>&amp;jm</sup> |
| Hg      | 0.014 $\pm$<br>0.0007*                    | 0.014 $\pm$<br>0.002*              | 0.019 $\pm$<br>0.005*                   | 0.017 $\pm$<br>0.0005*             | 0.017 $\pm$<br>0.001*                  | <LOD*                              | <LOD*                               | <LOD*                                  | <LOD*                                  | 0.024 $\pm$<br>0.002*              | <LOD*                              | <LOD*                                            | <LOD*                                   |
| Ni      | 66.34 $\pm$<br>13.81                      | 69.28 $\pm$<br>34.25               | 9.99 $\pm$<br>1.51* <sup>#&amp;\$</sup> | 42.45 $\pm$<br>13.63               | 22.69 $\pm$<br>0.76 <sup>ad</sup>      | 64.06 $\pm$<br>50.29               | 36.9 $\pm$<br>8.86 <sup>b</sup>     | 85.22 $\pm$<br>14.13* <sup>he</sup>    | 107.7 $\pm$<br>29.94* <sup>abc</sup>   | 8.47 $\pm$<br>2.5 <sup>cefg</sup>  | 46.61 $\pm$<br>5.0                 | 97.66 $\pm$<br>25.72 <sup>\$df</sup>             | 84.68 $\pm$<br>27.92 <sup>&amp;g</sup>  |
| Pb      | 27.19 $\pm$<br>1.85                       | 21.34 $\pm$<br>7.17                | 11.08 $\pm$<br>3.1                      | 25.29 $\pm$<br>10.24               | 8.95 $\pm$<br>1.54                     | 31.32 $\pm$<br>27.66*              | 6.78 $\pm$<br>2.39                  | 22.68 $\pm$<br>4.89                    | 9.2 $\pm$<br>3.31                      | 3.0 $\pm$<br>0.543*                | 12.22 $\pm$<br>1.47                | 10.89 $\pm$<br>1.07                              | 15.51 $\pm$<br>9.8                      |
| Zn      | 60.01 $\pm$<br>3.17*                      | 44.07 $\pm$<br>18.51 <sup>@</sup>  | 36.03 $\pm$<br>10.06* <sup>g</sup>      | 50.77 $\pm$<br>16.86 <sup>\$</sup> | 32.51 $\pm$<br>2.32 <sup>&amp;hk</sup> | 56.83 $\pm$<br>29.23 <sup>a</sup>  | 39.23 $\pm$<br>10.13 <sup>bi</sup>  | 87.44 $\pm$<br>13.68 <sup>ghij</sup>   | 55.6 $\pm$<br>22.8 <sup>c</sup>        | 19.42 $\pm$<br>7.65 <sup>djl</sup> | 42.34 $\pm$<br>1.18 <sup>e</sup>   | 125.4 $\pm$<br>21.74* <sup>@#&amp;\$abcdef</sup> | 80.79 $\pm$<br>20.07 <sup>fk</sup>      |

<LOD – Below the limit of detection, significant differences per element are indicated with common superscripts and letters.

Concentrations of trace elements (**Table 2.13**) in the Incomati River catchment ranged between 1.25 – 18.62 µg/g for As, 0.016 – 0.064 µg/g for Cd, 4.25 – 73.61 µg/g for Cr, 3.15 – 32.39 µg/g for Cu, 0.022 – 0.054 µg/g for Hg, 2.1 – 37.46 µg/g for Ni, 2.43 – 27.1 µg/g for Pb, and 11.75 – 39.22 µg/g for Zn. Site **SR KNP** had the highest concentrations for As, whereas **IR2** had the highest concentrations for Cr. Site **IR3** had the highest concentrations of Cd, Ni and Pb. The highest concentrations of Cr were 4 evident at **IR2** and the highest concentrations of Hg was evident at **LSR**. The highest concentrations of Cu and Zn was evident at **SR Moz**. There were no significant differences between any of the sites for Hg and Ni. Arsenic concentrations at **SR KNP** differed significantly from most of the sites, except for **SR Moz**, whereas the As concentrations at **SR Moz** differed significantly from most of the sites, except **IR2** and **SR KNP**. The Cd concentration at **SR Moz** differed significantly from most of the sites, except for **IR2**, and **IR3**, whereas **IR3** also differed significantly from most of the sites, except **IR2** and **SR Moz**. Chromium concentrations differed significantly between **IR2** and **LSR** ( $p = 0.0256$ ). The Cu concentrations at **SR Moz** differed significantly from **IR1** ( $p = 0.0074$ ), **SR KNP** ( $p = 0.0138$ ), and **VG** ( $p = 0.0095$ ). The Zn concentrations at **SR Moz** indicated a significant difference from the Zn concentrations at **VG** ( $p = 0.0253$ ), **LSR** ( $p = 0.0305$ ) and **IR1** ( $p = 0.0231$ ). The Pb concentrations between **SR Moz** differed significantly from most of the sites, except **IR2** and **IR3**, whereas the Pb concentrations differed significantly between **IR3** and **IR1** ( $p = 0.0066$ ), **IR3** and **SR KNP** ( $p = 0.0126$ ), as well as **IR3** and **VG** ( $p = 0.0113$ ).

**Table 2.13:** The mean  $\pm$  standard error of the trace element concentrations (µg/g) of sediment samples collected from the Incomati River catchment.

| Element   | LSR                                    | VG                                 | IR1                                  | SR Moz                                   | IR2                                 | IR3                                     | SR KNP                                   |
|-----------|----------------------------------------|------------------------------------|--------------------------------------|------------------------------------------|-------------------------------------|-----------------------------------------|------------------------------------------|
| <b>As</b> | 2.91 $\pm$<br>0.51 <sup>*b</sup>       | 3.65 $\pm$<br>2.02 <sup>@c</sup>   | 1.25 $\pm$<br>0.503 <sup>#d</sup>    | 14.22 $\pm$<br>3.79 <sup>bcde</sup>      | 7.24 $\pm$<br>5.61 <sup>&amp;</sup> | 2.95 $\pm$<br>0.92 <sup>ae</sup>        | 18.62 $\pm$<br>6.82 <sup>*@#&amp;a</sup> |
| <b>Cd</b> | 0.016 $\pm$<br>0.006 <sup>*&amp;</sup> | 0.027 $\pm$<br>0.009 <sup>@a</sup> | 0.016 $\pm$<br>0.004 <sup>#b</sup>   | 0.054 $\pm$<br>0.011 <sup>*@#&amp;</sup> | 0.043 $\pm$<br>0.02                 | 0.064 $\pm$<br>0.02 <sup>&amp;abc</sup> | 0.021 $\pm$<br>0.003 <sup>sc</sup>       |
| <b>Cr</b> | 4.25 $\pm$<br>1.26 <sup>*</sup>        | 34.11 $\pm$<br>15.05               | 13.81 $\pm$<br>5.24                  | 47.41 $\pm$<br>15.54                     | 73.61 $\pm$<br>50.06 <sup>*</sup>   | 64.12 $\pm$<br>42.73                    | 16 $\pm$<br>5.79                         |
| <b>Cu</b> | 12.14 $\pm$<br>16.34                   | 9.14 $\pm$<br>5.26 <sup>@</sup>    | 3.15 $\pm$<br>0.846 <sup>*</sup>     | 32.39 $\pm$<br>9.1 <sup>*@#</sup>        | 18.77 $\pm$<br>14.57                | 17.78 $\pm$<br>9.49                     | 5.07 $\pm$<br>1.6 <sup>#</sup>           |
| <b>Hg</b> | 0.054 $\pm$<br>0.054                   | 0.029 $\pm$<br>0.007               | 0.027 $\pm$<br>0.006                 | 0.022 $\pm$<br>0.001                     | 0.040 $\pm$<br>0.018                | 0.026 $\pm$<br>0.002                    | 0.023 $\pm$<br>0.001                     |
| <b>Ni</b> | 2.1 $\pm$<br>1.32                      | 13.67 $\pm$<br>5.46                | 5.85 $\pm$<br>1.59                   | 8.55 $\pm$<br>2.42                       | 33.78 $\pm$<br>25.25                | 37.46 $\pm$<br>33.75                    | 6.62 $\pm$<br>2.25                       |
| <b>Pb</b> | 2.53 $\pm$<br>1.61 <sup>*</sup>        | 6.45 $\pm$<br>2.0 <sup>@b</sup>    | 2.62 $\pm$<br>1.11 <sup>#&amp;</sup> | 19.81 $\pm$<br>7.11 <sup>*@#&amp;</sup>  | 10.26 $\pm$<br>5.68                 | 27.1 $\pm$<br>21.86 <sup>&amp;ab</sup>  | 4.29 $\pm$<br>0.508 <sup>sa</sup>        |
| <b>Zn</b> | 12.72 $\pm$<br>3.53 <sup>@</sup>       | 17.05 $\pm$<br>7.56 <sup>*</sup>   | 11.75 $\pm$<br>2.51 <sup>#</sup>     | 39.22 $\pm$<br>11.97 <sup>*@#</sup>      | 28.64 $\pm$<br>16.84                | 36.08 $\pm$<br>3.52                     | 26.32 $\pm$<br>7.06                      |

<LOD – Below the limit of detection, significant differences per element are indicated with common superscripts and letters.

There was clear spatial variance in the sediment quality variables of the sites on the Limpopo River catchment (**Figure 2.14**). Some of the sites (**CROC**, **LUV**, **MAM** & **BAL**) were characterised by higher concentrations of trace elements (As, Cr, Cu, Ni, Pb, Zn). Sites **GLET** and **LET** were characterised by high TOC, as well as mud and medium sediment particles. Some sites (**SHIN**, **MOG**, & **LR2**) were characterised by high Cd concentrations, as well as coarse to very coarse sediment particles. Sites with a higher SeQI score were characterised by high Hg concentrations.

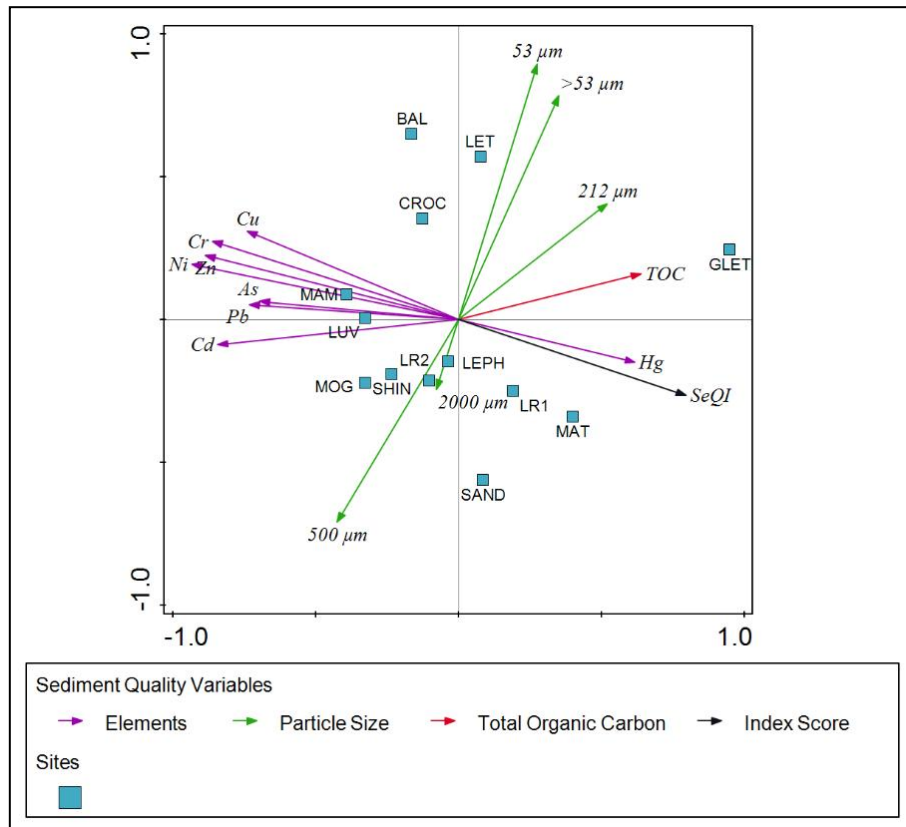


**Figure 2.14:** PCA biplot for the Limpopo River catchment indicating differences in element concentrations, particle size, as well as the total organic carbon at sample sites in 2021. This biplot describes 61.77% of the variation in the data, where 44.90% is displayed on the first axis, while 16.88% is displayed on the second axis.

Spatial variance in the sediment quality variables of the sites on the Incomati River catchment was evident (**Figure 2.15**). Sites that were situated inside the Kruger National Park (KNP) (**SR KNP** and **LSR**) were characterised by a high SeQI score. Sites **SR Moz**, **VG** and **IR3** were characterised by high trace element concentrations (Cd, Hg, Pb), as well as medium to very coarse sediment particles. Site **IR2** was characterised by high TOC concentrations, mud to fine sediment particles, as well as high trace element concentrations (Cr, Ni). The **IR1** site was characterised by coarse sediment particles, as well as high trace element concentrations (Cu, Zn).

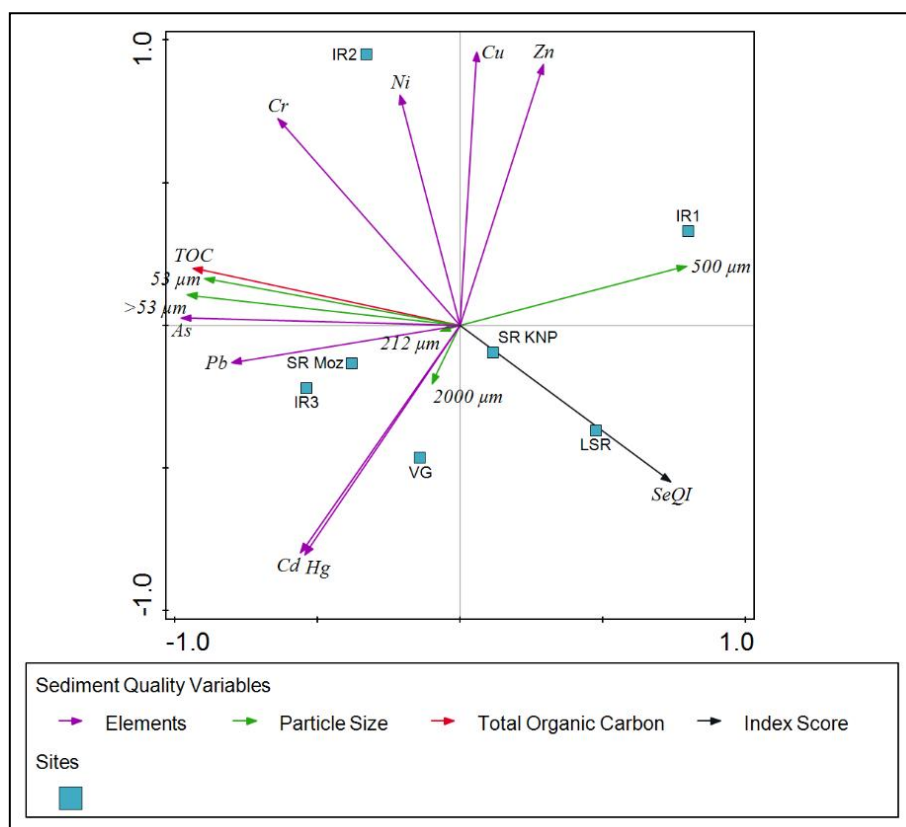


**Figure 2.15:** PCA biplot for the Incomati River catchment indicating differences in element concentrations, particle size, as well as the total organic carbon at sample sites in 2022. This biplot describes 74.81% of the variation in the data, where 43.46% is displayed on the first axis, while 31.35% is displayed on the second axis.

The PCA biplot representing the sediment quality comparison between the Limpopo River catchment and the Incomati River catchment (**Figure 2.16**) indicates a separation between the **LSR** and **IR1** from the other sites. Along the first component (explaining 42.25% of the variation) the trace element concentrations differentiate between the two systems. Along the second component (explaining 22.56% of the variation) the particle size and TOC differentiate between sites within the two systems. Most of the sites on the Limpopo River catchment were characterised by high trace element concentrations (Cr, Cu, Ni, Pb, Zn), as well as very coarse sediment particles. Sites (**LR1**, **SHIN** and **SAND**) from the Limpopo River catchment, as well as **IR1** from the Incomati River catchment, were associated with coarse sediment particles. Sites that were characterised by high TOC concentrations, fine to medium sediment particles, as well as high concentrations of trace elements (As, Cd) include **VG**, **SR Moz**, **IR2** and **IR3**. Sites **MAT** and **GLET**, as well as **LSR** and **SR KNP** were all characterised by high Hg concentrations, as well as a high SeQI score.

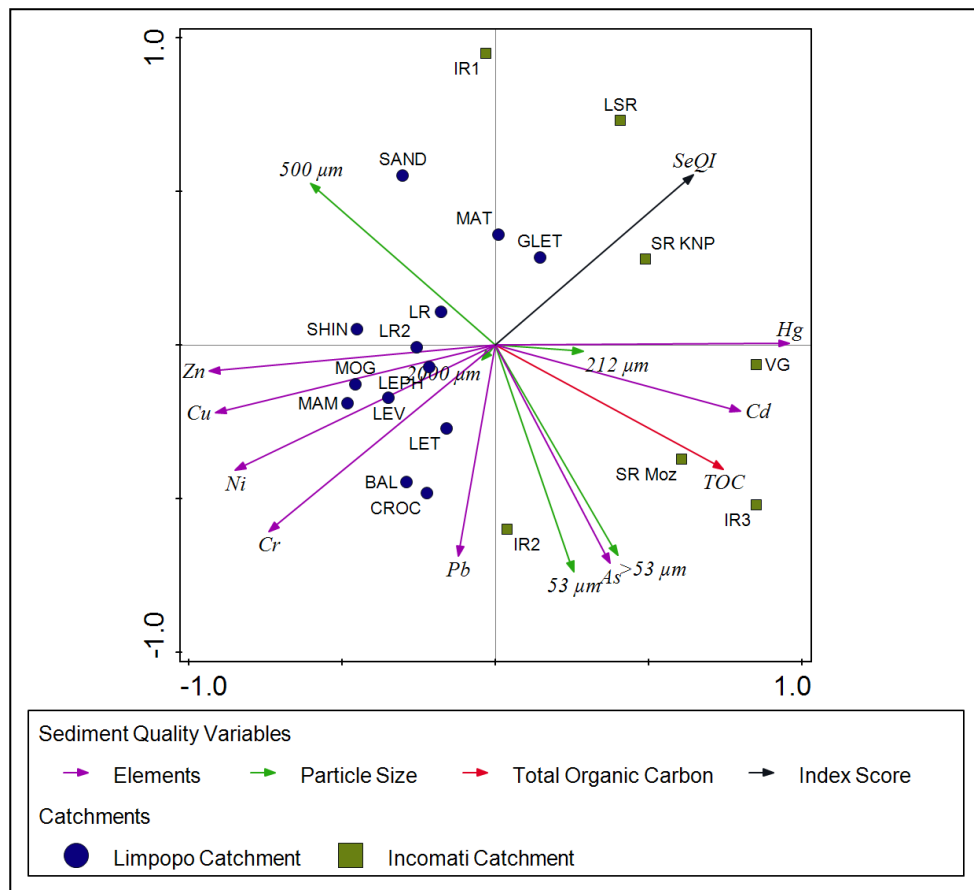


**Figure 2.16:** PCA biplot for the Limpopo River catchment and the Incomati River catchment indicating differences in element concentrations, particle size, as well as the total organic carbon at sample sites in 2021 and 2022. This biplot describes 64.80% of the variation in the data, where 42.25% is displayed on the first axis, while 22.56% is displayed on the second axis.

## 2.4 Discussion

The water and sediment quality of a catchment is important for monitoring the health of these ecosystems. The KNP is one of the largest protected areas in South Africa and is situated within the Limpopo and Incomati catchments (Riddel *et al.*, 2019). Both of these catchments have different stressors affecting them, which introduce different pollutants that affect the water and sediment quality of these systems. According to Riddel *et al.* (2019), the Sabie River is considered one of the most pristine and biodiverse rivers in South Africa, whereas the Crocodile occasionally observe cyanide spills from active mining in the Kaap River tributary, and salt enrichment in the Olifants River due to copper and phosphate mining along the banks of the Ga-Selati River, which flow into the Olifants River. The poor water quality of the Crocodile River, one of the tributaries of the Incomati River, could be attributed to a number of anthropogenic activities along the River as well as its tributaries. According to Madonsela *et al.* (2024), the Kaap River is dominated by mining and agricultural activities before its confluence with the Crocodile River, after which the water quality is generally characterised by high element contamination. There are multiple studies done on the water

and sediment quality of the Limpopo River catchment (Wepener *et al.*, 1992; Ashton *et al.*, 2001; Walters *et al.*, 2011; Vlok *et al.*, 2013; Gerber *et al.*, 2015a; Gerber *et al.*, 2015b; Kahinda *et al.*, 2016; Riddell *et al.*, 2019; Verhaert *et al.*, 2019; Mudaly & Van Der Laan, 2020), with only limited studies on the Incomati River catchment. Since the Incomati River catchment supports millions of people it is important to monitor the water and sediment quality.

#### **2.4.1 Water Quality**

The ATI scores for all the sites sampled on the Limpopo River catchment, as well as the Incomati River catchment were above 60. As stated by Wepener *et al.* (1992), ATI scores above 60 are regarded as acceptable for sustaining aquatic life. The variables that contributed to the lower ATI scores for the Limpopo River catchment included PO<sub>4</sub>, NH<sub>4</sub>, TDS, Cr, Cu and Mn, whereas variables contributing to the lower scores of the Incomati River catchment included F, NH<sub>4</sub>, PO<sub>4</sub> and TDS. Aquatic Toxicity Index scores from the Olifants, Letaba and Luvuvhu Rivers were similar compared to similar sites sampled in 2015, which were also affected by PO<sub>4</sub>, NH<sub>4</sub>, and TDS (Gerber *et al.*, 2015b), as well as Cr, Cu and Mn that affected sites sampled from 1990 to 1992 (Wepener *et al.*, 1999). According to Gerber *et al.* (2015b) the high TDS values could be attributed to land erosion, overgrazing, removal of riparian vegetation and ploughing, with the high nutrients being attributed to fertilisation during agricultural activities, as well as effluent from wastewater treatment plants.

According to Van Niekerk *et al.* (2014), Maucha diagrams summarise the ratios of the major ions present in a water sample. All the samples from both the catchments had high concentrations of anions compared to the cations that were assessed. Most of the sites indicated two or more ions that influence the chemistry of the water. The anion that was the biggest influence at most of the sites was total alkalinity with elevated Na and Mg concentrations. Vlok *et al.* (2013) stated that the geology of the KNP tends to weather easily and is rich in cations such as Na, Ca, Mg and K, thus the higher levels of Na and Mg in some of the sites could be attributed to the weathering of the rocks at these sites. The elevated trace element concentrations vary between these two catchments. The Limpopo River catchment has higher concentrations of Cr, Ni and Zn, whereas the Incomati River catchment has higher concentrations of As, Hg and Cd. Trace elements such as As, Hg, Cd and Cr could have health effects on the individuals who make use of these systems for their livelihoods. As mentioned, these elements are indicators of industrial activities. Anthropogenic sources of Hg along the Limpopo River catchment include cement as well as coal-fired power stations, whereas artisanal gold mining along the tributaries of the Incomati River contributes to the elevated Hg concentrations (Walters *et al.*, 2011). Many of the trace elements (As, Cu, Hg and Pb) that were elevated in the Incomati River could be attributed to tailings from gold mining (Mudyazhezha & Kanhukamwe, 2014).

The Phongolo River Floodplain and the Usuthu River are used in comparison to the Limpopo River catchment since these rivers are all influenced by similar stressors (agricultural activities, industrial

activities, as well as mining activities). The concentrations of As, Cr, Ni, and Zn in the water from the Limpopo River catchment were similar to or lower concentrations than water sampled from the Usuthu and Phongolo Rivers (Van Rooyen *et al.*, 2022). Although these element concentrations were lower in the Limpopo River catchment, the concentrations were still elevated. The element concentrations in the Incomati River catchment were compared to that of the Olifants River since both of these river systems are impacted by agricultural activities. When compared to element concentrations found in the water from the Olifants River which is not only impacted by agricultural activities but also many industrial activities (Gerber *et al.*, 2015a), concentrations of Cd, Cr, Ni, Pb, Cu and Zn detected in the Incomati River were similar to or lower. This could be attributed to the water quality of the Olifants River that is regarded as being mostly influenced by anthropogenic activities, whereas the Incomati River can be regarded as being less impacted by anthropogenic activity although some element concentrations were similar to that of the Olifants, thus also being polluted. Although the Olifants River is regarded as one of the most polluted rivers in South Africa, the water quality results from this study indicated that it is still able to sustain healthy aquatic life with an ATI score above 60. The majority of the sites were affected by  $\text{NH}_4$ ,  $\text{PO}_4$  and TDS with little to no influence from trace element concentrations on the ATI scores, similar to the findings by Gerber *et al.* (2015b) at sites in the KNP.

#### **2.4.2 Sediment Quality**

Sites on the Limpopo River catchment had a SeQI score between 43 and 100. Variables that affected the SeQI score of the sites on the Limpopo River catchment were As, Cr, Cu and Ni. This is in contrast with the ATI score which had little to no influence from trace element concentrations. This could indicate that trace elements precipitate from the water column and settle in the sediment where it can accumulate over time which could point to long-term problems in the catchments. The SeQI scores for sites on the Incomati River catchment are between 70 and 100. All the sites on the Incomati River catchment indicated an acceptable sediment quality ( $\text{SeQI} > 60$ ) whereas some of the sites on the Limpopo River catchment had a SeQI score below 60. The lower scores at most of the sites in the Limpopo River catchment are due to trace elements such as As, Cr, Cu, Ni, and Zn, whereas sites on the Incomati River catchment are only due to As, Cr and Ni. Lake Nyamithi and Shokwe River had similar SeQi scores and were also due to Cr, Cu and Ni (Van Rooyen *et al.*, 2022). According to Gerber *et al.* (2015a), these trace elements are all associated with combustion activities (smelters, urban areas, vehicular exhaust, burning of fossil fuels, electroplating and a by-product of copper mining) and agriculture (phosphate fertilisers and pesticides). All of these activities are in close proximity to the sites with the lower SeQI scores. These element concentrations also indicate an increase from previous studies done on the Olifants, Letaba and Luvuvhu Rivers (Gerber *et al.*, 2015a).

From the results of the present study, it seems like the major stressors in both systems are not the trace elements compared to other studies done on similar rivers in southern Africa which are affected by artisanal gold mining (Monjane-Mabuie *et al.*, 2022) activities as well as platinum mining (Erasmus *et al.*, 2020). When comparing the two catchments, it is evident that the Limpopo River catchment had higher trace element concentrations in both the water and sediment compared to the Incomati, with the exception of As and Cd in the water, as well as Hg in the sediment this could be attributed to the different geology of the two catchments.. The main stressor affecting these systems seems to be nutrient overload ( $\text{NH}_4$ ,  $\text{PO}_4$ ), which could be attributed to fertilisation of agricultural activities, as well as from effluent from wastewater treatment plants and other ions like F in the Incomati River catchment which could be attributed to the geology. According to Lukhele and Msagati (2024), approximately 70% of major river systems in South Africa are eutrophic to hypereutrophic. Wastewater treatment plant effluent generally has high concentrations of nutrients such as nitrogen and phosphorus and is one of the main contributors to eutrophication in South Africa (Phungela *et al.*, 2021; Lukhele & Msagati, 2024).

## **2.5 Conclusion:**

The research done on trace element concentrations in the water and sediment of the Incomati River catchment is the first of its kind and could be a guideline for future studies. The present study compared different water quality variables, as well as trace element concentrations of the Limpopo River catchment to that of the Incomati River catchment. Both of these catchments have similar general water quality and different trace elements affect the water quality of each catchment. The sites in the Limpopo River catchment had lower scores but all the sites were still above 60 with the lowest score being 61.26 at the Matlabas site. The ATI scores of the sites in the Incomati River catchment ranged from 67.31 to 75.52, which is an indication that the water quality of the catchment is suitable for sustaining aquatic biodiversity but is not in pristine conditions. Both catchments are influenced by more than one anion or cation as indicated by the Maucha diagrams. The similarity of the water quality is also supported by these diagrams indicating that most of the sites were influenced by cations. Trace element concentrations were also determined for both of these systems. Chromium, Ni and Zn concentrations were high in the Limpopo River catchment and could have adverse health effects if consumed. Higher concentrations of As and Cd in the water samples of the Incomati River could pose a health risk to anyone who makes use of the water from the river.

## Chapter 3: Element bioaccumulation in *Oreochromis mossambicus* from the Limpopo River and Incomati River catchments

### 3.1 Introduction

Trace elements are naturally occurring and can be introduced to the environment through different anthropogenic activities. As stated by Munyeshury *et al.* (2021), some of the anthropogenic sources include mining, smelting, electroplating, use of pesticides, phosphate-based fertilisers, and biosolids in agriculture, sludge dumping, coal combustion residues and industrial discharges. Aquatic organisms are susceptible to changes in their environment and can be used to determine the condition of their environment, as well as the ecosystem. According to Plessl *et al.* (2017), fish have been recognised as bioindicators of environmental contamination, providing an integrated insight into the status of their environment over longer periods of time. Trace elements bioaccumulate in the tissue of aquatic organisms, such as fish, through food, water, as well as sediments (Lebepe *et al.*, 2020).

The Mozambique tilapia (*Oreochromis mossambicus*) is abundantly distributed throughout both the Limpopo River catchment and Incomati River catchment and its feeding depends on the season and life stage of the species, but they generally feed on debris in reed beds, as well as algae (Mannzhi *et al.*, 2021). *Oreochromis mossambicus* is often used in environmental monitoring since they can be found in abundance throughout southern Africa and are tolerable in different habitats (Monjane-Mabuie *et al.*, 2022). Erasmus *et al.* (2022), determined that some elements do not biomagnify, but rather bioaccumulate in lower functional feeding groups thus meaning that organisms at a lower trophic level may accumulate more trace elements than organisms at a higher trophic level. This therefore makes the Mozambique tilapia an excellent indicator organism of element exposure in the Limpopo River and the Incomati River catchments.

Monitoring the trace element concentrations in the environment is crucial to determine if there is any contamination from anthropogenic sources. The concentrations in the water and sediment of a system are only a momentary snapshot of the pollution status of a system and do not take into account the variation in bioavailability due to fluctuating abiotic and biotic factors (Byns *et al.*, 2024). Some trace elements are essential for normal metabolic processes, but only at optimal concentrations (Vasanthi *et al.*, 2019). Increased concentrations of these essential trace elements, as well as non-essential trace elements, could have detrimental effects on the aquatic environment and the organisms that inhabit these systems (Alam *et al.*, 2023). According to Ahmed *et al.* (2020), the accumulation of these trace elements could lead to the development of abnormalities, an increase in fish mortality and also the extinction of the population near the contaminated area. Some trace elements such as Arsenic (As) and Mercury (Hg) are able to biomagnify from one trophic level to the next (Ghosh *et al.*, 2022).

There is a need to determine the effects of multiple stressors on a freshwater ecosystem. The monitoring of bioaccumulation of trace elements in fish in South Africa has predominantly been used to assess pollutant exposure, but that does not provide an indication of the effects that these pollutants have on the indicator organism. Contamination in aquatic ecosystems predominantly relies on measurements taken from the abiotic environment such as water and sediment, although this only provides a momentary snapshot of the pollution and does not account for the variation of bioavailability which is affected by fluctuating abiotic and biotic factors (Byns *et al.*, 2024). According to Bervoets *et al.* (2015), accumulated micro-pollutant levels can potentially be used to predict the ecological effects of micro-contamination on aquatic communities and to estimate safe pollutant levels. Bervoets *et al.* (2015), related trace element concentrations in macroinvertebrates to different indices to determine threshold values of trace elements in the tissues of these macroinvertebrates as a way to predict the effects of elements in aquatic ecosystems. Different indices were developed to determine the ecological state of a river system, with some focusing on the abiotic aspects of a river system and some on the biotic aspects. Relating ecosystem health indices to accumulated concentrations in indicator species could help better understand the effects on the aquatic ecosystem by taking into account both the biotic and abiotic aspects of an aquatic ecosystem. As stated by Evans *et al.* (2022), fish biological indices usually rely on an understanding of characteristics of the biology and ecology of species and their tolerance for environmental variables. The fish response assessment index (FRAI) is a South African biotic index that is used to evaluate a river system based on the fish community composition, the sensitivity of species to water quality, flow, habitat characteristics and potential competition (Evans *et al.*, 2022).

Therefore, this study aims to determine and compare the element bioaccumulation patterns in *O. mossambicus* from the Limpopo and Incomati River catchments. Secondly to study the relationship between bioaccumulation of element concentrations (in *O. mossambicus*) and water and sediment concentrations. Thirdly, the relationship between element bioaccumulation in fish and the ecological status indices, i.e. the ATI and SeQI (Chapter 2) and fish community responses in the form of FRAI values at the relevant sites, were determined. Based on the elevated trace element concentrations in the water and sediment (Chapter 2) of the Limpopo River it is predicted that the increased anthropogenic influence on the Limpopo River catchment would result in deteriorated ecological conditions with higher trace elements accumulated in the muscle tissue of *O. mossambicus* from the Limpopo River catchment.

### **3.2 Material and Methods:**

#### **3.2.1 Field sampling:**

*Oreochromis mossambicus* individuals (minimum of three (3) per site) were caught at six (6) of the sites on the Limpopo River from May – July 2021 (**Figure 3.1**) as well as from six (6) sites on the

Incomati River catchments from May – July 2022 (**Figure 3.2**). The Limpopo River catchment includes one site on each of the following tributaries: Crocodile River (CROC), Matlabas River (MAT), Lephalala River (LEPH), Shingwidzi River (SHIN), Letaba (LET), as well as on the mainstem Limpopo River (LR1). The Incomati River catchment sites are on the following tributaries: Sabie River (SR KNP, LSR, and SR Moz), and three sites on the mainstem Incomati River (IR1, IR2 and IR3). Different sampling techniques were used to catch *O. mossambicus* individuals for example, cast net, electrofishing and seine net after which specimens were weighed, measured, photographed and identified using the key by Skelton (2001) and were subsequently euthanized by severing the spinal cord. Muscle tissue was extracted from each individual and stored in pre-cleaned polypropylene tubes and frozen until further analysis could be done in the laboratory (Wepener *et al.*, 2011).

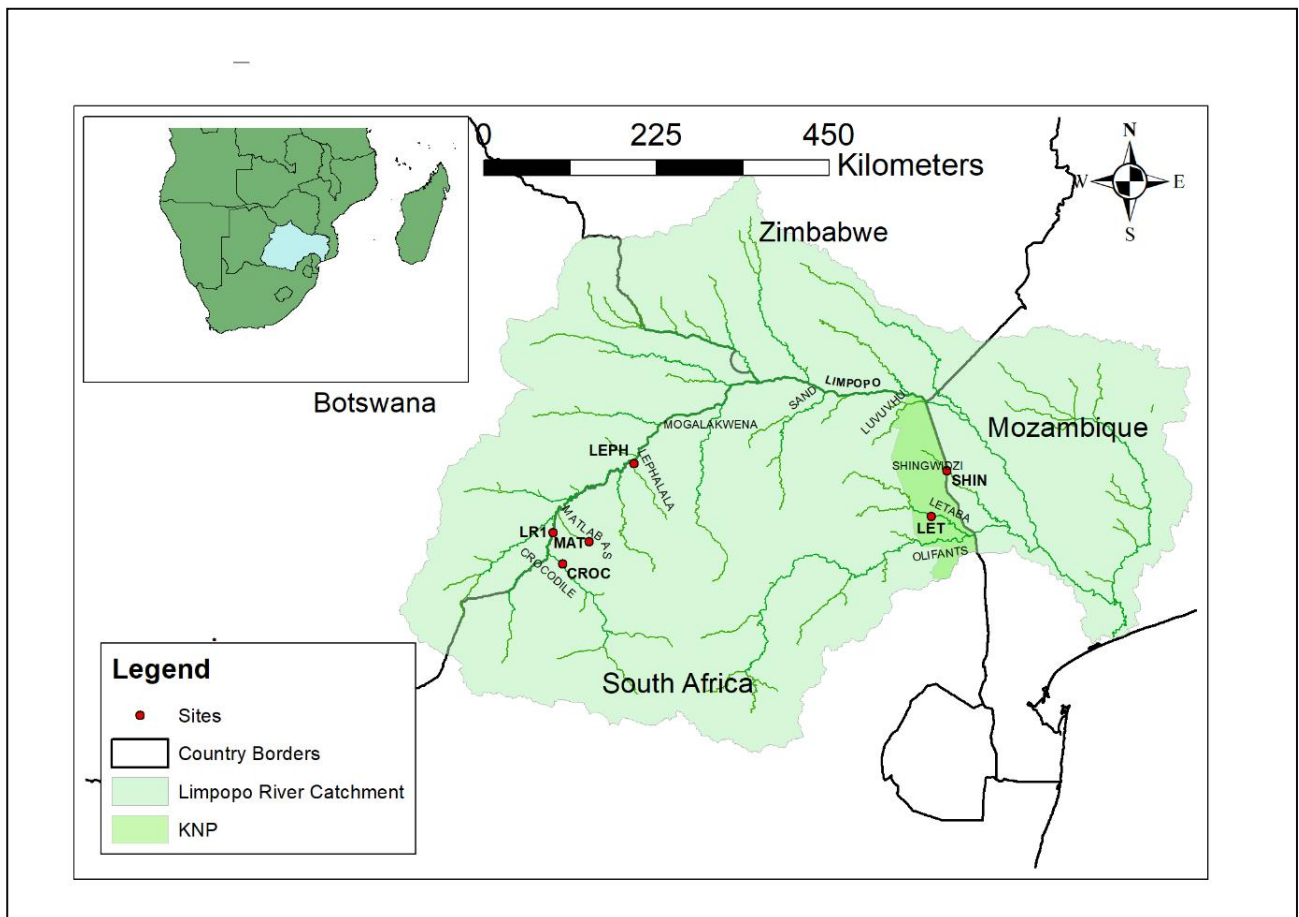


**Figure 3.1:** Sites where *Oreochromis mossambicus* specimens were sampled on the Limpopo River catchment.

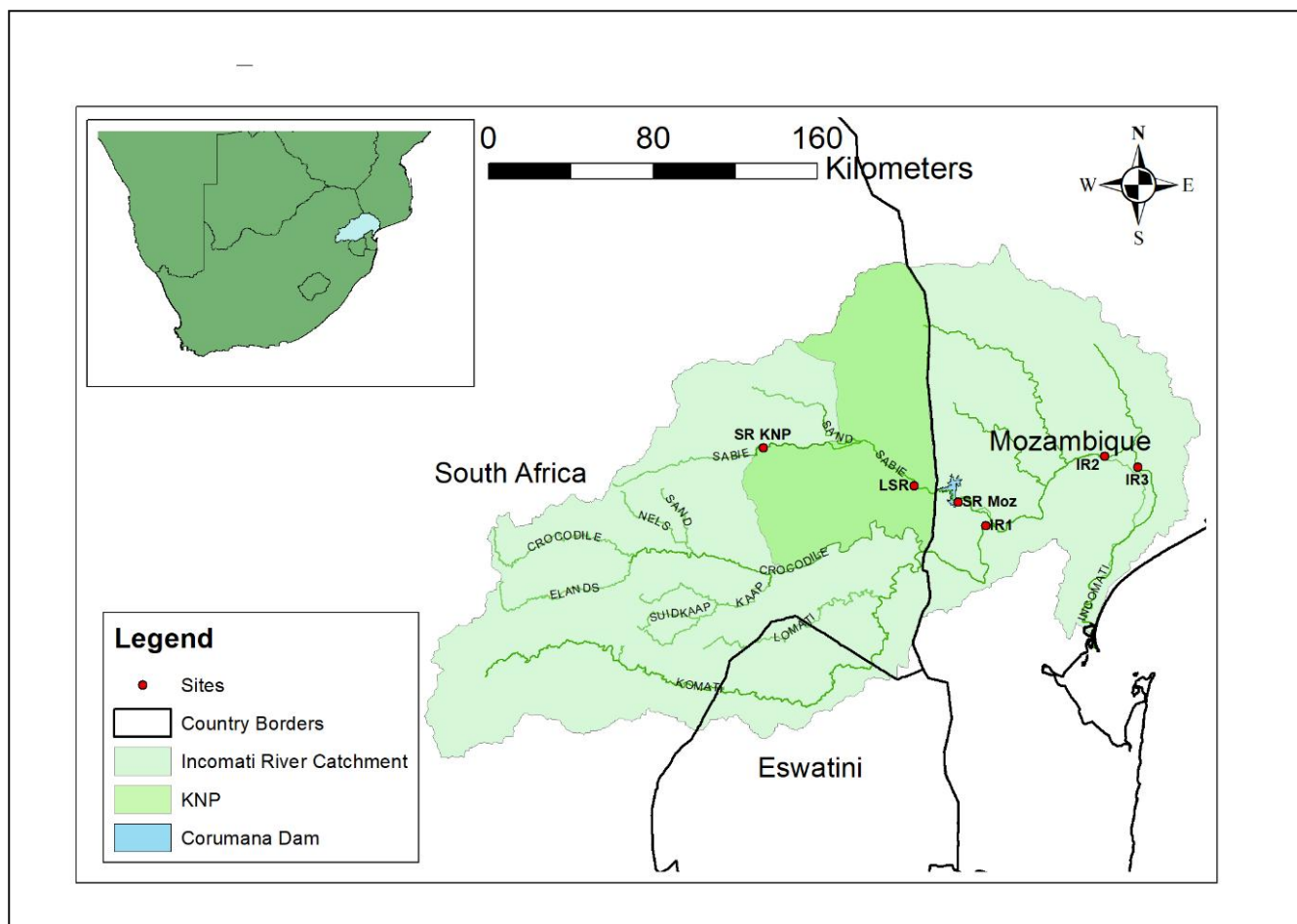


**Figure 3.2:** Sites where *Oreochromis mossambicus* specimens were sampled on the Incomati River catchment.

### 3.2.2 Laboratory Analysis:

The muscle tissue of *O. mossambicus* individuals was prepared for trace element analysis by freeze drying (FreeZone® 6, Labconco) the samples after which approximately 0.2 g (dry weight) of each sample was digested in a mixture of 7.5 mL HNO<sub>3</sub> (65%, supra pure quality, Merck) and 2.5 mL HCl (37%, supra pure quality, Merck) (Erasmus *et al.*, 2020). Digestions were done using an Ethos Easy advanced microwave digestion system (MAXI-44, Milestone), with 100 mL TFM® vessels (Milestone). After the samples had been digested the solution was decanted into 50 mL volumetric flasks diluted with MilliQ® water and stored at room temperature (Erasmus *et al.*, 2020). Samples were then analysed for trace elements (As, Cd, Cu, Ni, Pb and Zn) using a quadrupole inductively coupled plasma mass spectroscopy (ICP-MS) (Elan 6000, PerkinElmer) equipped with an autosampler system (AS-90, PerkinElmer), while concentrations of Cr were determined on an Atomic Absorption Spectrometer (AAS) (PinAAcle 900T, PerkinElmer) equipped with Zeeman-effect background correction. A cold vapour mercury atomic absorption flow injection mercury system (FIMS 400, PerkinElmer) was used to determine the Hg concentrations.

Quality assurance of the element analyses in fish was performed using reference material for biota (ERM-CE 278k; mussel tissue, Institute for Reference Materials and Measurements, European Commission) for all elements of interest. Recovery rates for all elements were recorded within a 20% deviation of the certified concentrations (**Table 3.1**).

**Table 3.1:** Limit of detection (LOD), limit of quantification (LOQ) reported in mg/kg dry weight (DW) in fish tissue samples, as well as recovery rates (%) of the elements of interest from the certified reference materials.

| Element   | Fish tissue |        | % recovery    |
|-----------|-------------|--------|---------------|
|           | LOD         | LOQ    | ERM – CE 278k |
| <b>As</b> | 0.022       | 0.067  | 87            |
| <b>Cd</b> | 0.0022      | 0.0065 | 104           |
| <b>Cr</b> | 0.068       | 0.203  | 95            |
| <b>Cu</b> | 0.106       | 0.318  | 116           |
| <b>Hg</b> | 0.008       | 0.025  | 114           |
| <b>Ni</b> | 0.067       | 0.202  | 118           |
| <b>Pb</b> | 0.079       | 0.238  | 102           |
| <b>Zn</b> | 0.013       | 0.038  | 85            |

The Aquatic Toxicity Index (ATI) and Sediment Quality Index (SeQI) were calculated for each of the sites in the previous chapter, with the Fish Response Assessment index (FRAI) for the Limpopo River were retrieved from the E-flows for the Limpopo River Basin: Report 5 (O'Brien *et al.*, 2022), and the FRAI for the Incomati were retrieved from the Water Research Commission (WRC) report no. TT 938/24 (O'Brien *et al.*, 2024).

### 3.2.3 Statistical Analysis

Statistical significance was determined at  $p < 0.05$  for the variation between element concentrations in muscle tissue within *O. mossambicus* individuals at different sites as well as between catchments. Following the tests for normality and homogeneity, one-way ANOVA Tukey's multiple comparison test, and Sidak multiple comparison tests were performed using GraphPad Prism v10. Redundancy Analysis (RDA) was constructed to assess the bioaccumulation patterns in the muscle tissues of *O. mossambicus* with water and sediment concentrations overlaid as supplementary variables. All the data used in the RDAs were log-transformed:  $\log(X+1)$ . Bioconcentration factors ( $BCF = C_{O.mossambicus}/C_{water}$ ) and biota-sediment accumulation factors ( $BSAF = C_{O.mossambicus}/C_{Sediment}$ ) were calculated as the ratio of the average trace element concentration in tilapia muscle tissue, per quantified element concentration of the filtered (0.45  $\mu m$ ) water and sediment, respectively. This was

done to evaluate the trace element transfer from water and sediment to the tilapia tissue. A quantile regression analysis was applied to determine the relationship between indices and trace element concentrations in the muscle tissue of *O. mossambicus* and Spearman rank correlation analysis was performed among the indices and the accumulated element concentrations.

### 3.3 Results

#### 3.3.1 Trace element bioaccumulation in the muscle tissue of *Oreochromis mossambicus*

Element bioaccumulation in the muscle tissue of *O. mossambicus* was determined between different sites in the two catchments. Concentrations of trace elements in the muscle tissue of *O. mossambicus* from the Limpopo River catchment ranged between 0.3 – 4.43  $\mu\text{g/g}$  for As, 0.02 – 0.81  $\mu\text{g/g}$  for Cd, 0.73 – 8.41  $\mu\text{g/g}$  for Cr, 1.33 – 69.04  $\mu\text{g/g}$  for Cu, 0.09 – 0.31  $\mu\text{g/g}$  for Hg, 0.41 – 2.85  $\mu\text{g/g}$  for Ni, 0.47 – 1.96  $\mu\text{g/g}$  for Pb, 23.28 – 66.13  $\mu\text{g/g}$  for Zn (**Table 3.2; Figure 3.3**). Site **LR1** had the highest concentrations of As, Ni, Pb and Zn, whereas **LEPH** had the highest concentrations of Cd and Cu, and site **MAT** had the highest concentrations of Hg. The highest concentrations of Cr were evident at **LET**. There were no significant differences between any of the sites for As, Ni, Pb or Zn. Chromium concentrations at **MAT** differed significantly from **LET** ( $p = 0.0441$ ). Site **LEPH** indicated significant differences between all the sites for Cd and Cu. The Hg concentrations at **LR1** differed significantly from that of **LEPH** ( $p = 0.0037$ ), **MAT** ( $p < 0.0001$ ) and **LET** ( $p = 0.0417$ ), whereas the Hg concentrations between **SHIN** and **MAT** ( $p = 0.0454$ ), and between **CROC** and **MAT** ( $p = 0.001$ ) also indicated significant differences.

**Table 3.2:** Trace element concentrations  $\pm$  standard deviation ( $\mu\text{g/g}$ ) of *Oreochromis mossambicus* muscle tissue collected from the Limpopo River catchment. Within rows concentrations with similar superscripts indicate significant differences between sites ( $p < 0.05$ ).

| Element   | CROC              | LR1               | MAT               | LEPH               | SHIN             | LET               |
|-----------|-------------------|-------------------|-------------------|--------------------|------------------|-------------------|
| <b>As</b> | 0.7 $\pm$ 0.33    | 4.43 $\pm$ 9.21   | 0.8 $\pm$ 0.8     | 0.34 $\pm$ 0.07    | 0.3 $\pm$ 0.06   | 0.32 $\pm$ 0.21   |
| <b>Cd</b> | 0.08 $\pm$ 0.19*  | 0.06 $\pm$ 0.06*  | 0.06 $\pm$ 0.06*  | 0.81 $\pm$ 0.53*   | 0.04 $\pm$ 0.02* | 0.02 $\pm$ 0.01*  |
| <b>Cr</b> | 1.8 $\pm$ 1.32    | 1.38 $\pm$ 1.11   | 0.73 $\pm$ 0.6*   | 1.41 $\pm$ 2.13    | 0.8 $\pm$ 0.61   | 8.41 $\pm$ 15.25* |
| <b>Cu</b> | <LOD*             | 7.02 $\pm$ 14.3*  | 2.63 $\pm$ 0.83*  | 69.04 $\pm$ 54.85* | 1.86 $\pm$ 0.91* | 1.33 $\pm$ 0.81*  |
| <b>Hg</b> | 0.15 $\pm$ 0.07\$ | 0.09 $\pm$ 0.04*  | 0.31 $\pm$ 0.07*# | 0.25 $\pm$ 0.12*   | 0.19 $\pm$ 0.08# | 0.25 $\pm$ 0.05*  |
| <b>Ni</b> | 0.58 $\pm$ 0.12   | 2.85 $\pm$ 6.01   | 0.74 $\pm$ 0.84   | 2.01 $\pm$ 0.84    | 0.63 $\pm$ 0.31  | 0.41 $\pm$ 0.14   |
| <b>Pb</b> | 0.47 $\pm$ 0.37   | 1.96 $\pm$ 2.31   | 1.22 $\pm$ 1.1    | 0.62 $\pm$ 0.41    | 1.57 $\pm$ 1.02  | 0.73 $\pm$ 0.43   |
| <b>Zn</b> | 23.28 $\pm$ 3.32  | 66.13 $\pm$ 121.9 | 53 $\pm$ 16       | 54 $\pm$ 18.9      | 31.48 $\pm$ 8.65 | 36.05 $\pm$ 8.9   |

<LOD – Below the limit of detection

Concentrations of trace elements in the muscle tissue of *O. mossambicus* from the Incomati River catchment ranged between 0.29 – 3.64 µg/g for As, 0.02 – 0.04 µg/g for Cd, 0.49 – 11.77 µg/g for Cr, 2.04 – 4.94 µg/g for Cu, 0.13 – 0.57 µg/g for Hg, 0.27 – 1.83 µg/g for Ni, 0.51 – 1.53 µg/g for Pb and 19.51 – 36.33 µg/g for Zn (**Table 3.3; Figure 3.3**). The site **SR KNP** had the highest concentrations for As, Cu and Hg, whereas **LSR** had the highest concentrations for Cd and Zn. Site **SR Moz** had the highest concentrations of Pb. The highest concentrations of Ni were evident at **IR2** and the highest concentrations of Cr was evident at **IR1**. There were no significant differences between any of the sites for Cr and Ni. The As concentrations at **IR1**, **IR2** and **IR3** differed from **SR Moz** ( $p < 0.0001$ ), **LSR** ( $p < 0.0001$ ) and **SR KNP** ( $p < 0.0001$ ). Cadmium concentrations at **LSR** differed significantly from **IR1** ( $p = 0.0076$ ) and **IR2** ( $p = 0.0005$ ). Copper concentrations differed significantly between most of the sites except between **SR Moz** and **IR1**, **IR3** and **IR2**, **LSR** and **IR2**, as well as **LSR** and **IR3**. The Hg concentrations at **LSR** differed significantly from the Hg concentrations at **IR1** ( $p = 0.0028$ ), **IR2** ( $p = 0.0242$ ), and **IR3** ( $p = 0.0314$ ). Lead concentrations differed significantly between **SR Moz** and **IR2** ( $p = 0.031$ ). The Zn concentrations at **IR1** indicated a significant difference from the Zn concentrations at **SR Moz** ( $p = 0.0016$ ), **IR2** ( $p = 0.0126$ ) and **LSR** ( $p < 0.0001$ ), as well as between **IR3** and **LSR** ( $p = 0.0174$ ).

**Table 3.3:** Trace element concentrations  $\pm$  standard deviation (µg/g) of *Oreochromis mossambicus* muscle tissue collected from the Incomati River catchment. Within rows concentrations with similar superscripts indicate significant differences between sites ( $p < 0.05$ ).

| Element   | SR KNP                             | LSR                             | SR Moz                        | IR1                              | IR2                              | IR3                              |
|-----------|------------------------------------|---------------------------------|-------------------------------|----------------------------------|----------------------------------|----------------------------------|
| <b>As</b> | 3.64 $\pm$ 0.06 <sup>\$</sup>      | 2.29 $\pm$ 1.13 <sup>#</sup>    | 2.14 $\pm$ 1.09 <sup>*</sup>  | 0.29 $\pm$ 0.1 <sup>*##\$</sup>  | 0.67 $\pm$ 0.26 <sup>*##\$</sup> | 0.43 $\pm$ 0.12 <sup>*##\$</sup> |
| <b>Cd</b> | 0.02 $\pm$ 0.01                    | 0.04 $\pm$ 0.02 <sup>*</sup>    | 0.03 $\pm$ 0.01               | 0.02 $\pm$ 0.01 <sup>*</sup>     | 0.02 $\pm$ 0.007 <sup>*</sup>    | 0.03 $\pm$ 0.02                  |
| <b>Cr</b> | 0.49 $\pm$ 0.01                    | 0.76 $\pm$ 0.48                 | 0.98 $\pm$ 0.94               | 11.77 $\pm$ 25.75                | 1.38 $\pm$ 1.39                  | 0.58 $\pm$ 0.25                  |
| <b>Cu</b> | 4.94 $\pm$ 0.83 <sup>*##\$@+</sup> | <LOD <sup>*\$@</sup>            | 2.56 $\pm$ 1.52 <sup>*</sup>  | 2.04 $\pm$ 1.42 <sup>*##\$</sup> | <LOD <sup>#</sup>                | <LOD <sup>*\$+</sup>             |
| <b>Hg</b> | 0.57 $\pm$ 0.39                    | 0.52 $\pm$ 0.21 <sup>*</sup>    | 0.32 $\pm$ 0.45               | 0.13 $\pm$ 0.04 <sup>*</sup>     | 0.24 $\pm$ 0.08 <sup>*</sup>     | 0.18 $\pm$ 0.09 <sup>*</sup>     |
| <b>Ni</b> | 0.81 $\pm$ 0.003                   | 0.36 $\pm$ 0.15                 | 0.84 $\pm$ 1.77               | 0.27 $\pm$ 0.09                  | 1.83 $\pm$ 6.29                  | 0.3 $\pm$ 0.1                    |
| <b>Pb</b> | 0.58 $\pm$ 0.03                    | 0.98 $\pm$ 1.16                 | 1.53 $\pm$ 1.18 <sup>*</sup>  | 0.9 $\pm$ 0.35                   | 0.61 $\pm$ 0.51 <sup>*</sup>     | 1.1 $\pm$ 0.92                   |
| <b>Zn</b> | 29.82 $\pm$ 2.46                   | 36.33 $\pm$ 8.72 <sup>*##</sup> | 32.75 $\pm$ 6.05 <sup>*</sup> | 19.51 $\pm$ 9.13 <sup>*</sup>    | 29.98 $\pm$ 10.05 <sup>*</sup>   | 23.67 $\pm$ 5.08 <sup>#</sup>    |

<LOD – Below the limit of detection

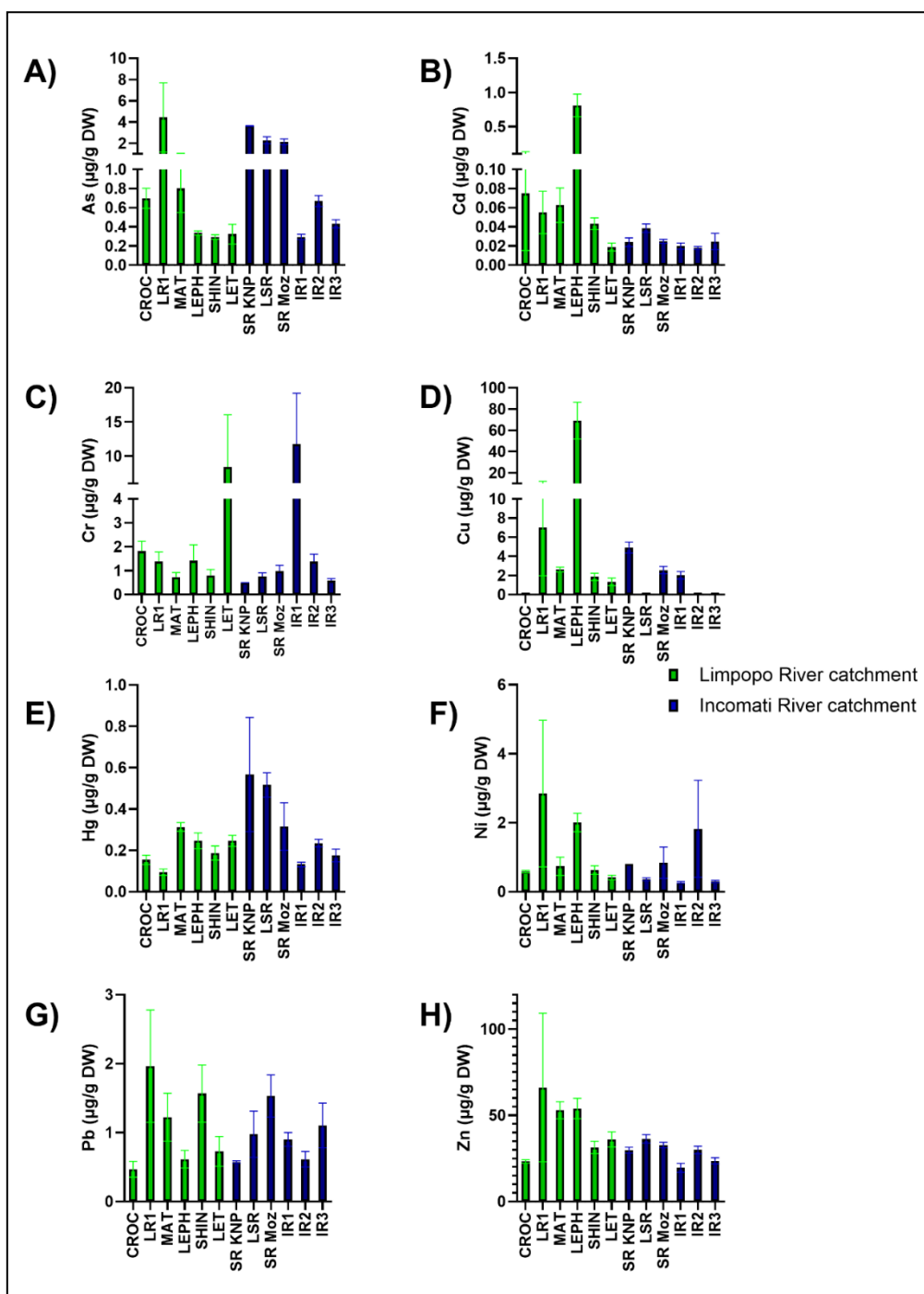


**Figure 3.3:** Mean concentrations (µg/g DW) of As (A), Cd (B), Cr (C), Cu (D), Hg (E), Ni (F), Pb (G) and Zn (H) with standard error of the mean in *Oreochromis mossambicus* muscle tissue sampled at different sites on the Limpopo River catchment and the Incomati River catchment.

There was clear spatial variance in the element concentrations in the muscle tissue of *O. mossambicus* sampled from the Limpopo River catchment (**Figure 3.4**). Sites such as **LET**, **SHIN** and **CROC** were characterised by Cd, Cr, Cu, Ni, and Zn concentrations in sediment, as well as As and Pb in water and Cr concentrations in fish muscle tissue. Site **LR1** was mostly characterised by element concentrations in the water, as well as As and Pb concentrations in fish muscle tissue. The

site **LEPH** was characterised by As and Pb concentrations in sediment, as well as Cd and Hg concentrations in fish muscle tissue. Site **MAT** was characterised by Hg concentrations in sediment, as well as Cu, Ni and Zn concentrations in fish muscle tissue.

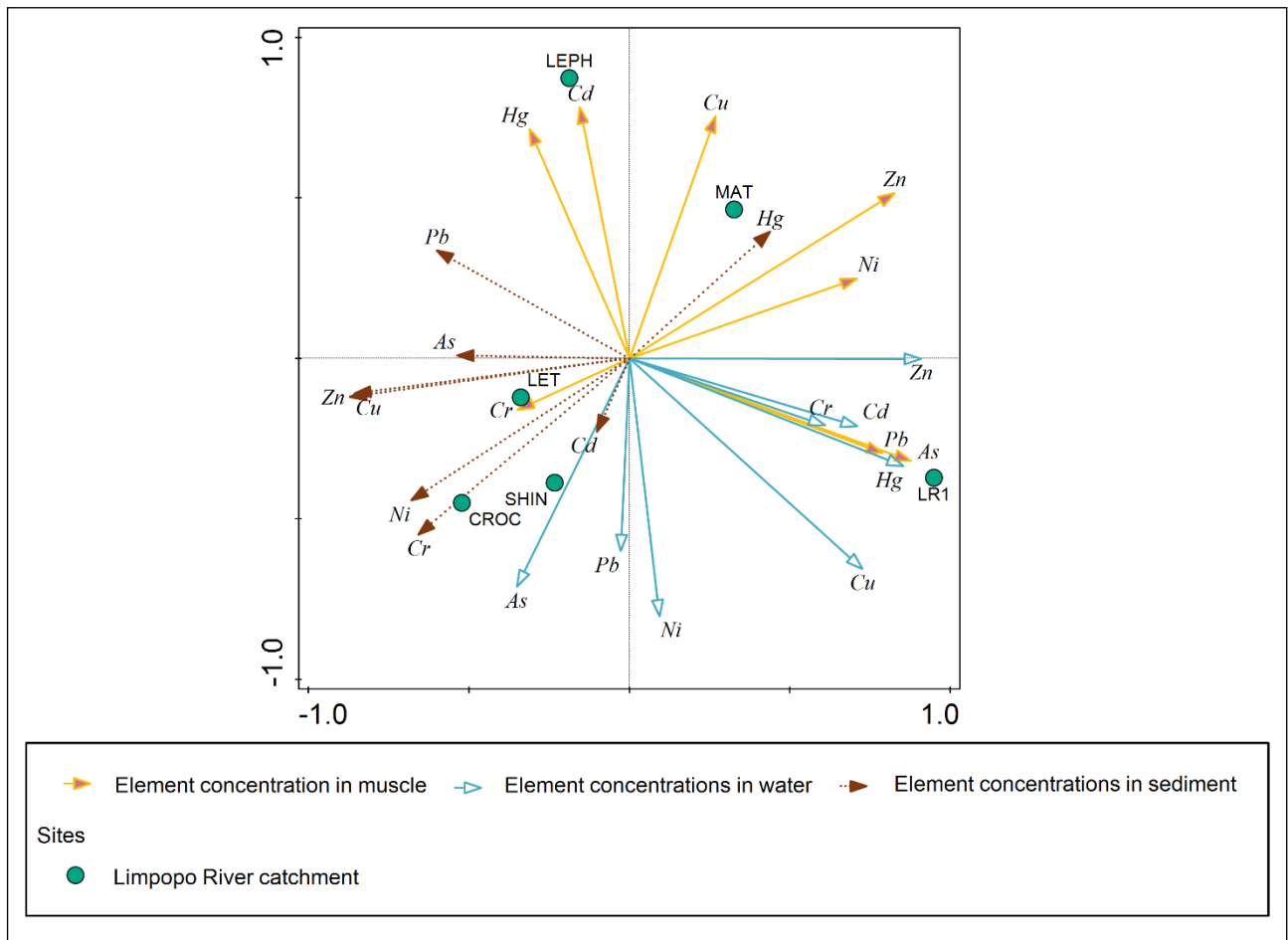


**Figure 3.4:** RDA triplot for the Limpopo River catchment indicates differences in element concentrations in the muscle tissue of *Oreochromis mossambicus*, as well as the water and sediment at sample sites in 2021. This biplot describes 60.22% of the variation in the data, where 38.47% is displayed on the first axis, while 21.75% is displayed on the second axis.

There was clear spatial variance in the element concentrations in the muscle tissue of *O. mossambicus* sampled from the Incomati River catchment (**Figure 3.5**). Sites such as **SR KNP** and **SR Moz** were characterised by As, Cu, Hg and Zn concentrations in fish muscle tissue, as well as Pb in sediment and Zn concentrations in water. Site **IR1** was mostly characterised by Cd, Cr, Hg and Ni concentrations in the water, as well as Cr concentrations in fish muscle tissue. Site **IR2** was characterised by Cr, Cu, Ni and Zn concentrations in sediment, as well as Cr concentrations in water and Ni concentrations in fish muscle tissue. The sites **LSR** and **IR3** were characterised by As, Cd and Hg concentrations in sediment, as well as Cd and Pb concentrations in water and Cd and Pb concentrations in fish muscle tissue.

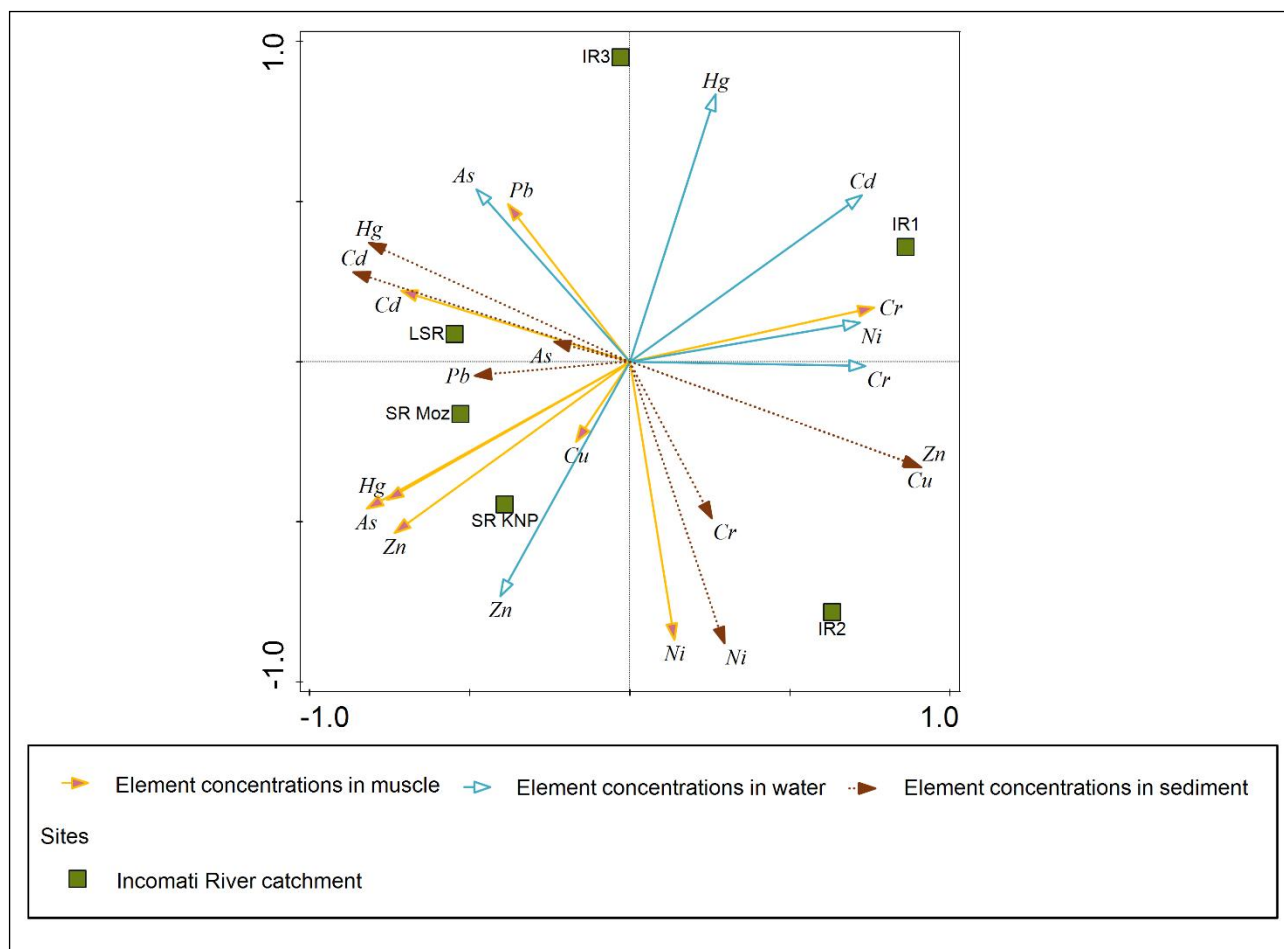


**Figure 3.5:** RDA triplot for the Incomati River catchment indicates differences in element concentrations in the muscle tissue of *Oreochromis mossambicus*, as well as the water and sediment at sample sites in 2022. This biplot describes 62.30% of the variation in the data, where 39.23% is displayed on the first axis, while 23.07% is displayed on the second axis.

The RDA triplot representing the element concentrations in the water, sediment and *O. mossambicus* muscle tissue comparison between the Limpopo River catchment and the Incomati River catchment (**Figure 3.6**) indicates a separation between the sites on the Limpopo River catchment and the sites on the Incomati River catchment. Along the first component (explaining 42.45% of the variation) trace element concentrations differentiate between the two systems. Along the second component (explaining 14.86% of the variation) the variables differentiate between sites within a system. Sites on the Sabie River (**SR KNP**, **LSR** and **SR Moz**) were characterised by As, Cd and Hg concentrations in sediment, as well as As and Hg concentrations in *O. mossambicus* muscle tissue, whereas sites on the Incomati River (**IR1** and **IR3**) were characterised by As, Cd and Hg concentrations in water. Site **IR2**, as well as **LET**, **CROC** and **SHIN** were characterised by Cr, Cu, Ni and Zn concentrations in sediment, as well as Pb concentrations in water and Cr concentrations in *O. mossambicus* muscle tissue. Sites such as **MAT**, **LEPH** and **LR1** were characterised by Pb concentrations in sediment, as

well as Cr, Cu, Ni and Zn concentrations in water and Cd, Cu, Ni and Zn concentrations in *O. mossambicus* muscle tissue.

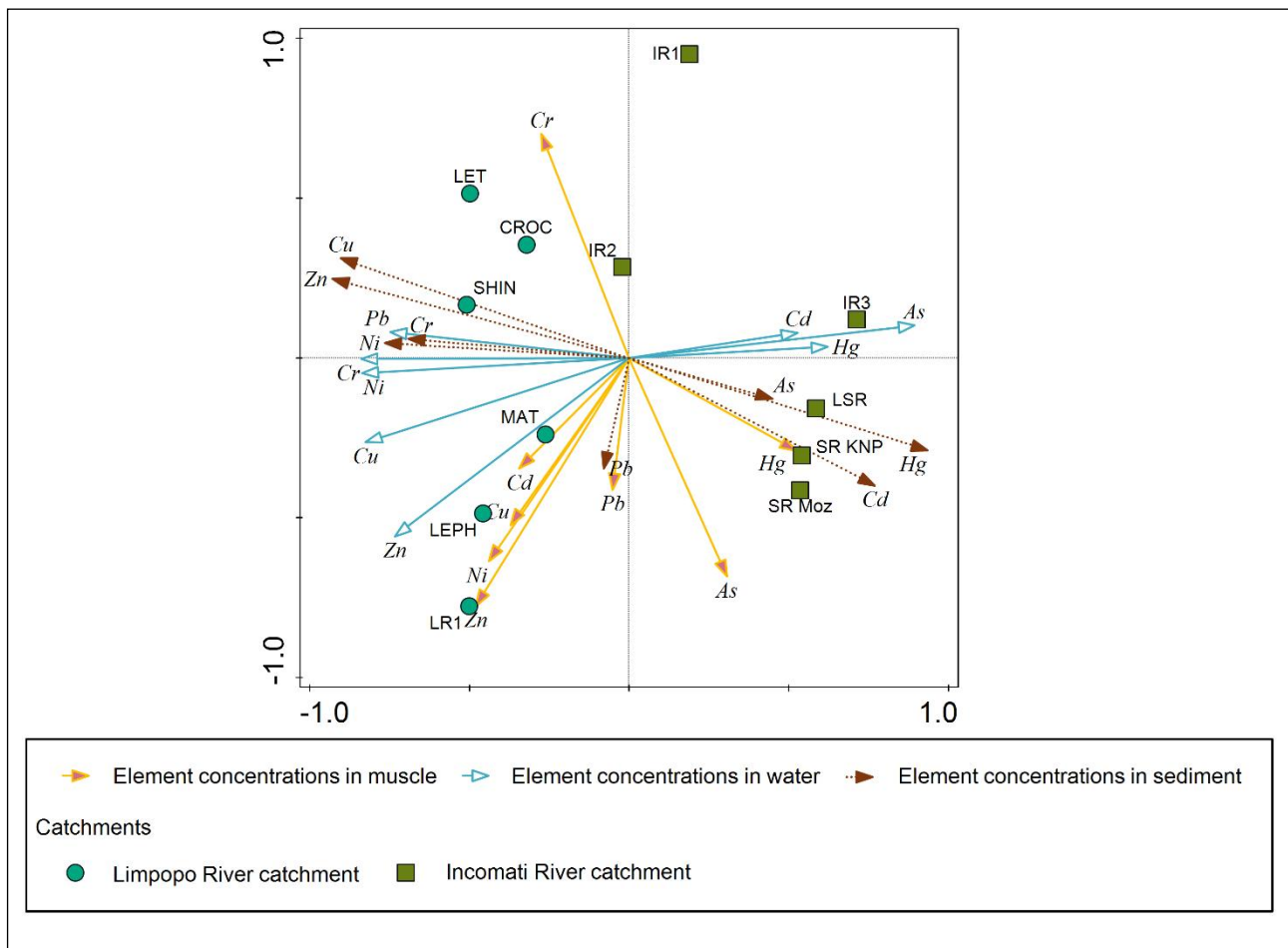


**Figure 3.6:** RDA triplot for the Limpopo River catchment and Incomati River catchment indicating differences in element concentrations in the muscle tissue of *Oreochromis mossambicus* at sample sites in 2021 and 2022. This biplot describes 57.31% of the variation in the data, where 42.45% is displayed on the first axis, while 14.86% is displayed on the second axis.

### 3.3.2 Bioconcentration factor and biota-sediment accumulation factor in fish

The  $BCF_{\text{Limpopo River catchment}}$  ranged from 0.14 (Ni at **CROC**) to 90.85 (Cu at **LEPH**) and the  $BCF_{\text{Incomati River catchment}}$  ranged from 0.06 (As at **IR3**) to 95.49 (Cr at **IR1**), while the  $BSAF_{\text{Limpopo River catchment}}$  ranged from 0.01 (Cr at **CROC**, **LR1** and **SHIN**, as well as Ni at **CROC**, **SHIN** and **LET**) to 16.65 (Hg at **MAT**) and the  $BSAF_{\text{Incomati River catchment}}$  ranged from 0.01 (Cr and Hg at **IR3**) to 45.61 (Ni at **IR2**) (**Tables 3.4 and 3.5**). The mean BCF for the Limpopo River catchment decreased in the order of Cu > Cd > Pb > Zn > Cr > As > Ni > Hg, while for the Incomati River catchment, it was Hg > Zn > Cr > Ni > Pb > Cd > As > Cu. The order of the mean BSAF for the Limpopo River catchment was Hg > Cd > Zn > Cu > As > Pb > Ni > Cr, while for the Incomati River catchment, it was Ni > Zn > Cd > Cu > As > Cr > Hg > Pb.

**Table 3.4:** Bioconcentration factors (BCF) are calculated as the ratio of the element concentration in *Oreochromis mossambicus* muscle tissue and element concentrations in water collected from the Limpopo River catchment and the Incomati River catchment.

|                          | Site   | As   | Cd    | Cr    | Cu    | Hg    | Ni   | Pb    | Zn    |
|--------------------------|--------|------|-------|-------|-------|-------|------|-------|-------|
| Limpopo River catchment  | CROC   | 0.42 | 3.75  | 9.07  | -     | -     | 0.14 | 7.82  | 5.65  |
|                          | LR1    | 2.84 | 5.51  | 9.22  | 4.56  | -     | 0.72 | 49.07 | 24.68 |
|                          | MAT    | 2.97 | 2.09  | 2.51  | 1.62  | -     | 0.44 | 15.29 | 6.03  |
|                          | LEPH   | 2.4  | 81.09 | 5.87  | 90.85 | -     | 1.73 | 0.53  | 10.09 |
|                          | SHIN   | 0.54 | 2.16  | 3.07  | 1.06  | -     | 0.22 | 0.54  | 5.96  |
|                          | LET    | 0.88 | 1.9   | 21.57 | 0.76  | -     | 0.32 | 0.56  | 18.11 |
| Incomati River catchment | SR KNP | 1.54 | 0.82  | 5.99  | -     | 55.61 | 26.3 | -     | 18.48 |
|                          | LSR    | 0.82 | 1.68  | 14    | -     | 41.14 | 4.59 | -     | 32.15 |
|                          | SR Moz | 0.32 | 1.02  | -     | -     | 51.97 | 2.22 | -     | 15.86 |
|                          | IR1    | 0.14 | 0.51  | 95.49 | -     | 7.74  | 0.56 | -     | 20.1  |
|                          | IR2    | 0.33 | 0.64  | 5.99  | -     | 55.2  | 4.19 | 25.33 | 24.02 |
|                          | IR3    | 0.06 | 0.71  | 3.42  | -     | 5.85  | 0.95 | -     | 44.53 |

**Table 3.5:** Biota-sediment accumulation factors (BSAF) are calculated as the ratio of the element concentrations in *Oreochromis mossambicus* muscle tissue and element concentrations in sediment collected from the Limpopo River catchment and the Incomati River catchment.

|                          | Site   | As   | Cd   | Cr   | Cu   | Hg    | Ni    | Pb    | Zn    |
|--------------------------|--------|------|------|------|------|-------|-------|-------|-------|
| Limpopo River catchment  | CROC   | 0.09 | 0.5  | 0.01 | -    | 10.96 | 0.01  | 0.02  | 0.39  |
|                          | LR1    | 0.44 | 0.43 | 0.01 | 0.3  | 6.56  | 0.04  | 0.09  | 1.5   |
|                          | MAT    | 0.21 | 0.67 | 0.02 | 0.57 | 16.65 | 0.07  | 0.11  | 1.47  |
|                          | LEPH   | 0.09 | 4.71 | 0.02 | 2.57 | 14.64 | 0.05  | 0.02  | 1.06  |
|                          | SHIN   | 0.11 | 0.3  | 0.01 | 0.03 | -     | 0.01  | 0.17  | 0.57  |
|                          | LET    | 0.12 | 0.12 | 0.07 | 0.05 | -     | 0.01  | 0.06  | 0.85  |
| Incomati River catchment | SR KNP | 0.2  | 1.12 | 0.03 | 0.97 | 0.13  | 34.91 | 0.02  | 4.5   |
|                          | LSR    | 0.78 | 2.4  | 0.18 | -    | 0.2   | 6.63  | 0.077 | 17.27 |
|                          | SR Moz | 0.15 | 0.46 | 0.02 | 0.08 | 0.02  | 37.57 | 0.04  | 3.83  |
|                          | IR1    | 0.24 | 1.25 | 0.85 | 0.65 | 0.05  | 10.1  | 0.08  | 3.33  |
|                          | IR2    | 0.09 | 0.42 | 0.02 | -    | 0.06  | 45.61 | 0.02  | 0.89  |
|                          | IR3    | 0.15 | 0.39 | 0.01 | -    | 0.01  | 11.6  | 0.03  | 0.63  |

Different indices were compared to trace element concentrations in the muscle tissue of *O. mossambicus* from the Limpopo River and Incomati River catchments (**Figures 3.7 – 3.9**). The relationship between trace element concentrations in *O. mossambicus* from the Limpopo and Incomati catchments was plotted against the aquatic toxicity index (ATI), sediment quality index (SEQI) and fish response assessment index (FRAI).

No significant negative relationship between the element concentrations in the fish muscle tissue from the Limpopo River or the Incomati River catchments was observed for the ATI, SEQI or FRAI. There was a significant correlation between Cr ( $p = 0.03333$ ) and the ATI scores for the Limpopo River catchment, whereas only Pb ( $p = 0.01667$ ) indicated a significant correlation with the FRAI. No significant correlations were evident for the element concentrations in the fish and the different indices for the Incomati River catchment.

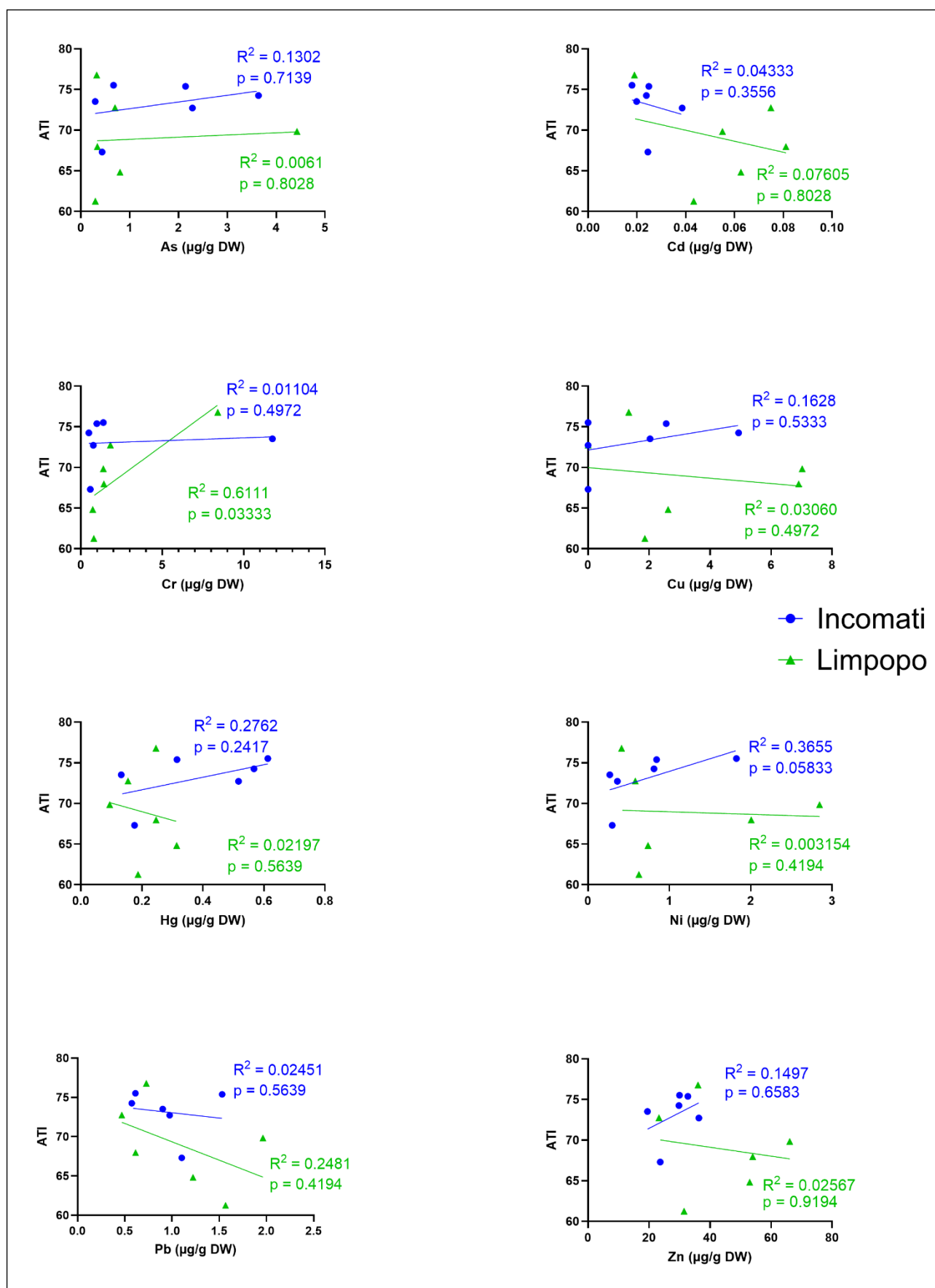


**Figure 3.7:** Correlations of the ATI as a function of the mean accumulated elements in *Oreochromis mossambicus* in the Limpopo River and Incomati River catchments.

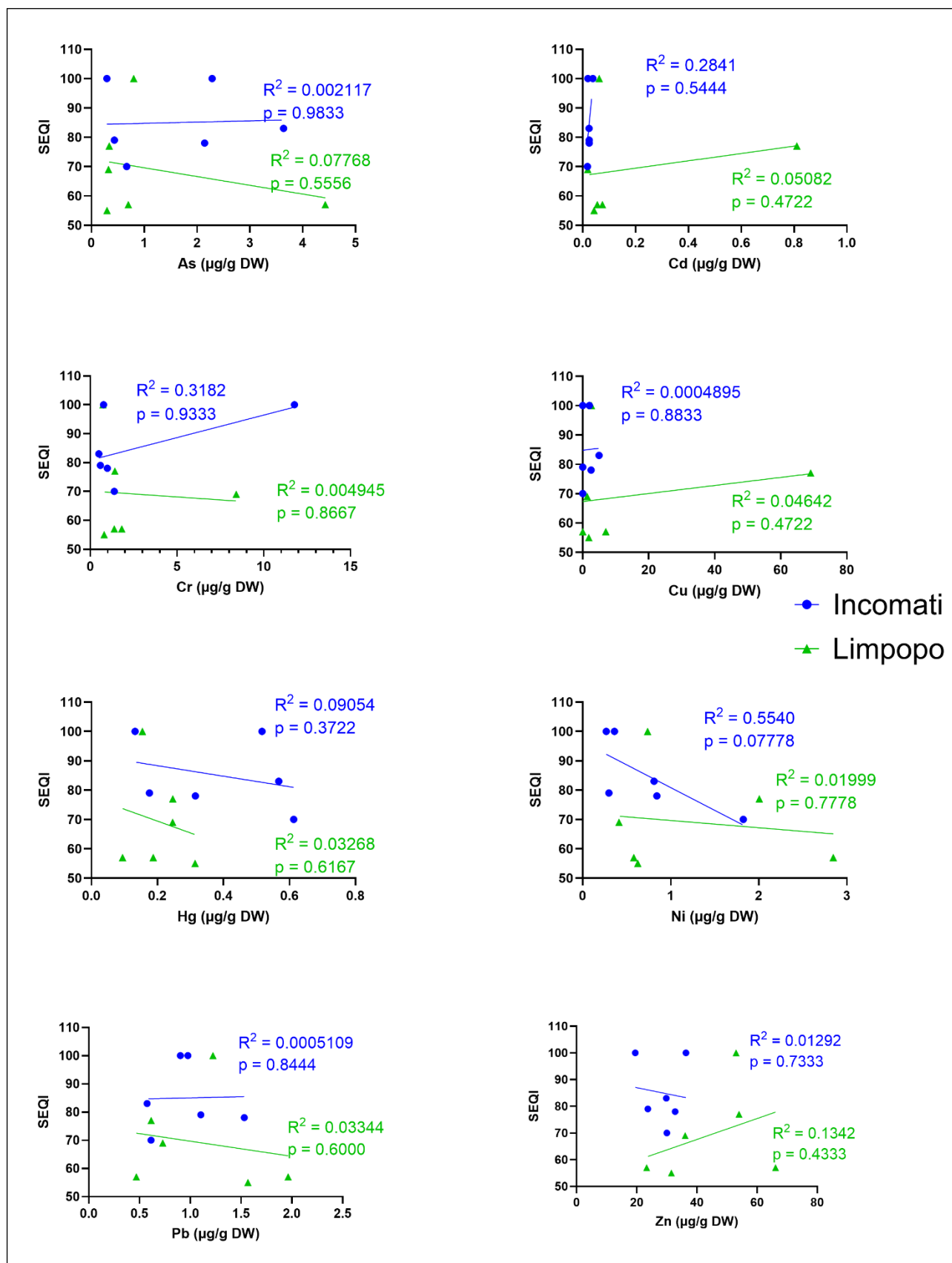


**Figure 3.8:** Correlations of the SEQI as a function of the mean accumulated elements in *Oreochromis mossambicus* in the Limpopo River and Incomati River catchments.

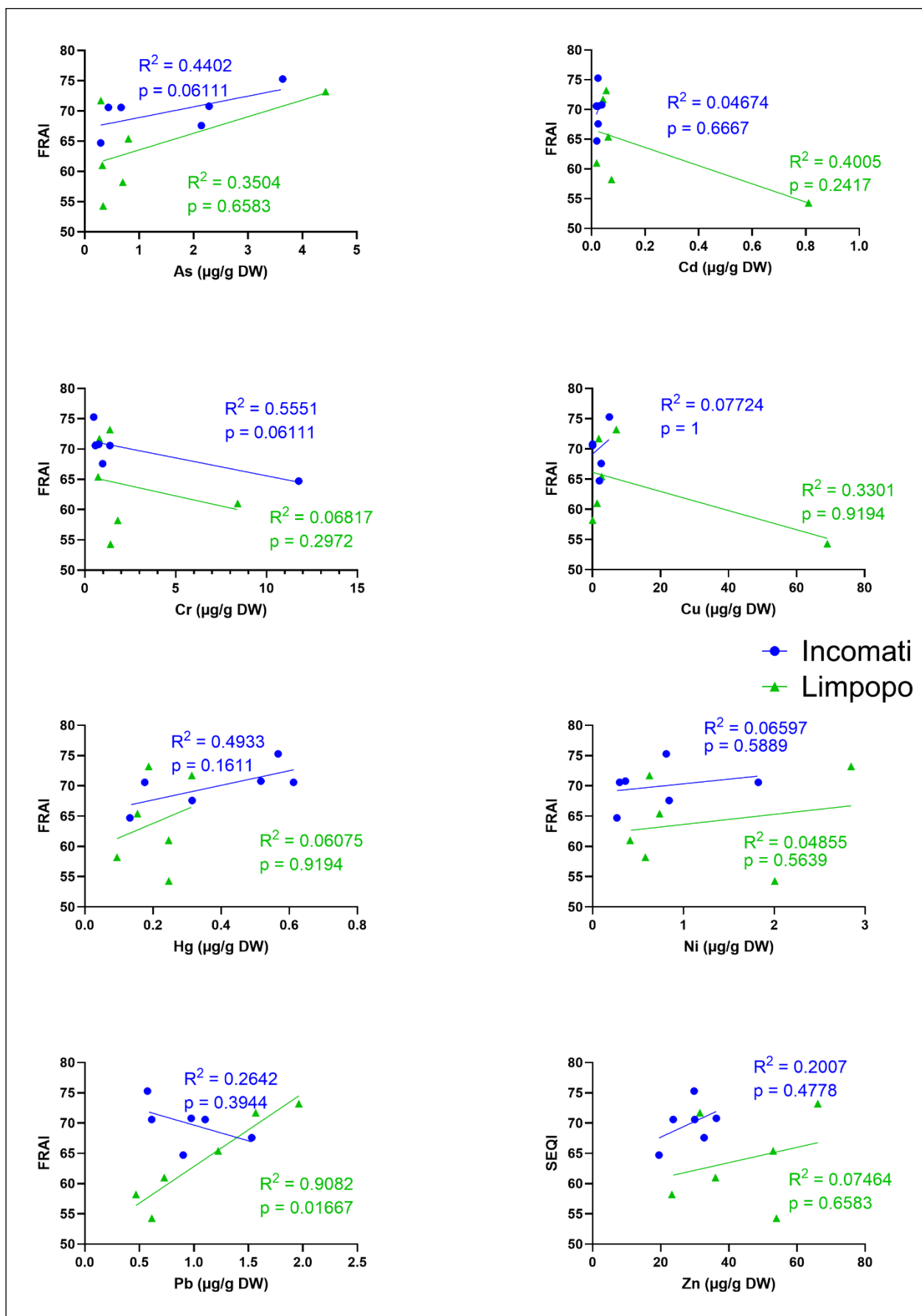


**Figure 3.9:** Correlations of the FRAI as a function of the mean accumulated elements in *Oreochromis mossambicus* in the Limpopo River and Incomati River catchments.

### 3.4 Discussion:

This study was done to determine the accumulation of trace elements in *O. mossambicus* from different sites on these catchments, as well as to determine the trace element transfer from water and sediment to *O. mossambicus* individuals. The trace element concentrations were also compared to different indices to determine if there is a correlation between the mean concentration of each element assessed in *O. mossambicus* and index scores that consider the water quality, sediment quality and fish assemblage at each of the sites. There are multiple studies done on the accumulation of trace elements in *O. mossambicus* (Chatterjee *et al.*, 2006; Arain *et al.*, 2007; Addo-Bediako *et al.* 2014; Yap *et al.*, 2015; Plessl *et al.*, 2017; Lebepe *et al.*, 2020; Mannzhi *et al.*, 2021; Erasmus *et al.*, 2022; Lebepe *et al.*, 2024). Fishes living in contaminated catchments tend to accumulate trace elements from their environment, as well as from their diet (Lebepe *et al.*, 2020). Trace elements accumulate in different organs of fish and could indicate short-term or long-term exposure.

#### 3.4.1 Trace element bioaccumulation in muscle tissue of *Oreochromis mossambicus*.

The As concentrations at CROC, LR1 and MAT were similar or higher than what was reported for the two impoundments on the Olifants River (Addo-Bediako *et al.*, 2014), whereas Cd concentrations at all the sites were similar or higher. The copper and zinc concentrations in at all the sites on the Limpopo River catchment were similar to the Cu and Zn concentrations that were detected in the muscle tissue of *O. mossambicus* at sites on the Olifants River as reported by Kotze *et al.* (1999). Chromium and Pb concentrations reported by Erasmus *et al.* (2022) were similar to all the sites in the Limpopo catchment, whereas the Zn concentrations were higher in the present study. The Cu and Hg concentrations at most of the sites reported by Mannzhi *et al.* (2021), were lower than the Cu and Hg concentrations at all the sites on the Limpopo River catchment. These rivers are impacted by agricultural activities, as well as mining and industrial activities, similar to the impacts affecting the Limpopo River catchment.

The As concentrations from the muscle tissue of *O. mossambicus* at sites on the Limpopo River catchment (CROC, LR1 and MAT) and the Incomati River catchment (SR KNP, LSR, SR Moz and IR2) were similar to or higher than what was reported by Lebepe *et al.* (2020) for Loskop Dam, whereas the As concentrations at the rest of the sites were lower than what was reported. Chromium concentrations from the muscle tissue of fish from the Limpopo River were similar to or higher than what was reported at Loskop Dam (Lebepe *et al.*, 2020), but a lot lower than what was reported at Mamba Weir (Robinson & Avenant-Oldewage, 1997). The Pb concentrations from the current study were similar or higher than that which was reported by Lebepe *et al.* (2020) for both catchments.

Chromium concentrations at SR KNP, LSR and IR3 were similar to that reported by Lebepe *et al.* (2020), whereas the rest of the sites were much higher. Both the Incomati River catchment and the

uMgeni River are impacted by agricultural activities as well as characterised by rural settlements (Misra *et al.*, 2024). The arsenic concentrations at all the sites on the Incomati River catchment was much higher than what was reported for the uMgeni River (Misra *et al.*, 2024), whereas the Hg concentrations were similar or higher. The accumulation of trace elements in the muscle tissue of the Limpopo River indicated higher concentrations for most metals compared to the Incomati River catchment, whereas the Incomati River indicated higher concentrations of Hg. The same trend could be seen in the previous chapter where most of the element concentrations in the water and sediment of the Limpopo River catchment were higher than that of the Incomati River catchment.

The bioconcentration factor (BCF) and biota-sediment accumulation factor (BSAF) for both catchments indicate that the main route of exposure is water with sediment only contributing to Cd, Cu, Hg and Zn similar to that reported by Erasmus *et al.* (2023). This could be attributed to the way these fish feed since they feed predominantly on debris in reed beds and not near the bottom of the river. The BCF and BSAF also indicated that at some of the sites, non-essential elements such as Hg and Cd are higher than essential elements (Cu and Zn), which means that these non-essential elements are more bioavailable than essential elements and could thus be accumulated. It was observed that although the scatter plots (**Figures 3.7 – 3.9**) at some of the sites indicated a low index score, it was not directly correlated to the accumulation of trace elements. According to Byns *et al.* (2024), this is likely due to the influence of other factors such as environmental stressors as well as the presence of other contaminants. Other studies have demonstrated that relating metal-tolerant biomonitoring with assemblage-based metrics, are able to identify metal stressors even in situations where other stressors are present (Rainbow *et al.*, 2012). Bervoets *et al.* (2015), stated that the general water characteristics such as pH, conductivity, oxygen level as well as nutrients might affect the community structures of an aquatic ecosystem, which could affect the biological indices as well as the accumulation of trace elements. with the index at both catchments. The indices that were assessed did not indicate a significant correlation with most of the elements except for Cr indicating a significant correlation with the ATI at the Limpopo River catchment and Pb indicating a significant correlation with the FRAI.

### **3.5 Conclusion:**

The research done on the trace element concentrations in the muscle tissue of *O. mossambicus* of the Incomati River catchment is the first of its kind and could be a guideline for future studies. Most trace element concentrations were higher in the muscle tissue of *O. mossambicus* from the Limpopo River catchment than from the Incomati River catchment, whereas Hg was higher in the Incomati River catchment. Both of these catchments indicated that *O. mossambicus* accumulate trace elements from the water with only some of the elements being accumulated from the sediment. The present study also determined that there are correlations between some of the trace element concentrations found in the muscle tissue of *O. mossambicus* and the different indices. It is important

to note that these concentrations found in the water and sediment do not reflect the concentrations found in the muscle tissue of *O. mossambicus* since the concentrations found in *O. mossambicus* could be an indication of long-term exposure. There is still a need for biomonitoring to be able to gather sufficient information on the element concentrations found in these systems.

## **Chapter 4: General conclusions and recommendations**

### **4.1 Conclusion**

Freshwater systems, such as rivers and streams, are under immense pressure from increased demand due to population growth, deforestation, climate change, as well as water extraction for agricultural, mining and industrial activities. Both the Limpopo River and the Incomati River catchments support large populations, which make them very important as they provide not only water for drinking but also other ecosystem services.

#### **4.1.1 Water and sediment quality assessment**

The health of aquatic ecosystems such as rivers is dependent on the quality of the water and sediment of the catchment. Different activities in the Limpopo River and Incomati River catchments have far-reaching influences on the water and sediment quality of the rivers that flow through the KNP and into the down-stream neighbouring Mozambique. This study determined the water and sediment quality of these catchments and highlighted the variation in the water and sediment quality between the different river systems. Each of the rivers that form the two catchments are influenced by unique combinations of stressors like mining, agriculture, and industrial activities. It is therefore important that the overall ecological integrity, quality, and safekeeping of the rivers and associated catchments are prioritised, monitored, and managed.

The Aquatic Toxicity Index (ATI) was used to evaluate the quality of the water from the catchments in terms of its ability to support aquatic life. All the sites sampled from both catchments had an ATI score above 60, which concluded that these systems were able to support aquatic life. Some of the sites had lower ATI scores that was attributed to higher concentrations of trace elements or elevated nutrients. The water quality variables that affect the rivers in the Limpopo River catchment are elevated concentrations of ortho-phosphates ( $\text{PO}_4$ ), ammonium ( $\text{NH}_4$ ), and total dissolved solids (TDS). The possible sources of these contaminants are agricultural runoff, wastewater effluent, and land erosion, with agricultural fertilisers and overgrazing playing significant roles in nutrient enrichment. Elevated concentrations of trace elements like chromium (Cr), copper (Cu), and manganese (Mn) are associated with mining activities and power generation, which are common in the upper Limpopo River catchment. The geology of the Limpopo River catchment could be the reason for the elevated concentrations of sodium (Na) and magnesium (Mg).

The Incomati River catchment showed similar challenges with nutrients such as  $\text{NH}_4$  and  $\text{PO}_4$ , as well as TDS, but to a lesser degree than the Limpopo River. Trace elements that had an effect on the Incomati River catchment include arsenic (As), cadmium (Cd), and mercury (Hg), which are all

associated with historical, and current artisanal and commercial gold mining along the Kaap River (a tributary of the Crocodile River) and the Sabie River. The water quality of the Incomati River was generally better than that of the Limpopo River catchment with most of the variables that were analysed being lower in the Incomati River than in the Limpopo River. These findings emphasise the input of trace elements and nutrients from natural, as well as anthropogenic activities. While nutrient enrichment was a major stressor in both catchments, the differences in trace element concentrations indicated the impact of mining and industrialisation in selected rivers (e.g. Crocodile River (West), Olifants River, and Letaba River). The Sabie River and the Lower Sabie River are both located inside of the KNP and indicated similar concentrations of physico-chemical variables, as well as nutrient concentrations compared to historical data. The water quality of the rivers inside of the KNP was generally better than that of the rivers outside or on the borders of the KNP as they are less impacted by anthropogenic activities this was also supported by the ATI scores of these rivers.

The sediment quality index (SeQI) was used to assess the sediment quality at each of the sites in the Limpopo River and Incomati River catchments. The SeQI from different sites in the Limpopo River catchment ranged from 43 to 100, with multiple sites being below 60, which is an indication of a decline in sediment quality. Trace elements that affected the sediment quality at the different sites include As, Cr, Cu, and Ni, which are typically linked to industrial activities, mining and agricultural activities. Accumulation of trace elements in the sediments could lead to detrimental effects on the ecosystem. In contrast, the Incomati River catchment had overall higher SeQI scores ranging between 70 and 100, indicating acceptable sediment quality. Trace element concentrations that affected the sediment quality were As, Cr, and Ni but these were present in lower concentrations compared to the Limpopo River catchment. This suggests that the Incomati River may be less affected by the intense industrial activities that characterise the upper catchments of the tributaries such as the Crocodile and Olifants Rivers in the Limpopo River catchment. When compared to other river systems in southern Africa, the Limpopo River and Incomati River catchments had similar trace element concentrations. These comparisons suggest that while the Limpopo River and the Incomati River are impacted by anthropogenic activities, the severity of contamination varies depending on the scale of the activities that affect each site. The sediment quality of most of the rivers inside of the KNP was overall better than the sediment quality of the rivers that are closer to the edge of the KNP with most of the sites with the highest SeQI score being located inside the KNP.

Based on the above, the data support the hypothesis that trace element concentrations in the water and sediment of the Limpopo River and Incomati River catchments are influenced by the unique anthropogenic activities in their respective tributaries. The hypothesis on the degree of element pollution at different sites will be reflected in the water and sediment quality indices was not supported by the data.

#### 4.1.2 Element bioaccumulation in *Oreochromis mossambicus*

In the present study the bioaccumulation of trace elements in *O. mossambicus* (Mozambique tilapia), from selected sites in the Limpopo River and Incomati River catchments, and the relationship with elements in water and sediments were assessed. The study highlighted the importance of monitoring trace element concentrations in aquatic organisms such as fish, and the use of these organisms as bio-indicators to determine the quality of the ecosystem.

As reflected in the concentrations of trace elements in the water and sediments from the two catchments, the trace element concentrations in the muscle tissue of *O. mossambicus* were higher in sites from the Limpopo River catchment. Fish from the Limpopo River catchment had elevated concentrations of As, Cd, Cr, and Pb, whereas the Incomati River had higher concentrations of Hg, which could be attributed to artisanal gold mining in the catchment as well as aerial deposition of particles from coal combustion power stations on the Mpumalanga highveld. Due to different anthropogenic activities affecting these catchments the concentrations of trace elements in the muscle tissue of *O. mossambicus* from the Limpopo River differed from that of the Incomati River. Exposure to trace elements can be through the water column or from the sediments. The present study determined that trace elements in *O. mossambicus* are primarily accumulated through water-borne exposure, with the bioconcentration factor (BCF), and to a lesser extent from the sediments using the biota-sediment accumulation factor (BSAF). Non-essential elements like Cd and Hg were found to be more bioavailable than essential elements like Cu and Zn, suggesting that these non-essential elements pose a greater risk to aquatic organisms. While sediment quality did contribute to the accumulation of certain elements, particularly Cd, Cu, Hg, and Zn, the primary concern remains the accumulation from the water column.

Furthermore, the correlation between trace element concentrations and environmental indices, such as the ATI, SeQI and the fish response assessment index (FRAI), provided valuable insights into the health of the ecosystem. The ATI and FRAI were significantly correlated to the concentration of most trace elements, demonstrating that poor water quality and higher concentrations of trace elements in fish muscle tissue are often linked. The SeQI is a good indication of sediment quality but did not show a significant correlation to trace element concentrations in fish muscle tissue, which may indicate the more direct role of accumulation of trace elements from water.

The hypothesis tested in this study, namely that the bioaccumulation of elements in fish will reflect the degree of element contamination of water and sediment of the different tributaries of the two catchments, was supported by the data. The data also supported the hypothesis that environmental

element exposure, indicated by element bioaccumulation in *O. mossambicus*, will reflect the ecological status as indicated by water, sediment and biotic (fish) indices was partially supported by the data since only the water and biotic (fish) indices reflected a relationship to the environmental exposure to elements.

In conclusion, the accumulation of trace elements in *O. mossambicus* from the Limpopo River and Incomati River catchments serves as a valuable indicator of environmental quality, pointing to significant pollution risks from industrial, agricultural, and mining activities. The stressors affecting these catchments need to be addressed, to help preserve these both the aquatic organisms that inhabit these catchments, as well as the communities that are dependent on these water resources.

## **4.2 Recommendations**

Based on the findings of the present study, the following aspects should be considered for future research:

- There is a need for further studies to be done on these catchments to determine the effects of climate change on the water flow and temperature and therefore the effect it had on the element exposure patterns in these catchments.
- While the current study demonstrated a relationship between element accumulation in fish and the FRAI biotic index score, further studies could be conducted to assess the relationship of other biotic indices with the trace element accumulation in fish.
- The present study provided valuable information on the current water and sediment quality of the Limpopo River and Incomati River catchments, as well as the accumulation of trace elements in *O. mossambicus* from these catchments. Human exposure studies are also needed for these catchments to determine the effects of exposure to trace elements through the consumption of fish as well as through direct contact with these contaminated waters.
- Further studies should be conducted on the effects of other contaminants such as pesticides on the water and sediment quality of these catchments, as well as the accumulation of these contaminants in fish.
- In future studies, more sites could be sampled to get a better understanding of the overall water and sediment quality of these catchments, as well as the inclusion of reference sites.
- Studies could be conducted to determine the long-term distribution of trace elements in these catchments. It could give valuable information and help set guidelines for these catchments to prevent further deterioration of these catchments. This could be done by doing multiple surveys a year over a few years to account for seasonal variation, which is important to determine how contaminants accumulate or dissipate over time within the catchment.

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