Health assessment of fishes from coastal lakes on the east coast of South Africa

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ABSTRACT

Kosi Bay located on the subtropical east coast of South Africa bordering the south of Mozambique is a unique Ramsar system that is composed of four interconnected, roughly circular lakes that is considered the most pristine system left in KwaZulu-Natal. Kosi Bay is classified as an estuarine wetland which includes mangrove swamps, tidal marshes and deltas that are considered as an important nursing area and feeding source for marine and estuarine fish. According to the Water Research Commission (WRC), a workshop in 2013 indicated there is a general lack of aquatic biodiversity in selected Ramsar sites in South Africa. Limited sampling efforts have been done in Kosi Bay referring to more detailed health investigations. Studies on the fish of Kosi Bay have been done with limited detailed investigations on the fish health. The aim of the study was thus to assess the health on *Oreochromis mossambicus, Rhabdosargus sarba* and *Terapon jarbua.* This investigation is important because Kosi Bay is an important nursing and breeding area for fishes and the local community rely on the system to catch these fish. Fish collection surveys took place during the wet and dry seasons, August 2015, December 2015 and February 2016, using hand line and rod and reel. Water quality and sediment samples were also collected in the Kosi Bay and Lake Sibaya systems to determine the metals present in these areas. Only water and sediment samples of Lake Sibaya were collected during this study. The Fish Health Assessment Index protocol (FHAI) was used on the selected fish to determine the health of the fish with a detailed investigation of the abnormalities that may be present in the fish. Metal bioaccumulation, metallothioneins inductions and human consumption hazard of selected fish species in Kosi Bay were investigated. The fish of Kosi Bay was in a relatively good condition with no serious abnormalities present in the fish and the methods used on these surveys were successful with positive results.

Keywords: metals, Kosi Bay, Lake Sibaya, Ramsar, bioaccumulation, *Oreochromis mossambicus*, *Rhabdosargus sarba*, *Terapon jarbua*

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LIST OF ABBREVIATIONS

°C	Degrees Celsius
µm	Micrometres
ADD	Average daily dose
Al	Aluminium
ANOVA	One-way analysis of variance
ANZECC	Australian and New Zealand environmental and conservation council
As	Arsenic
BCF	Bioconcentration factors
CCME	Canadian Council of Ministers of the Environment
Cd	Cadmium
CF	Condition Factor
Co	Cobalt
Cr	Chromium
CRM	Certified Reference Material
Cu	Copper
DDT	Dichlorodiphenyltrichloroethane
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EBI	Estuarine Biotic Integrity Index
EFCI	Estuarine Fish Community Index

ERL	effects range-low
ERM	effects range-medium
Fe	Iron
FHAI	Fish Health Assessment Index
FHI	Estuarine Fish Health Index
FRI	Estuarine Fish Recruitment Index
FS	Fisherman’s Spot
g	Gram
GCF	Gutted Condition Factor
GSI	Gonadsomatic Index
ha	Hectares
HAI	Health Assessment Index
Hg	Mercury
HI	Hazard index
HSI	Hepatosomatic Index
IBI	Index of Biotic Integrity
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
kg	Kilograms
km	Kilometres
KUSH	Kushengeza
KZN	KwaZulu - Natal
L	Litres
L1	Lake 1
L2	Lake 2

L2+3C	Lake 2+3 (Channel)
L3C	Lake 3 (Campsite)
L3E	Lake 3 (Entrance)
L3SE	Lake 3 (South East)
L4	Lake 4
LS1	Lake Sibaya 1
LS2	Lake Sibaya 2
LS3	Lake Sibaya 3
LS4	Lake Sibaya 4
m	Metres
M	Mouth
MAL	Malangeni River
mg	Milligrams
min	Minutes
ml	Millilitres
mm	Millimetres
Mn	Manganese
MS	Mouth (Sea)
MT	Metallothionein
MU	Mouth Upper
Ni	Nickel
NWA	National Water Act
OSI	Total Organ Weight
Pb	Lead

PT	Protein
Se	Selenium
SL	Standard Length
SQG-I	Sediment Quality Guideline Index
SQI	Sediment Quality Index
Sr	Strontium
SSI	Spleenosomatic Index
TL	Total length
TWQR	Target Water Quality Range
WMA	Water management area
WRC	Water Research Commission
Zn	Zinc

1. Introduction

Wetlands have extraordinary biodiversity and natural productivity that play an important role within ecosystems, but over time wetlands have been drained and used for construction (Matthews, 1993). Conservation of the natural environment and especially wetlands became more prominent in the 1980's and 1990's (Matthews, 1993). Wetlands are responsible for ground water protection, purification, water storage and retention of pollutants, sediment and nutrients (Ramsar Secretariat, 2013). They act as biological and mechanical filter systems (which clean the water that runs through it) and form the habitat of many plant and animal species (Ramsar Secretariat, 2013). These plant and animal species rely on wetlands for various life stages and survival (Kross and Richter, 2016). Certain wetland habitats, such as estuarine wetlands, are especially important to fish for breeding as well as providing a nursery function (Meynecke *et al*., 2008).

According to the National Water Act (NWA) (Act No 36 of 1998) of South Africa, a wetland can be defined as land which is transitional between terrestrial and aquatic systems where the water table is near the surface, or land that is periodically covered with shallow water, and in normal circumstances supports or would support vegetation typically adapted to life in saturated soil (NWA, 1998). According to the Ramsar Convention, a wetland can also be classified as an area with peatland, marsh or water, either natural or artificial, permanent or temporary that has fresh, brackish or salt water that is either flowing or static (Matthews, 2013). It also includes areas of marine water where the low tide does not exceed six metres (Matthews, 2013).

The Ramsar Convention recognised five major wetland types: marine, estuarine, lacustrine, riverine and palustrine (Ramsar Secretariat, 2013). Ollis *et al*. (2013) developed the *Classification System for Wetlands and Other Aquatic Ecosystems in South Africa* to classify different wetland areas in South Africa. There are six levels of classification with the first level distinguishing marine, estuarine and inland wetlands (Ollis *et al*., 2013). Estuarine wetlands include mangrove swamps, tidal marshes and deltas (Ramsar Secretariat, 2013). An estuary is a transition zone between freshwater and seawater (brackish) and occur abundantly over all the world's coastlines (Cooper *et al*., 1995). They are shallow productive systems that offer abundant food and shelter (Cooper *et al*., 1995). Estuaries are important to most fish as they serve as nursing grounds and have direct or indirect commercial and recreational importance to man (Brinda *et al*., 2010).

Disappearances of wetlands are caused by: accumulation of pollutants, shoreline destruction, (Kross and Richter, 2016); as well as water demand from people and urban development (Matthews, 1993). Thus, wetland degradation causes economic pressure which can lead to a decline in clean and reliable water for the community (Ramsar Secretariat, 2013).

Water birds migrate each year and depend on wetlands to rest, feed and breed. The loss of wetlands results in the disappearance of unique species with it (Ramsar Secretariat, 2013). One species that is under pressure due to the loss of wetlands is *Sarothrura ayresi* (White-winged flufftail). Ornithologists were the first to support the conservation of wetlands to maintain the diversity of migrating water birds and thus the proposal for an international treaty, known today as the Ramsar Convention, came predominately from the ornithological community (Ramsar Secretariat, 2013).

The Ramsar Convention

The official name of the treaty is "The convention on wetlands of international importance especially as waterfowl habitat" (Ramsar Secretariat, 2013). The Ramsar Convention was adopted on 2 February 1971 in the Iranian town of Ramsar. It is an intergovernmental treaty for the protection of sustainable natural resources and wetlands. This treaty entered into force in 1975 (Ramsar Secretariat, 2013) and as of 2017 has 169 contracting parties in all parts of the world and a list of more than 2282 wetlands of international importance for special protection (Ramsar Secretariat, 2017). The official name of this treaty reflects the emphasis upon the conservation for wetlands primarily as habitat for water birds. The treaty also recognises wetlands as ecosystems vital for biodiversity and human communities. The main message of the Ramsar convention is thus that of the sustainable use of wetlands globally (Ramsar Secretariat, 2013).

South African Ramsar sites

The convention entered into force in South Africa on 21 December 1975 and currently has 23 Ramsar sites designated as wetlands of international importance with a total combined surface area of 555,678 hectares (ha) (Ramsar Secretariat, 2013) with the Bot-Kleinmond system was added and declared in January 2017 (Ramsar Secretariat, 2017). The areas of international importance on the east coast of South Africa are Lake St. Lucia, Turtle Beaches/Coral Reefs of Tongaland, Lake Sibaya and Kosi Bay, all lying within the iSimangaliso Wetland Park (Ramsar Secretariat, 2013) (Figure 1.1).

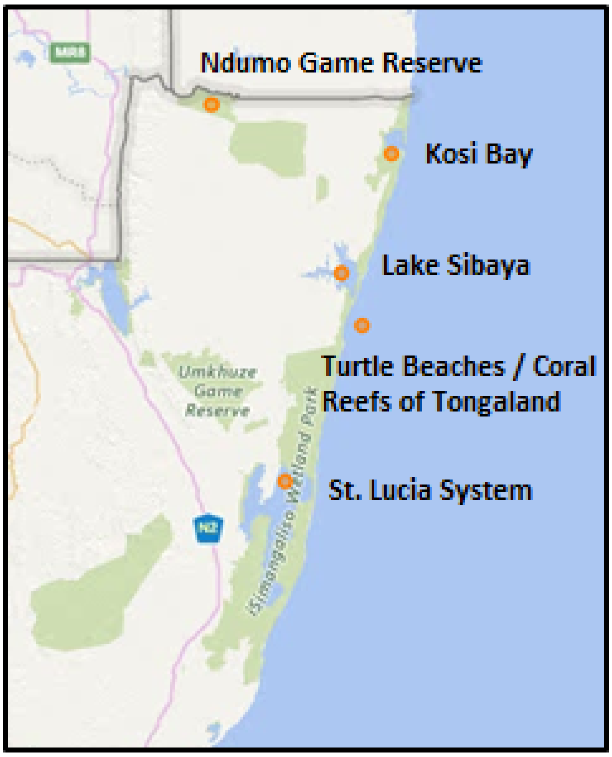

Figure 1.1. Ramsar sites on the east coast of South Africa.

The east coast of southern Africa has several coastal lakes situated close to the sea on the coastal plain (Figure 1.2). Some of these coastal lakes are temporarily connected to the sea (Hill, 1975) while others are temporarily connected to estuaries. Few of the estuaries are closed off during the dry seasons. Many of these coastal lakes are shallow (ranging from 1 – 5 m), whereas more northern lakes (Lagoa Poelela, Lake Nhlange (Lake 3) and Lake Sibaya are deeper (ranging from 5 – 30 m) (Hill, 1975).

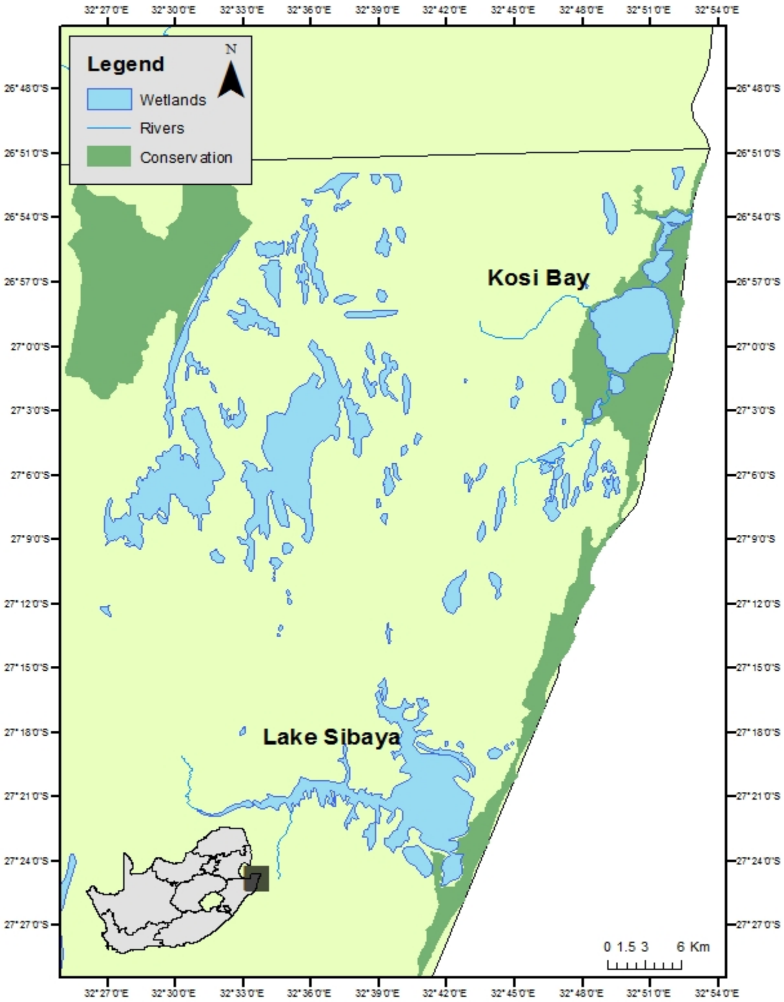

Figure 1.2. Map of the Kosi Bay and Lake Sibaya systems along the east coast of South Africa.

Kosi Bay

The Kosi Bay system is 470 km north east of Durban and has an area of approximately 10982 ha with a unique estuary-linked lake system (Kyle and Kwangwanase, 1995). Kosi Bay lies on the subtropical east coast of KwaZulu-Natal (KZN) (32°50'S; 29°50'E) (Pedersen *et al*., 2003; Green *et al*., 2006), bordering to the north with Mozambique, and is considered to be one of the most pristine estuarine-lacustrine systems in South Africa (Green *et al*., 2006) (Figure 1.2). This estuarine-lacustrine system forms part of the South African east coast's iSimangaliso Wetland Park (Green *et al*., 2006). The iSimangaliso Wetland Park was listed in 1990 as South Africa's first UNESCO World Heritage site and South Africa's third largest protected area (Carbutt and Goodman, 2013). The Kosi Bay system is composed of four interconnected, roughly circular lakes with water channels leading to an estuary which opens to the Indian Ocean (Kyle and Kwangwanase, 1995). These lakes are Makhawulani (Lake 1), Mpungwini (Lake 2), Nhlange (Lake 3) and Amanzimnyama (Lake 4) (Figure 1.3). The maximum water depths reached in the lakes of Kosi Bay are 3 m in the estuary, 8 m in

Makhawulani, 18 m in Mpungwini (Kyle and Kwangwanase, 1995), 31 m in Nhlange and 3 m in Amanzimnyama (Holbach *et al*., 2012). Due to the very shallow tidal basin of Lake Makhawulani the surface area of the lake can be 70% exposed during low tide. The mouth is generally 20 - 50 m wide and varies in size due to seasonal changes (Kyle and Kwangwanase, 1995).

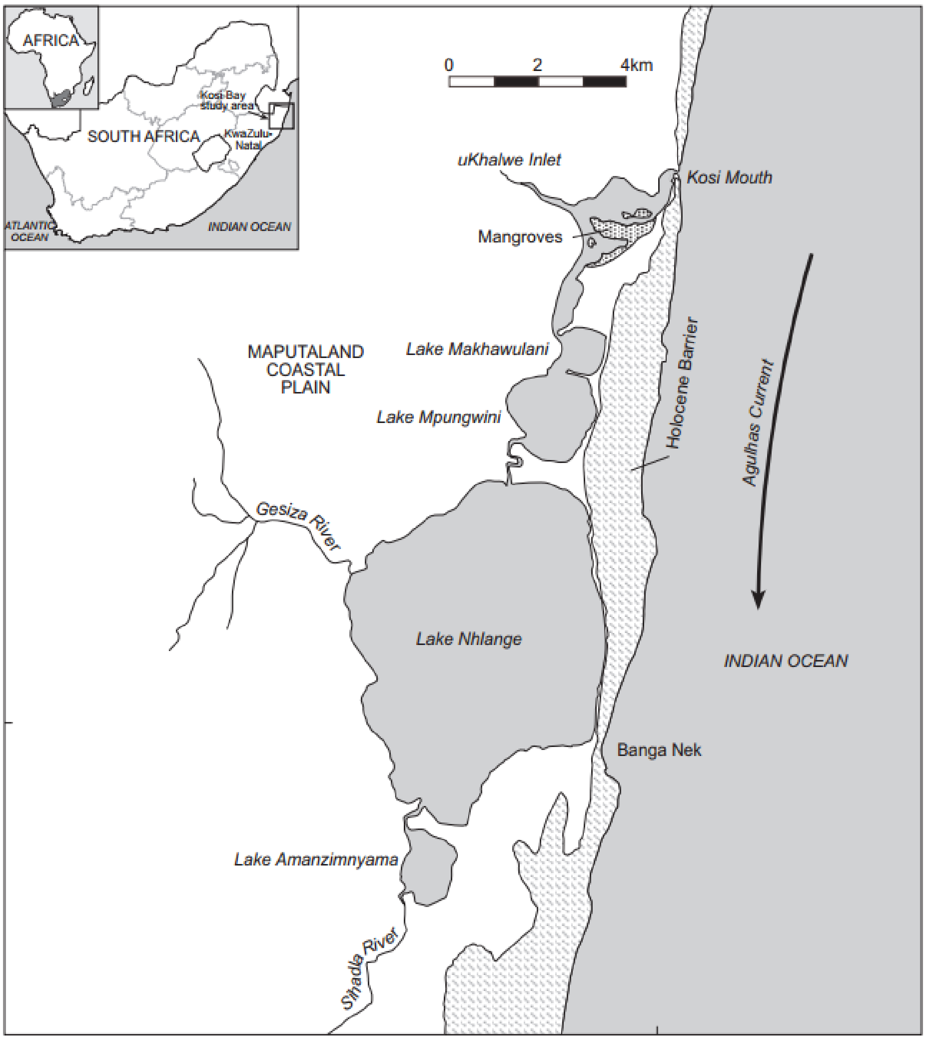

Figure 1.3. Map of the various lakes in the Kosi Bay system along the east coast of South Africa. Obtained from Green *et al*., (2006).

The vegetation habitat types of the Kosi Bay system include swamp forest, *Phragmites* beds, mangrove forest (32 ha), coastal grassland and dune forests (Kyle and Kwangwanase, 1995). These forests are coastal forest (*Raphia australis)*, sand forest (*Hymenocardia ulmoides)* and swamp forest (*Ficus trichopoda)* (Cowling *et al*., 2004). Kosi Bay also consists of open woodland/palm communities and aquatic ecosystems which are scattered lakes, pans, streams, marshes and swamp forests (Kyle and Kwangwanase, 1995). The mangrove communities are scattered throughout the system but do not extend past Makhawulani (Green *et al*., 2006).

The lakes are separated from the ocean by a strip of forested sand dunes approximately 600 - 2000 m in width (Kyle and Kwangwanase, 1995). Kosi Bay has warm summers with a humid subtropical climate with average annual rain records of 980 mm (Holbach *et al*., 2012); variation in rainfall of 1200 mm in the south-east region and 700 mm in the west region (Green *et al*., 2006) have also been recorded.

The demand for tourism observed in 1990 has resulted in increased tourism development in the area which could possibly lead to anthropogenic pressure on the system (Odendal and Schoeman, 1990). These increased infrastructures are transport, water supply, electricity to these new infrastructures as well as other facilities which can increase anthropogenic pressure (Odendal and Schoeman, 1990). More recent observations indicated that there are minimal anthropogenic disturbances to the system (Green *et al*., 2006). Tourism development did increase in the area over the years and was noted during the study but there was no clear evidence of anthropogenic pressure on the system.

Lake Sibaya and Kushengeza

A small coastal pan called Kushengeza was also briefly investigated near Kosi Bay along with Lake Sibaya in the current study.

Lake Sibaya is an isolated coastal freshwater lake (Kyle and Ward, 1990) south of Kosi Bay with a higher diversity of ichthyofauna than other freshwater coastal lakes (Allanson, 1979). It is isolated from the sea by a large sand dune that is vegetated and has an area of 7750 ha (Kyle and Ward, 1990). The lake is situated within a rural area and is 430 km north east of Durban and lies in a south eastern direction from Kosi Bay (27°15’S; 32°44’E) - (Kyle and Ward, 1990) (Figure 1.2). In 2015, the ecological status, importance and sensitivity assessments were investigated in the Lake Sibaya system by scientists from the Department of Water and Sanitation (DWS) (DWS, 2015). The variables studied were water quality, vegetation, sediment, molluscs/crustaceans, fish, mammals and birds. Sedimentary processes of the system were studied (Wright *et al*., 1997) along with the sedimentology of the Kosi Bay system (Walther and Neuman, 2011). According to Kyle and Kwangwanase (1995), low levels of metals within the water were recorded in August 1976 throughout the estuary. There are also future possibilities where mining along the dunes of Kosi Bay can occur due to the high titanium levels which are present within the dunes (Kyle and Kwangwanase, 1995). Fortunately, these mining activities have not been implemented yet. Holbach *et al*. (2012) determined the levels of metals present within the water from each lake of the system. The mean metal concentrations in the water of the different lakes were: manganese (Mn) (0.0035 mg/L), iron (Fe) (0.1024 mg/L), nickel (Ni) (0.00.7 mg/L), copper

(Cu) (0.00132 mg/L), zinc (Zn) (0.00648 mg/L), strontium (Sr) (1.706 mg/L) and cadmium (Cd) (0.00001 mg/L). The aim of the study by Holbach *et al*. (2012) was to investigate the otolith chemistry of the fishes as a multiple analytic method to reconstruct fish migrations in the Kosi Bay system.

The importance of fish

Fish are considered important for pollution studies and are used to determine the aquatic health of an ecosystem as they can reflect environmental conditions (Whitfield, 1997). If an aquatic ecosystem shows deterioration, it would be expected that the fishes of that particular system would also be affected. Environmental factors that cause fish to reflect environmental conditions include habitat degradation, disturbance of essential ecological processes and environmental pollution (Whitfield, 1997). There are several fish species that occur within an estuarine system, with some species restricted to certain zones; namely, sea water and freshwater zones (Cooper *et al.,* 1995). Approximately 20% of the 1500 species of fish recorded from the seas of southern Africa occur in estuaries at certain stages in their life cycles (Cooper *et al*., 1995).

The rural community of Kosi Bay is dependent on the fish they catch from the system by using constructed fish traps throughout the estuary. The fish traps have been used by traditional Zulu fishermen in the Kosi Bay system for many generations (Kyle, 2013). The higher demand for fish resulted in the local community moving closer to the lakes and the population near the lakes has therefore increased (Kyle and Kyle, 2003). Investigating metal exposures in the tissues of the selected fish species will indicate what the current concentrations of the metals are and if these concentrations can result in alterations to the fishes. The Fish Health Assessment Index (FHAI) protocol was used to determine the overall health of any fish species.

A rapidly increasing population and the tendency to ignore environmental concerns are the reasons for the disruption in the balance between maintaining a stable ecosystem and meeting human needs (Kaya and Akbulut, 2015). Kosi Bay is a clear water system with a large variety of fish species (Kyle and Kwangwanase, 1995) where a number of surveys have been undertaken but most have been non-quantitative with limited sampling (Blaber, 1978). These surveys where undertaken during 1975, 1976 and 1977. The aims of those studies were to establish the important characteristics and seasonal variations of the fish fauna of each part of the system (Blaber, 1978). The results recorded a total of 124 species (which were marine fish species), 70% of which are restricted to the estuary and the remaining 30% consisted of estuarine species that move between the marine and estuarine environment. The aim of another study by Kyle and Robertson (1997) was to tag fish in the Kosi Bay system to provide valuable information on fish movements, growth and mortality rates and population estimates. A total of 500 *Acanthopagrus berda* were tagged of which 279 were caught on rod and reel, 157 purchased from local fisherman and the remainder caught by seine and gill netting. James *et al*. (2001) conducted a study on the analysis of recreational angling within the Kosi Bay system. This study was based on the catch card data that recreational anglers filled out from 1986 to 1999 at the Nhlange campsite (James *et al*., 2001). These cards were analysed to determine total catches, catch composition and seasonality of the catches. Angling outings increased from 510 to a peak of 2379 in 1994 and then declining to 892 in 1999. Not only were fish diversities, characteristics and seasonal variations looked at but the impact of fish traps was also investigated within the system. The number of fish traps increased from 66 traps in 1981 to 158 traps in 2001 (Green *et al*., 2006). Fish caught in the traps increased from 40 000 fish in 1981 to 93 000 fish in 1993 (James *et al*., 2001). The rural community of Kosi Bay has been building fish traps in the system for centuries and have been using fish traps long before the system was declared as a Ramsar site (James *et al*., 2001).

Threats to the Kosi Bay system

The “slash-and-burn” method was practiced by the local community on the dune forest of Kosi Bay which was a major problem and lead to the destruction of the dune and swamp forests (Kyle and Kwangwanase, 1995). The technique is destructive and results in unproductive cultivation (Kyle and Kwangwanase, 1995). Freshwater supply to the lake system from the wetland areas is also being threatened by the effect of afforestation, which could cause salinity levels to rise in the lakes, thereby affecting the ecological processes (Kyle and Kwangwanase, 1995). Another pressure to the area and the catchment is domestic sewage and water supply schemes. Furthermore, increasing population pressure in the area could result in more demand for land and cultivation. This will lead to more fertilisers and chemicals being used in the catchment.

During the investigation of the Lake Sibaya system destruction of forests and over grazing were noted. There were big herds of local cattle in the area and next to the lake. Lake Sibaya is also threatened by population pressure that causes more cultivation in the area.

Other threats include the in-filling of sediment into the lakes of the system and over-fishing (Kyle and Kwangwanase, 1995). Due to the population pressure, the fish of the system are being over fished by fish traps and the use of gill nets that begun in 1992 (Kyle and Kwangwanase, 1995). The increase in fish traps over the years have led to a decline in fish populations. The gillnetting scheme allowed the local community to use gillnets in Lake

Nhlange on a controlled basis. These gillnetting methods caused damage to the fish populations and are now illegal to use. However, they are still being used illegally and without being monitored (Kyle and Kwangwanase, 1995). Other factors besides gillnetting that cause fish to become threatened in general are mostly habitat degradation, environmental pollution and ecological process disruption (Whitfield, 1997).

1.1 Hypothesis, aim and objectives

1.1.1 Hypothesis:

Due to limited anthropogenic impacts in the Kosi Bay system metal levels in water and sediment will be lower than the DWS proposed water quality levels and therefore fish from this system will be healthy and pose no risk to human health following consumption.

1.1.2 Aim:

The aim of the project was to assess *Rhabdosargus sarba* (Forsskål, 1775), *Oreochromis mossambicus* (Peters, 1852) and *Terapon jarbua* (Forsskål, 1775) of the Kosi Bay system to determine if metal concentrations in water and sediment affected their health and posed a risk to human health following consumption (Skelton, 2001).

1.1.3 Objectives:

The objectives of the study:

- To determine the current metal concentrations in the water and sediment of the Kosi Bay and Lake Sibaya systems.
- Sample and determine the health of the selected fishes following the Fish Health Assessment Index (FHAI) protocol.
- To determine the current metal concentrations in the muscle tissues from the selected fishes of the Kosi Bay system.
- Determine the metallothioneins in the fish liver tissues from the selected fishes.
- To determine the bioconcentration factors (BCF) between the muscle tissues and the environment (water and sediment).
- To determine consumption hazard and risks.

1.2 Chapter breakdown:

This section provides a brief breakdown of the contents of each of the different chapters in this dissertation.

Chapter 1: Introduction:

This is a general introduction of what a wetland is and why it is important to the environment and how it links nature to humans. It provides a brief description of how the Ramsar Convention was first adopted as well as a general background about study area with the aim, objectives and hypothesis given for the project.

Chapter 2: Site description:

This chapter provides a detailed background of the Kosi Bay and Lake Sibaya system with information about the fish of the system and a detailed discussion about the selected fish species used for this study. The various sites that were included in each system are also presented and discussed.

Chapter 3: Water and sediment:

This chapter provides the results of the assessment of water and sediment quality variables in the Kosi Bay and Lake Sibaya systems. Water and sediment quality variables in the Kushengeza pan were also analysed.

Chapter 4: Fish health assessment index:

This chapter gives details about the Fish Health Assessment Index and how it is used to determine the health of fish. The seasonal fish health results are presented for *Terapon jarbua*, *Rhabdosargus sarba* and *Oreochromis mossambicus*.

Chapter 5: Bioaccumulation and biomarkers:

This chapter gives information on bioaccumulation, what bioaccumulation is and what was used to determine bioaccumulation. Bioaccumulation of the metals in the tissue from the fish results is also presented and discussed.

Chapter 6: Conclusion and recommendations:

The final conclusion of this study is provided in this chapter and integrates all of the results generated in the preceding chapters. Some recommendations on future studies in the Kosi Bay system and Lake Sibaya system are also proposed.

Chapter 7: References:

Complete list of all the references cited in the preceding chapters are provided.

2. Site description and selections

The four lakes in the Kosi Bay system connect to a narrow channel leading to an estuary on the north eastern side of the South African coast. The lakes are interlinked and drain three large swamp areas through the Malangeni (Sihadhla), Swamanzi (Gesiza) and Sifazanene streams which are normally perennial (Harrison and Whitfield, 2006). The Kosi Bay Estuary is usually a permanently open estuary (Blaber, 1978) and it is the first part of the entire lake system, running from the ocean to the beginning of Lake Makhawulani. The following sections will describe the Kosi Bay area as well discuss the selection of sites within the study area. A brief description of the fish species used for the health assessment in the systems will also be presented.

Geology

Kosi Bay is part of the Mozambique coastal plain which opens to the sea 2 km south of the Mozambique/South African border (James *et al.,* 2001) that consists of sandy soils with Cretaceous beds. The coastal dunes of the system are composed of both Holocene and Pleistocene sand deposits. The Kosi Bay system is not a rocky system although some rock ledges, shelves and outcrops occur. The geology in the area is an important factor that could indicate what metals can occur in the water of the system. Some of the metals that could occur from the local geology include chromium (Cr), copper (Cu), iron (Fe) and zinc (Zn) (Singh *et al*., 1997). There is a vegetated sand dune area over 130 meters (m) high on the eastern side of the coast (Walther and Neumann, 2011). There is one rock outcrop near the mouth of the Kosi Bay Estuary which forms a natural reef system inside the estuary rather than in the marine environment (Kyle and Kwangwanase, 1995). Circular lakes form in the system through a process known as segmentation which divides certain sections of the lake. Each lake is separated from the other lakes by a shallow beach barrier (Kyle and Kwangwanase, 1995).

Hydrology and Origin

Kosi Bay obtains most of its freshwater input through local drainage and the high ground water table that is characteristic of the coastal plain. The high water table in the area causes stagnant water to form with wet and muddy ground i.e. coastal wetlands (Kyle and Kwangwanase, 1995). Only 5% of the annual precipitation can be expressed as surface runoff into the Kosi Bay system. Surface drainage in the area is also low due to the high porosity of the cover sand and with only the Malangeni (Sihadhla) and Swamanzi (Gesiza) Rivers being perennial (Wright *et al*., 2000). The origin of the system is formed by two principal rivers which enter the system. The Malangeni (Sihadhla) River is approximately

30 kilometres (km) long and rises in the Mtombeni pans. This river receives contributions from twelve principal tributary systems and then enters into Lake Amanzimnyama (Kyle and Kwangwanase, 1995). The other river that contributes to the Kosi Bay system is the Swamanzi (Gesiza) River which is approximately 15 km long and collects water from nine principal tributaries and enters Lake Nhlange (Figure 1.2). The hydrology of the Kosi Bay system has a fairly strong seasonal inflow of fresh water (Kyle and Kwangwanase, 1995) and due to the porous sand in the area most of the freshwater input into the system is from ground water inputs (Walther and Neumann, 2011).

Sediment type

The bottom sediment in the system is mainly clear white sand that is a result of tidal influences on the northern side of the system. Accumulation of metals into the sediments from the overlaying waters is dependant of the surface area for adsorption which is caused by the variation in grain size distribution (Binning and Baird, 2001). Increased metal concentrations are associated with finer grained sediments (Binning and Baird, 2001). Silt can be found in deeper waters with thin overlaying sand in certain shallow areas. Sandy substrates in the system lack fine particles and it has a low nutrient content. Unconsolidated organic debris collects on the bottom of deeper waters and gradually becomes anoxic with high volatile nutrient values and hydrogen sulphide. These materials collected in the deeper waters originate alongside the marshes and swamps of the system and gravitates towards the deeper waters (Kyle and Kwangwanase, 1995).

Vegetation

Vegetation along the coast of Kosi Bay includes grasslands, mangrove forests, subtropical dune thickets, subtropical freshwater wetlands and timber plantations. The subtropical dune thickets consist of dense shrubs, vines and small trees (Walther and Neumann, 2011). Mangrove forests in Kosi Bay have increased from 59 hectares (ha) to 60.7 ha in recent years (Rajkaran and Adams, 2011). There are six mangrove species that occur in South Africa (*Avicennia marina*, *Bruguiera gymnorrhiza*, *Rhizophora mucronata*, *Lumitzera racemosa, Ceriops tagel* and *Xylocarpus granatum*) but only *Ceriops tagel*, *Lumitzera racemosa* and *Xylocarpus granatum* are found at Kosi Bay (Rajkaran and Adams, 2011). The vegetation around Lake Amanzimnyama has very dense and tall coastal palm tree populations (*Raffia palms*).

Climate

Kosi Bay has a warm and humid subtropical climate with humid predominantly summer rainfall conditions (Harrison and Whitfield, 2006). It has an average annual rainfall of 980 millimetres (mm) (see attached Appendix A for the seasonal rainfall in the area) (Holbach *et al*., 2012) with maximum average temperatures that vary from 28 degrees Celsius (°C) in January to 22 °C in July and averages minimum temperatures are 19 °C in January and 9 °C in July (Kyle and Kwangwanase, 1995). There are several thermoclines that develop in the lakes during the summer because of strong northerly or southerly winds.

In winter, Lake Nhlange (Figure 2.1) tends to develop a homothermal temperature of 18.5 °C to 19 °C and exhibits a complex pattern of stratification (Kyle and Kwangwanase, 1995). Lake Makhawulani (Figure 2.1) and Lake Mpungwini (Figure 2.1) have temperature layering causing bottom temperatures to be significantly warmer than the surface waters of the lake. Water temperatures in the channels of the system do not fall below 20 °C in the winter and can reach temperatures of 30 °C in the summer months (Kyle and Kwangwanase, 1995).

Fish have to deal with many environmental stressors, one being fluctuating temperatures. The fluctuating temperatures within a system can cause physiological stress and impair their health (Adams *et al*., 1993). These thermoclines within a system can cause fish to move around in search of favourable temperatures. The increase or decrease of temperatures affects the release of metals within the environment, (the release rate of metals is associated with higher temperatures). Higher temperatures can thus cause higher metal accumulation by fish (Li *et al*., 2013). An increased temperature increases the metabolic process which subsequently increases the uptake of metals by fish. Thus, the drop in temperature changes the metabolic processes which could lead to the release of metals (Avenant-Oldewage and Marx, 2000).

Water quality

The Kosi Bay system has mostly clear waters and a classical transition from sea water, which enters at the mouth, to fresh water in Lake Amanzimnyama. Due to this connection to the sea, a mixture of sea water and fresh water occurs along a salinity gradient in the system (Harrison and Whitfield, 2006). Salinity levels in the tidal basin come close to salinity levels of the sea and vary naturally with the tides (can drop remarkably at low tide). Lake Makhawulani and Lake Mpungwini both exhibit salinity layering whereas Lake Nhlange is not similarly arranged, and is predominantly a freshwater lake. The water in Lake Nhlange appears to be well mixed and has a different ionic composition to the sea water (Kyle and Kwangwanase, 1995). This different composition is because of different ion ratios between sea water and that of Lake Nhlange. The low salinities of Lake Nhlange adversely affect the osmoregulation of many marine fish species that would enter the lake (Kyle and Kwangwanase, 1995).

The water in Kosi Bay is mostly well oxygenated (Kyle and Kwangwanase, 1995). Lake Mpungwini oxygen levels can fall to zero at 9 – 13 metres (m) depending on the season. A depth below 10 m causes bottom waters to become anoxic in winter and may contain hydrogen sulphide. This decrease in oxygen levels is caused by abrupt temperature and salinity layering at this depth (Kyle and Kwangwanase, 1995). These characteristics can act as a barrier to juvenile fish and other small organisms, affecting their movement through the system. Shallow areas represent an important migration route and very few fish are caught at depths deeper than 6 m (Kyle and Kwangwanase, 1995).

Fish community

The changes in the different *in situ* variables in a system place considerable physiological stress and demands on fishes that make use of estuaries for breeding (Harrison and Whitfield, 2006). There is no estuary that is identical to another estuary and this is due to their different biotic and abiotic characteristics (Harrison and Whitfield, 2006). Thus, the ichthyofauna of a certain estuary will differ from another estuary based on the fish community structures (Harrison and Whitfield, 2006). Many studies (Begg, 1984a; Bennett, 1989; Whitfield *et al*., 1994; Harrison and Whitfield 1995; Vorwerk *et al*., 2001, 2003) have been completed on estuarine systems with an emphasis on the fish community structures and functional differences between different fish communities and different estuary systems (Harrison and Whitfield, 2006). Many of these studies on fish community structures have been implemented in the Kosi Bay system (Harrison and Whitfield, 2006). A factor influencing the fish diversity and occurrences in South Africa is the latitude of the estuary. The tropical fish species decline as one moves more to the western coast of South Africa (Harrison and Whitfield, 2006). Tropical waters along the coast of Kosi Bay and the absence of local silt-laden river systems result in a diverse fish fauna (Blaber, 1978). A total of 155 species of fish are associated with southern African estuaries of which 40% are marine species that use estuaries as nursing and feeding areas, 27% live and breed in estuaries, and 25% are marine species that occur in the estuary but do not depend on the system (Ramm *et al*., 2000). The fish species depend on estuaries to live and breed, providing shelter and food, as well as a less stressful environment.

There are a number of factors that determine estuarine fish diversity. Estuary size plays a vital role in the species richness of a system, where larger estuaries will have a bigger degree of marine influence than smaller estuaries (Harrison and Whitfield, 2006). Geomorphology, the width and depth of the estuary mouth, as well as the runoff all have an effect on species richness too. Larger estuaries, such as Kosi Bay have a more diverse habitat than smaller estuaries which also leads to an increased species richness in the system (Harrison and Whitfield, 2006). Estuary mouth formation plays an important role in the community structure of fishes between predominantly open and closed estuaries. A total of 8% of freshwater species also uses estuaries for transit routes (Ramm *et al*., 2000). A sand bar formation can occur across the opening of the mouth and blocks off the pathway for migrating marine fish species (Harrison and Whitfield, 2006).

A total of 124 marine species have been recorded in Kosi Bay where 70% are present in the estuary and on the reef (Blaber, 1978). Some of the common marine species that are found in the system are the Scomberoides (queenfish), *Caranx ignobilis* (kingfish), *Pomadasys commersonni* (spotted grunter), *Rhabdosargus sarba* (Natal stumpnose), *Acanthopagrus berda* (river or sly bream) and various Mugilidae (mullet) (Blaber, 1978). Freshwater species mostly occur in the freshwater lake of Lake Amanzimnyama. Some of the freshwater species that occur in the system are *Oreochromis mossambicus* (Mozambique tilapia), *Tilapia sparmanii* (banded tilapia), *Coptodon rendalli* (red breasted tilapia) and *Pseudocrenilabrus philander* (southern mouth brooder) (Blaber, 1978). Although these species are mostly restricted to Lake Nhlange and Lake Amanzimnyama, *Oreochromis mossambicus* occurs throughout the system (Blaber, 1978).

2.2 Fish species used in the project

2.2.1 Rhabdosargus sarba

Rhabdosargus sarba (Figure 2.1), commonly known as the Natal stumpnose or yellowfin bream is common in subtropical and tropical inshore estuaries throughout the Indo-West Pacific (James *et al*., 2004). It occurs along coastal lakes and estuaries along East Africa and is one of the more common fish species present in KwaZulu-Natal estuaries (Blaber, 1984). This fish species has 6 – 8 incisor teeth followed by 3 – 5 series of strong molars (Heemstra and Heemstra, 2004) and feeds during the morning and early afternoon (Blaber, 1984). The juvenile’s diet consists of aquatic macrophytes, filamentous algae and amphipods (Blaber, 1984). Adults feed on hard-shelled molluscs, sea urchins, sand dwelling crabs and barnacles (Heemstra and Heemstra, 2004). During the late winter and early spring (July to November) *R. sarba* spawns close to the mouth of the estuary and juveniles (approximately 15 – 20 mm standard length (SL) at 2 – 3 months at age) enter the estuary and use the estuary as a nursing area. The juveniles remain in estuaries until they reach maturity before migrating back into the ocean (James *et al*., 2004) where adults are frequently found in coastal waters less than 50 m in depth (Radebe *et al*., 2002). *Rhabdosargus sarba* is a protandrous fish species born male and will change into a female later on in its life (Garratt, 1993). When they reach a total length (TL) of 260 mm they are at

50% sexual maturity (Radebe *et al*., 2002) and as adults they can reach a TL of 750 mm (James *et al*., 2004).

Rhabdosargus sarba are targeted by spear fishermen, shore-anglers and estuarine anglers for recreational fishing (Radebe *et al*., 2002) along the east coast of KwaZulu-Natal (James *et al*., 2004). According to James *et al*. (2001), little research has been done on recreational angling in the Kosi Bay system. Thus, they conducted such a study, based on catch cards filled in by anglers after each angling trip (James *et al*., 2001). Results from this study indicated that 7045 *Rhabdosargus* specimens were caught in the system from 1986 to 1999.

The local community in Kosi Bay are subsistence fishermen that rely on their fish traps throughout the estuary system to catch *R. sarba* (Radebe *et al*., 2002). They are also found in Lake Makhawulani and Lake Mpungwini. Currently, there is no particular closed season in which this fish species is not allowed to be caught and there is no verification that the marine reserves are being sustained or depleted (Radebe *et al*., 2002). Even though this fish species can be caught at any time of the year, there might be some protection in some national reserve parks where fishing is prohibited (Radebe *et al*., 2002). Another protection implemented by the Marine Living Resources Act No. 18 of 1998 is that the bag limit for recreational fishing is set at five per person per day with a minimum size of 250 mm TL (Radebe *et al*., 2002).

According to Radebe *et al*. (2002), some aspects of the biology of *R. sarba* have been studied, but no published information on the age and growth of this species has been recorded. Radebe *et al*. (2002), focused on the estimation of the age of *R. sarba* from KwaZulu-Natal, using otoliths to determine longevity, growth rate and age-at-maturity. A study by Blaber (1984) investigated the feeding ecology of *R. sarba* in Natal estuaries. There was a low concentration of plant materials in the stomach contents of *R. sarba* of the Kosi Bay system, with the most important prey item being the bivalve *Brachidontes virgiliae* followed by a wide variety of benthic invertebrates.

Figure 2.1. *Rhabdosargus sarba*. Common name: Natal stumpnose.

2.2.2 Oreochromis mossambicus

Oreochromis mossambicus (Figure 2.2), commonly known as the Mozambique tilapia is widely distributed (Uchida *et al*., 2000) occurring in the Zambezi tributaries, reaches down into the Bushman's river in the Eastern Cape, and also in the Limpopo and Luvhuhu Rivers which runs next to and through the Kruger National Park (Skelton, 2001). It can be found inland to the lower Orange River and several rivers in Namibia (Skelton, 2001) but do not naturally occur here. This fish species also occurs along the east coast of South Africa in eastward-flowing rivers and occurs along the reed banks in the upper reaches of estuaries and coastal lagoons. Although this fish species adapts well, the *O. mossambicus* prefers slow, standing waters but can be found in faster flowing waters (Skelton, 2001). They adapt well in shallow waters with their movements between shallow and deeper waters being influenced by temperature changes. *Oreochromis mossambicus* prefers warmer waters above 22 °C and can tolerate temperatures up to 42 °C (Skelton, 2001), however, it cannot tolerate temperatures below 15 °C (Skelton, 2001). It is a hardy fish species with a rapid growth rate and has a high tolerance to various environmental changes (Uchida *et al*., 2000).

Oreochromis mossambicus is a maternal mouth-brooder (Barata *et al*., 2007) and may mature and breed within a year of age. Sexual maturity within a year is prone to stunting under adverse or crowded conditions (Skelton, 2001). This fish species grows to about 380 mm; 150 - 160 mm in females and 170 - 180 mm in males, and can attain a SL of 400 mm (Skelton, 2001). Adults have an olive to deep blue grey colour with red margins on the dorsal and caudal fins (Figure 2.2) (Skelton, 2001). During breeding seasons males change colour to a deep greyish black with a white lower head and throat (Figure 2.2) (Skelton, 2001). Spawning of *O. mossambicus* occurs in the summer months when temperatures are suitable and enough food is available. It feeds on algae, insects and diatoms (Skelton, 2001). During the breeding seasons the females visit pits that the males form and use to spawn (Barata *et al*., 2007). The juveniles grow rapidly and form shoals in shallow waters (Skelton, 2001).

A study by Weyl and Hecht (1998) on the biology of *O. mossambicus* in a subtropical lake in Mozambique focussed on age and growth of this species by examining the otoliths. Another study by de Moor *et al.* (1986) focused on the food and feeding habits of *O. mossambicus* in Hartebeespoort Dam. This study examined in detail the feeding behaviour of these species and found that the stomach contents consisted mostly of detritus. Other components within the stomach consisted of plant matter, zooplankton and zoobenthos. A histology based health assessment by Nibamureke *et al.* (2016) was done on *O. mossambicus* from a Dichlorodiphenyltrichloroethane (DDT) - sprayed area in the Limpopo province. Results showed that most values were within normal ranges and only a few pathology indications were present within *O. mossambicus*.

Figure 2.2. *Oreochromis mossambicus*. Common name: Mozambique tilapia. Breeding male with deep greyish black colour and a white lower head and throat.

2.2.3 Terapon jarbua

Terapon jarbua (Figure 2.3), commonly known as the thornfish or target fish, is a small fish with a silvery body and has three or four longitudinal, brownish black stripes along each flank of the fish with black markings on the dorsal and caudal fins (Van der Elst, 1993). From a dorsal view, the stripes present itself as a target; hence the common name for this fish. *Terapon jarbua* has rough ctenoid scales, sharp spines on the dorsal and anal fin, and hard spines on each gill cover (Van der Elst, 1993). It is abundant all year round and has a wide distribution in the estuarine environment where they feed, grow and reproduce (Vijayavel *et al*, 2006). It reaches a mature length of 130 mm and a max length of 360 mm (Froese and Pauly, 2016) and spawns in KwaZulu-Natal in the late spring and summer (Heemstra and Heemstra, 2004).

Terapon jarbua is mostly found in brackish waters and uses estuaries as nursing areas. The thornfish is hardy and is a predator with a very unique feeding strategy. It eats small crabs and shrimps and the scales of other fish which consists mostly of keratin (protein) (Van der Elst, 1993). It waits in shallow depressions for a suitable fish to approach, then creates a cloud of mud by using its caudal fin and ambushes the fish through the cloud, biting pieces of scales off the fish (Van der Elst, 1993). This ambush usually occurs in groups of two to seven. (Heemstra and Heemstra, 2004). *Terapon jarbua* also has a unique defence strategy where it will try to fold its body in half when caught in order to expose its sharp spines, thus making the fish hard to swallow (Van der Elst, 1993).

Whitfield and Blaber (1978) conducted a study on the feeding habits of the marine teleost *Terapon jarbua*. This fish species were collected from St Lucia, Sodwana and the Kosi Bay Estuary. The stomach contents were examined to determine their diet variety. The dominant food items of *T. jarbua* from these regions were fish scales and crustaceans. The fish swallows whole scales and relies on the element of surprise when attacking other fishes (Whitfield and Blaber, 1978).

Figure 2.3. *Terapon jarbua*. Common name: Thornfish or target fish.

2.3 Site selection

The dark green highlighted area on the map (Figure 2.4) is an indication of the whole Kosi Bay system along the east coast of South Africa bordering Mozambique. The same sites were used during each survey and were easily accessible, making sampling more efficient. The sites were chosen in different parts of the system to see if any variations were present between sites and surveys.

Sites chosen for this study were picked throughout the system to show comparisons between the different lakes and channels in the system and for seasonal variations. A total of 12 sites were sampled, one in Lake Amanzimnyama, one on the Malangeni River as it flows into Lake Amanzimnyama, three sites in Lake Nhlange, one site in the channel that connects Lake Mpungwini and Lake Nhlange, one site in Lake Mpungwini, two sites in Lake Makhawulani and three sites throughout the estuary (Figure 2.4). Sediment and water samples were sampled at all of these 12 sites and the fish were caught in the channel between Lake Mpungwini and Lake Nhlange and close to Lake Makhawulani due to a higher success rate and easier access. Two other sites where also sampled for sediment and water close to Lake Amanzimnyama. The first site at the Kushengeza pan and the other in the Malangeni River that flows into Lake Amanzimnyama (Figure 2.4).

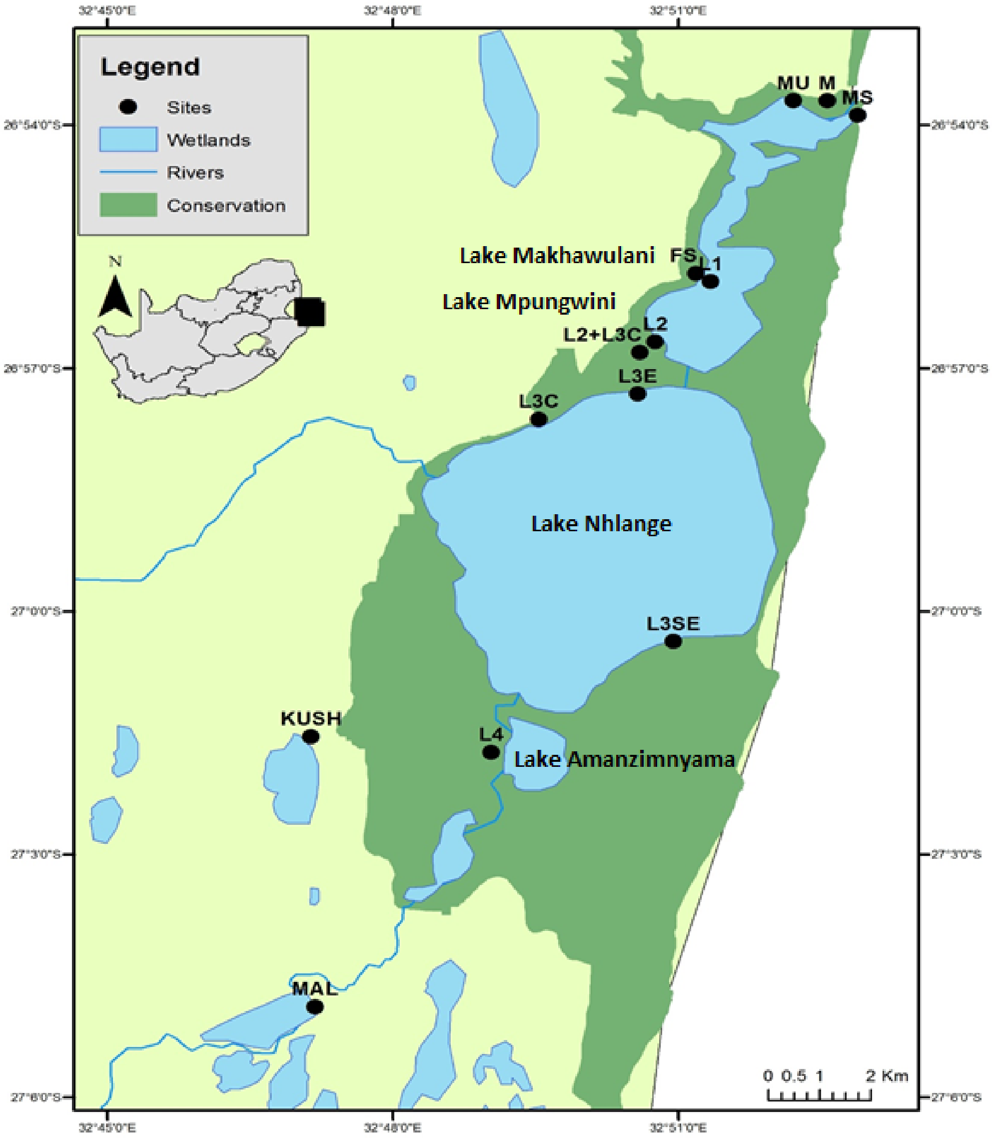

Figure 2.4. Map of the Kosi Bay system and Kushengeza, with the selected sites used during the study. MS – Mouth sea, M – Mouth, MU – Mouth upper, FS – Fisherman's spot, L1 – Lake 1, L2 – Lake 2, L2+L3C – Channel linking Lake 2 and 3, L3E – Lake 3 entrance, L3C – Lake 3 campsite, L3SE – South east of Lake 3, L4 – Lake 4, KUSH – Kushengeza and MAL – Malangeni.

Kushengeza and Lake Sibaya (Figure 2.4 and Figure 2.5) are not part of the Kosi Bay system but were sampled due to the fact that these coastal lakes are close to Kosi Bay and have similar anthropogenic pressures than seen at Kosi Bay. A total of four sites were sampled at Lake Sibaya and one in Kushengeza (Figure 2.4 and Figure 2.5).

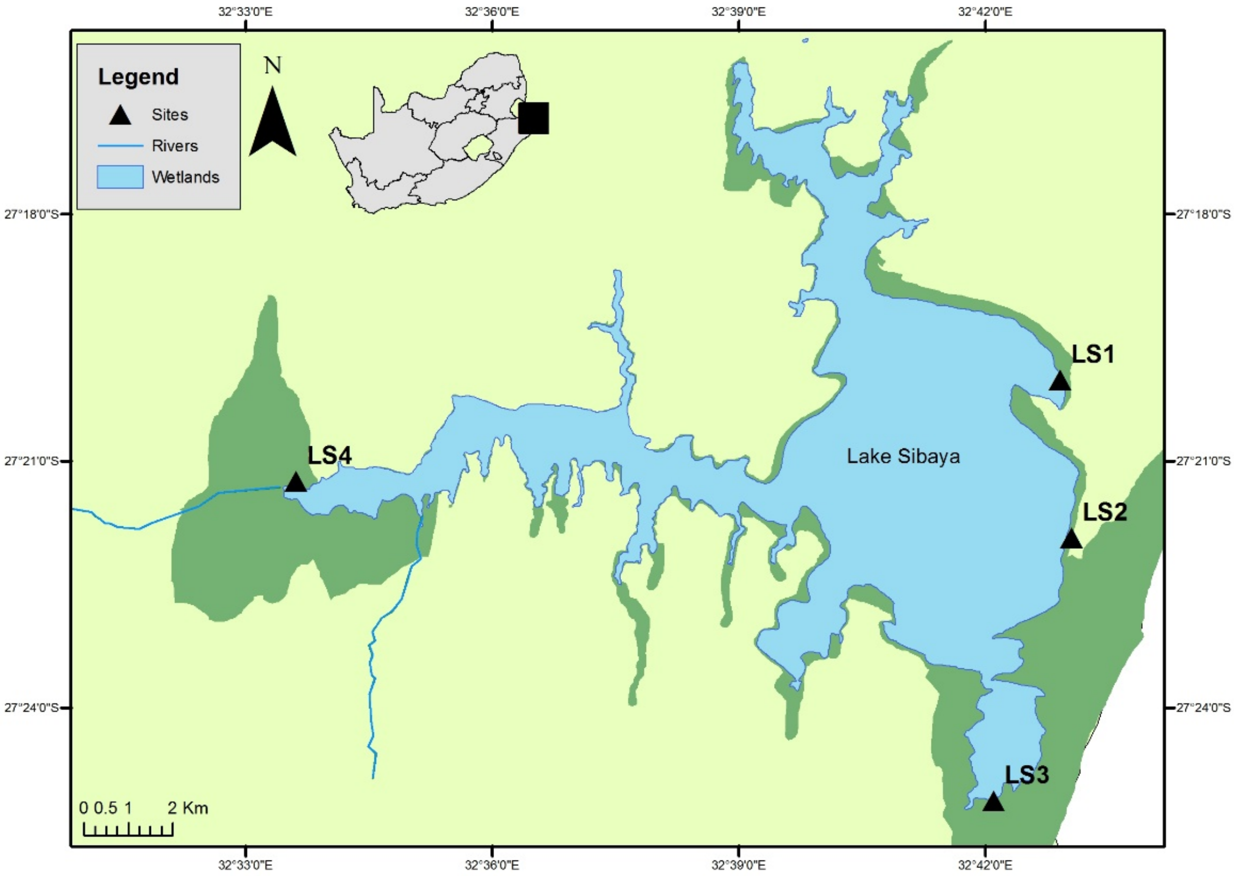

Figure 2.5. Map of the Lake Sibaya system and selected sites. LS1 – Lake Sibaya 1, LS2 – Lake Sibaya 2, LS3 – Lake Sibaya 3 and LS4 – Lake Sibaya 4.

2.3.1 Site names with coordinates:

2.3.1.1 Kosi Bay

The MS site (sea sample at mouth) is situated at the entrance of the system in the most north-eastern part of the system (Figure 2.6 and Table 2.1). The M site is close to the MS site and has a reef outcrop next to the forest dune along the coast (Figure 2.6 and Table 2.1). The MU site consists mostly of fish traps (this is where the majority of the fish traps start) with very few fish habitats except open water and benthic sediments (Figure 2.6 and Table 2.1). The FS site also consists of numerous fish traps with reeds along the banks and it is the main entrance for the local community to reaching the fish traps (Figure 2.6 and Table 2.1).

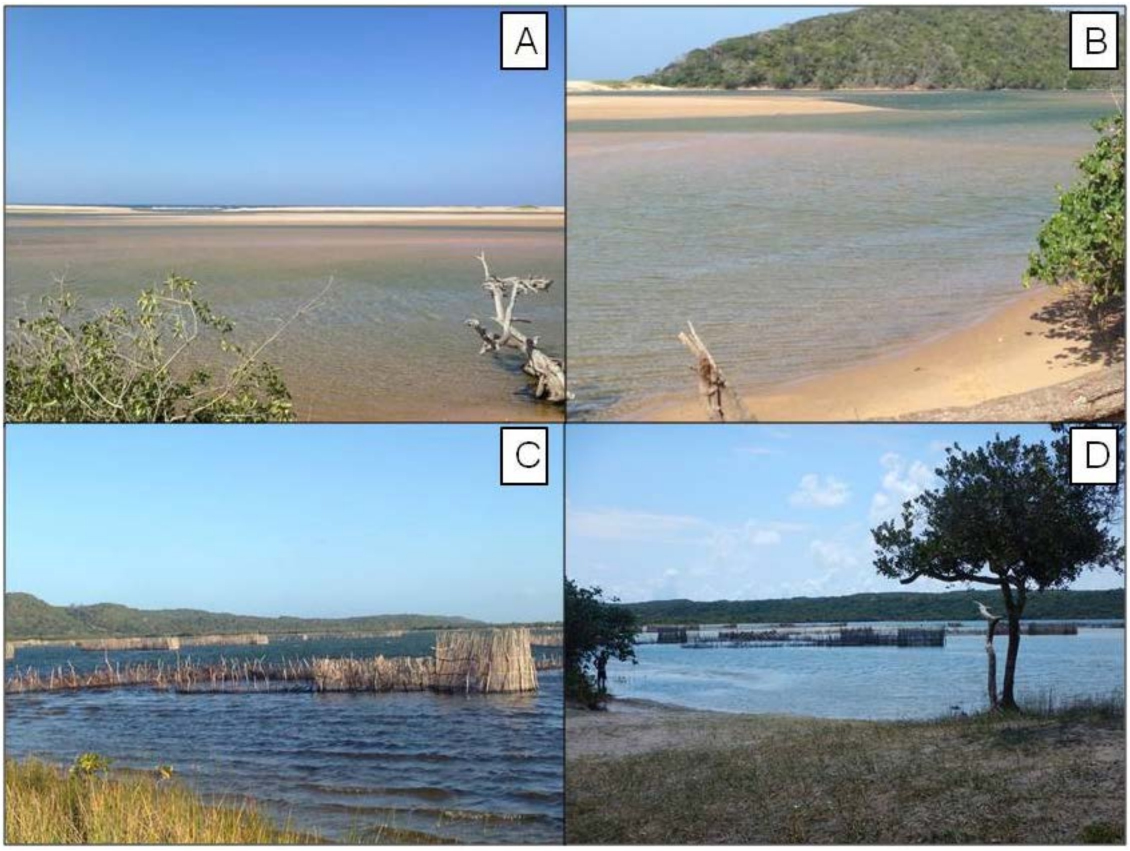

Figure 2.6. Selected sites sampled in the Kosi Bay system during the August 2015, December 2015 and February 2016 surveys. (A): MS – Mouth sea, (B): M - Mouth, (C): MU – Mouth upper, (D): FS – Fisherman's spot.

Table 2.1. GPS coordinates of all the sampled sites (site abbreviations used further are indicated in bold) in the Kosi Bay system during the August 2015, December 2015 and February 2016 surveys.

Site Name	Lake	South	East
Mouth (Sea) - **MS**	Estuary (sea)	S26.89797°	E32.881408°
Mouth - **M**	Estuary	S26.89494°	E32.87612°
Mouth upper - **MU**	Estuary	S26.89497°	E32.87014°
Fisherman's Spot - **FS**	Between upper estuary and Lake Makhawulani	S26.93049°	E32.85312°
Lake 1 - **L1**	Lake Makhawulani	S26.93220°	E32.85567°
Lake 2 - **L2**	Lake Mpungwini	S26.94457°	E32.84597°
Lake 2+3 (Channel) - **L2+L3C**	Channel between Lake Mpungwini and Lake Nhlange	S26.94678°	E32.84328°
Lake 3 (Entrance) - **L3E**	Entrance to Lake Nhlange	S26.95531°	E32.84292°
Lake 3 (Campsite) - **L3C**	North Western bank of Lake Nhlange	S26.96048°	E32.82555°
Lake 3 (South East) – **L3SE**	South eastern bank of Lake Nhlange	S27.00617°	E32.84913°
Lake 4 – **L4**	Lake Amanzimnyama	S27.02898°	E32.81723°
Malangeni - **MAL**	Malangeni River	S27.08135°	E32.78638°

The L1 site had extensive mangrove communities present on the banks of the lake (Figure 2.7 and Table 2.1). Fish traps are also present at this site. The L2+L3C site is situated within the channel between Lake Mpungwini and Lake Nhlange where increased marginal vegetation is present (Figure 2.7 and Table 2.1). The channel is not very deep (approximately 3 m at maximum) and it includes a high variety of habitats for fish living in the channel or passing through. The L2 site is a big open lake with marginal vegetation on the banks of the lake with a few fish traps present (Figure 2.7 and Table 2.1). The L3E site is the entrance to Lake Nhlange from the channel between Lake Mpungwini and Lake Nhlange and it is where the fish trap structures stop (Figure 2.7 and Table 2.1).

Figure 2.7. Selected sites sampled in the Kosi Bay system during the August 2015, December 2015 and February 2016 surveys. (A): L1 – Lake 1, (B): L2+L3C - Channel linking Lake 2 and 3, (C): L2 – Lake 2, (D): L3E – Lake 3 entrance.

The L3C site is in Lake Nhlange at the Ezemvelo KZN Wildlife campsite (Figure 2.8 and Table 2.1). There are mangrove communities with reeds along the campsite area and around the rest of the lake. The L3SE site is on the south eastern side of Lake Nhlange close to Lake Amanzimnyama with marginal vegetation on the banks (Figure 2.8 and Table 2.1). The L4 site is a freshwater lake with aquatic vegetation (mostly reeds) and high coastal palm trees (Raffia palms) around the lake (Figure 2.8 and Table 2.1). This lake is home to many *Hippopotamus amphibius* (hippopotamus) and *Crocodylus niloticus* (crocodile). Site MAL is a freshwater stream that enters Lake Amanzimnyama with aquatic and marginal vegetation along the banks of the stream (Figure 2.8 and Table 2.1).

Figure 2.8. Selected sites sampled in the Kosi Bay system during the August 2015, December 2015 and February 2016 surveys. (A): L3C – Lake 3 campsite, (B): L4 – Lake 4, (C): L3SE - South east of Lake 3, (D): MAL - Malangeni.

2.3.1.2 Other coastal lakes

The LS1 site in Lake Sibaya was on the north-eastern side of the system which had tall reeds and grass on the banks (Figure 2.9 and Table 2.2). The LS2 site was on the eastern side of the system with almost no vegetation in the water (Figure 2.9 and Table 2.2). The LS3 site was on the south-eastern side of the system and had also no vegetation in the water. The LS2 and LS3 sites only had vegetation higher up on the banks (Figure 2.9 and Table 2.2). The final site was LS4 which was in the far western side of the system and had lots of reeds and grass with aquatic vegetation (Figure 2.9 and Table 2.2).

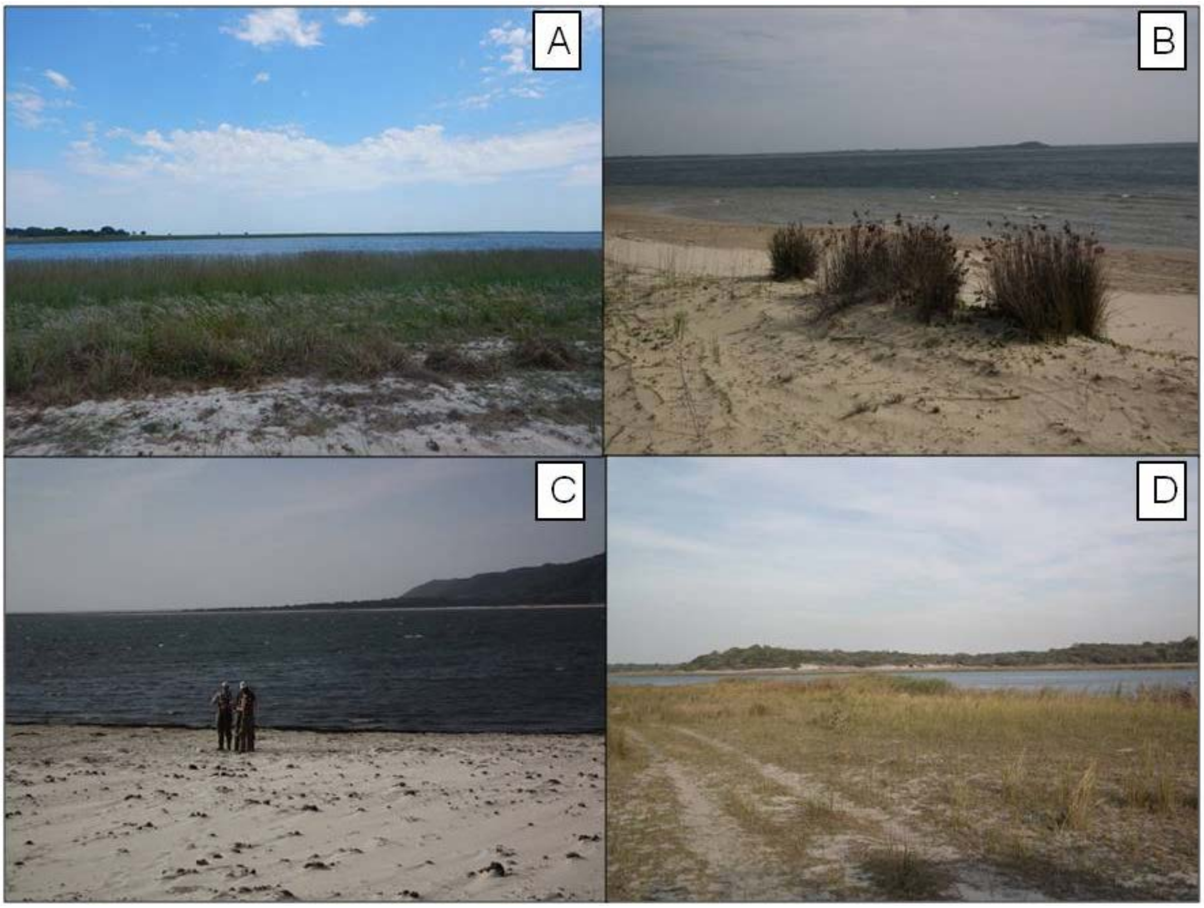

Figure 2.9. Selected sites sampled in the Lake Sibaya system during the August 2015, December 2015 and February 2016 surveys. (A): LS1 – Lake Sibaya 1, (B): LS2 – Lake Sibaya 2, (C): LS3 – Lake Sibaya 3, (D): LS4 – Lake Sibaya 4.

The KUSH site (Figure 2.10) is a pan on the western side outside the Kosi Bay system with limited vegetation in the area (Figure 2.4). The only vegetation growing on the banks and edge of the water is tall grasses.

Figure 2.10. (A): Kushengeza site (KUSH) sampled near the Kosi Bay system during the August 2015, December 2015 and February 2016 surveys.

Table 2.2 GPS coordinates of all the sampled sites (site abbreviations used further are indicated in bold) in other coastal Lakes near Kosi Bay in the August 2015, December 2015 and February 2016 surveys.

Site Name	**Lake**	**South**	**East**
Lake Sibaya 1 - **LS1**	Lake Sibaya site	S27.33361°	E32.71512°
Lake Sibaya 2 - **LS2**	Lake Sibaya site	S27.36564°	E32.71747°
Lake Sibaya 3 - **LS3**	Lake Sibaya site	S27.41886°	E32.70162°
Lake Sibaya 4 - **LS4**	Lake Sibaya site	S27.35416°	E32.56028°
Kushengeza - **KUSH**	Kushengeza pan (west of Lake Amanzimnyama)	S27.02577°	E32.78570°

3. Water and sediment quality

3.1 Introduction

Water quality is a term used to describe the chemical, physical and biological characteristics of water (DWA, 2009) and can be affected by dissolved or suspended substances in water. Water characteristics are influenced by different factors, namely: natural factors such as seasonal changes, geological changes from types of soils and human actions including development, mining and recreation. The effects of polluted water on an ecosystem and specifically on biota can have negative impacts on the aquatic ecosystem (DWA, 2009).

Water quality can change over short periods of time but this does not always pose a major threat to fish in a system as the fish are mobile and can avoid areas that are deteriorating (Ramm and Cooper, 2000). The long term water quality changes are often more detrimental to fish communites. Metals in the environment can accumulate to a toxic concentration and can cause ecological damage (Karadede and Ünlü, 2000). Kosi Bay is a major water resource which falls under the Usutu to Mhlathuze water management area (WMA).The Swamanzi and Malangeni are the main contributing rivers to the Kosi Bay system within the WMA (DWA, 2014).

Kosi Bay receives its sediment from various sources in the area, including sand entering into the estuary mouth on the flood tide, floods that bring in sediment from the catchment, sediment being deposited from biological activity and from bank erosion (Wright *et al*., 1997). Kosi Bay has a completely sandy substrate throughout the system with very little habitat modification although the channels that link the lakes are modified due to speedboats that use these channels (Wright *et al*., 1997). Almost all of the sediment throughout the system is fine to medium size grains (Wright *et al*., 1997). Grain size distributions are an indication of the physical characteristic of the sediment and these characteristics determine the biological assemblage (Venter and van Vuren, 1997). Coarse sediment can be an indicator of sediment transport in a system as smaller particles are more easily transported. Sediment can accumulate pollutants and have an effect on biota, especially fish that feed off the benthic ecosystem. Fish in the ecosystem are exposed to these pollutants from direct contact or via pollutants that leach into the aqueous phase (Chen and Chen, 1999).

The assessment of water and sediment quality can provide a general indication of what the health of that specific environment is or might be in the future. The target water quality range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems is used by the Department of Water and Sanitation (previously Department of Water Affairs and Forestry) for the protection of freshwater ecosystems (DWAF, 1996). These guidelines are used as a source of reference information for the management and protection of aquatic ecosystems in South Africa. As this project takes place in an estuarine habitat, the TWQR was only used for Lake Amanzimnyama, Lake Sibaya and Kushengeza pan which are all freshwater. The TWQR is for freshwater and thus is only used for these lakes. The Canadian water and sediment quality guidelines for the protection of marine aquatic life (CCME) were used in this project as a source of reference information (CCME, 2016). The CCME provides science-based goals for aquatic and terrestrial ecosystems and is an intergovernmental forum that has a goal in addressing environmental issues of national and international concern (CCME, 2016). The ERL (effects range-low) and ERM (effects range-medium) guidelines were used to show if any biological effects within the ranges of chemical concentrations in marine and estuarine sediments were present. These guidelines will be discussed later in this chapter.

The Australian and New Zealand environmental and conservation council (ANZECC) guidelines for water quality were used in this project as another source of reference information. The goal of the ANZECC is to protect their water resources and enhancing their quality while maintaining economic and social development. The ANZECC incorporates scientific, international and national information documents which are easy to read and understandable (ANZECC, 1994).

The aim of this part of the project was to compare the water and sediment quality between the different sites and surveys, as well as to determine any potential impact of metals on the fish of the Kosi Bay system. To achieve this aim, nutrients and trace metal concentrations were determined in the Kosi Bay system as well as trace metals from sediments. The results of these assessments were then compared to the relevant guidelines. In addition, nutrients and trace metal concentrations from the water and sediment were also assessed for Lake Sibaya.

3.2 Material and methods

3.2.1 Water Quality

3.2.1.1 Sampling protocol

The assessment of water quality in the Kosi Bay system was completed at 12 sites; four sites at Lake Sibaya and one from Kushengeza were also assessed as described in Chapter 2. Sampling took place during August 2015, December 2015 and in February 2016. These sampling months were chosen to determine temporal variation within the system. The water samples were collected from below the surface (approximately 15 cm) in polyethylene 500 mL bottles. The method for collecting water samples was adapted from the Gilbert and Avenant-Oldewage (2014) study. The water samples were frozen for transport back to the laboratory for further analyses.

3.2.1.2 Laboratory analyses

The water from the selected sites was filtered with a 0.45 µm vacuum assisted filter (Demirak *et al*., 2006) (Figure 3.1) to determine the dissolved metals in the samples. Filtered samples were analysed with an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500ce). The water samples from L1, L2, L2+L3C, L3E, L3SE and L3C (first six sites) were diluted 20 times to reduce the salinity in the samples. The nutrients of the samples were analysed in the laboratory using various nutrient test kits and a Merck SpectroQuant (Pharo 300). Nutrients tested for included ammonium (1.14752.0001), chloride (1.14897.0001), nitrate (1.09713.0001), nitrite (1.1476.0001), sulphate (1.14791.0001) and phosphate (1.14848.0001).

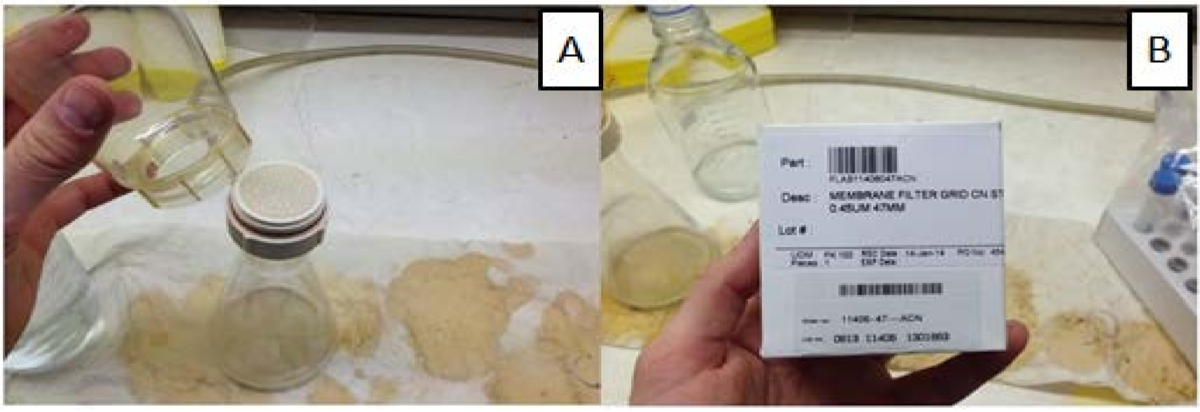

Figure 3.1. Water filtering procedure. (A): Glass test tube used to filter water. (B): filters (0.45 µm) used for water filtration.

3.2.1.3 Statistical analysis

Using GraphPad Prism 5, a one-way Analysis of variance (ANOVA) was performed on the nutrient results and the metals within the water. Significant differences between the sites were determined using the Tukey posthoc analysis with a value of $p < 0.05$ (Gerber *et al*., 2015).

3.2.2 Sediment quality

3.2.2.1 Sampling protocol

Sediment from the Kosi Bay system was collected with a Petit Ponar sediment grab and placed in polyethylene jars. Collections were made during August 2015, December 2015 and February 2016 at 12 Kosi Bay sites, four Lake Sibaya sites and one Kushengeza site (see Chapter 2). Collected samples were frozen for transport back to the laboratory for further analyses.

3.2.2.2 Laboratory analyses

In the laboratory, sediment samples were defrosted and oven dried for four days at 60 °C. Sediment samples were digested in a Milestone Microwave Digestion system following an adapted method from Gerber *et al.* (2015). Sediment samples were weighed to 0.2 gram (g) and added to the Teflon digestion vessel. Seven ml of 65% nitric acid was added to the vessel and left for 10 minutes (min) for digestion to start. Following the 10 min waiting period, the vessels were closed and placed into the microwave. The digestion run was set at 40 min with a maximum temperature of 200 °C. The samples were transferred into 50 milliliters (ml) volumetric flasks and made up to 50 ml with 1% nitric acid. Before the ICP-MS reading, the samples were first filtered with a 0.45 Micrometre (µm) filter. Filtered samples were analysed for metal concentrations using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500ce). A certified reference material (CRM) of sediment was used as a reference.

A total of 30 g of the remaining dried sediment for each site was weighed and used to determine the grain size distribution. A Clear Edge Test sieve system (ISO 3310-1) was used with the various sieves, ranging from 53 µm to 4000 µm.

Table 3.1 Sediment grain size classification system (from Cyrus *et al*. 2000).

Grain size (µm)	Sediment type
>4000 µm	Gravel
4000 µm – 2000 µm	Very coarse sand
2000 µm – 500 µm	Coarse sand
500 µm – 212 µm	Medium sand
212 µm – 53 µm	Very fine sand
< 53 µm	Mud

Grain sizes were determined with the aid of a classification table (Table 3.1). The value of 30 g was chosen for this study to keep percentage calculations consistent. After the sediment sample was added to the sieve system, it was placed in the King Test VB 200 300 sieve shaker for 20 min. After the 20 min, each sieve was weighed and the percentage of each sieve was calculated (Cyrus *et al.,* 2000).

3.2.2.3 Statistical analysis

Using GraphPad Prism 5, a one-way analysis of variance (ANOVA) was performed on the metals within the sediment. Significant differences between the sites were determined using the Tukey posthoc analysis with a value of $p < 0.05$ (Gerber *et al*., 2015).

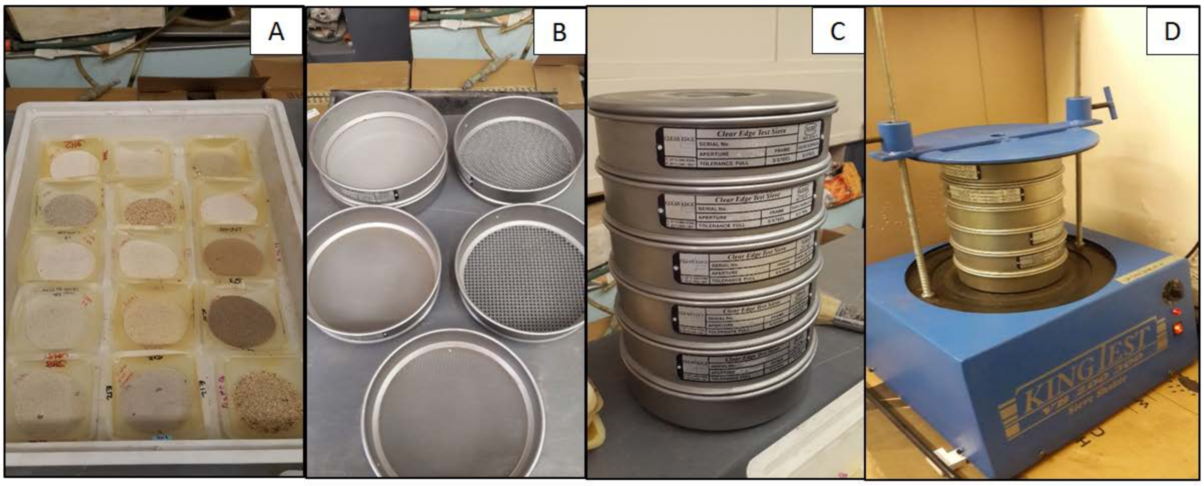

Figure 3.2. Procedure for determining grain size. (A): Sediment weighed to 30 g for each site. (B): Different sieve sizes. (C): The Clear Edge Test sieve system fitted together ranging from 4000 μm to 53 μm (Table 3.1). (D): Clear Edge Test sieve with the added sediment

3.3 Results

3.3.1 Water quality

Water samples from Kosi Bay system (12 sites) and other coastal lakes (four Lake Sibaya sites and one Kushengeza site) were tested for ammonium, chloride, nitrate, nitrite, sulphate and phosphates (Figures 3.1).

Mean nutrient concentrations for the Kosi Bay system are presented in the Appendix B. There were no significant ($p > 0.05$) differences between the sites for ammonium. Chloride had a significant difference between the first ten sites and the last two sites (L4 and MAL) (Figure 3.3). There was also a significant difference between L4 and MAL (Figure 3.3). Nitrate levels were higher in the M and MU site but it was not significant to the other sites (Figure 3.3). Nitrite levels were higher in L3SE (0.055 mg/L) and in L4 (0.051 mg/L), but had no significant difference to the other sites (Figure 3.3). Sulphate levels were > 300 mg/L for the first six sites and had significant differences between these six sites and the last three sites; L3SE (169.3 mg/L), L4 (31.67 mg/L), MAL (23.3 mg/L) (Figure 3.3). Phosphate levels were the highest in L3SE (0.69 mg/L) and had a significant difference between L3SE and the rest of the sites but had no significant difference to L4 (0.45 mg/L) (Figure 3.3).

Mean nutrient concentrations for the Lake Sibaya system and Kushengeza are presented in the Appendix B. Ammonium levels for LS2 and KUSH were higher than the rest of the sites but had no significant difference ($p < 0.05$) between these sites (Figure 3.4). Chloride levels had the same trend with no significant difference between these sites (Figure 3.4). Nitrate levels for KUSH were higher than the Lake Sibaya sites and had a significant difference to all of the Lake Sibaya sites (Figure 3.4). Nitrite levels in LS1 were higher than the rest of the sites of Lake Sibaya and KUSH but there was no significant difference between all of these sites (Figure 3.4). Lake Sibaya sites had higher levels of sulphates than KUSH with only LS3 and KUSH having a significant difference ($p < 0.05$) (Figure 3.4). Phosphate levels of KUSH were lower than the Lake Sibaya sites but had no significant difference between these sites (Figure 3.4).

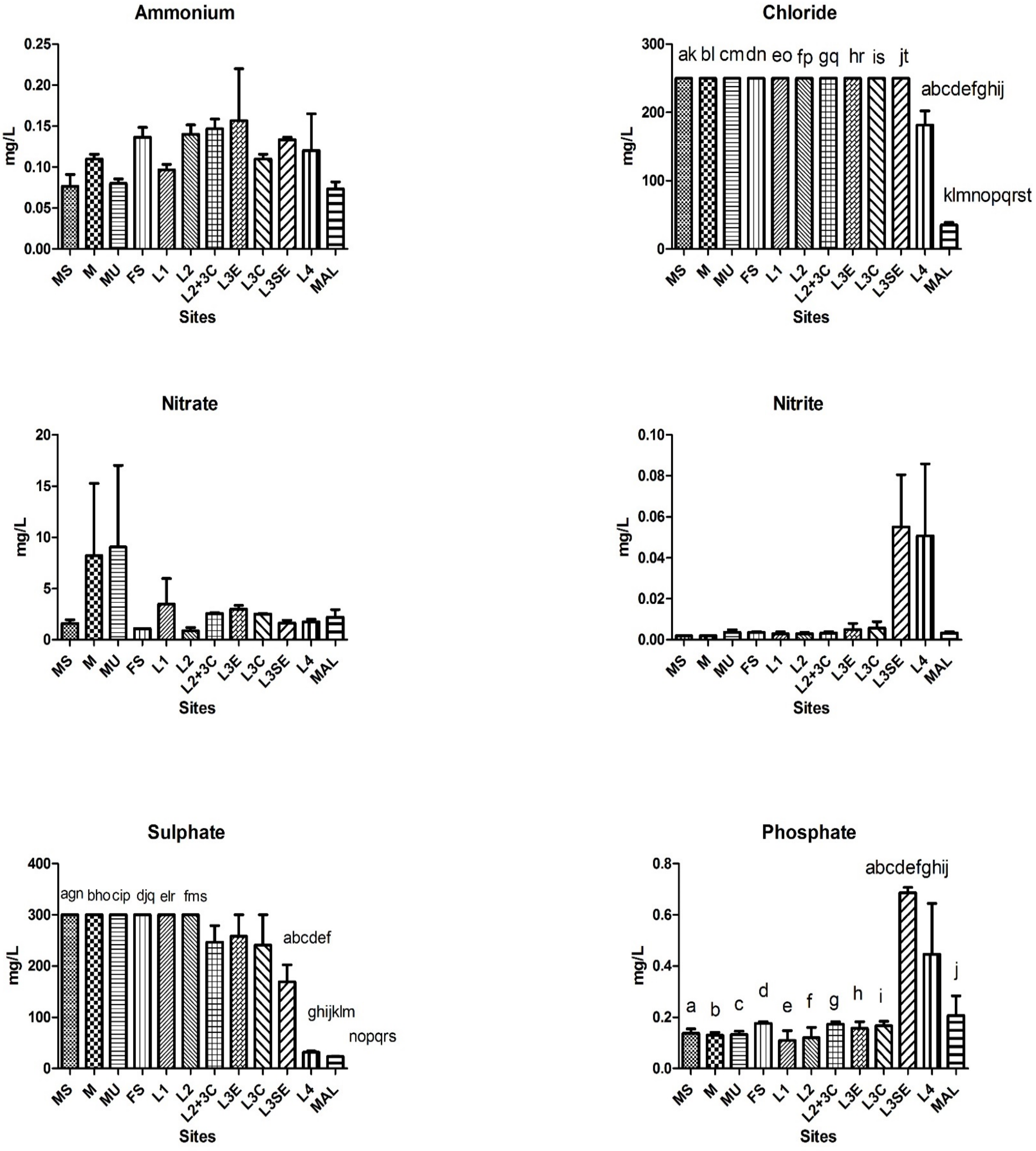

Figure 3.3. Mean concentrations (mg/L) of ammonium, chloride, nitrate, nitrite, sulphates and phosphates present in the water samples of the Kosi Bay system for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. Similar letters indicate significant differences ($p < 0.05$) between those sites.

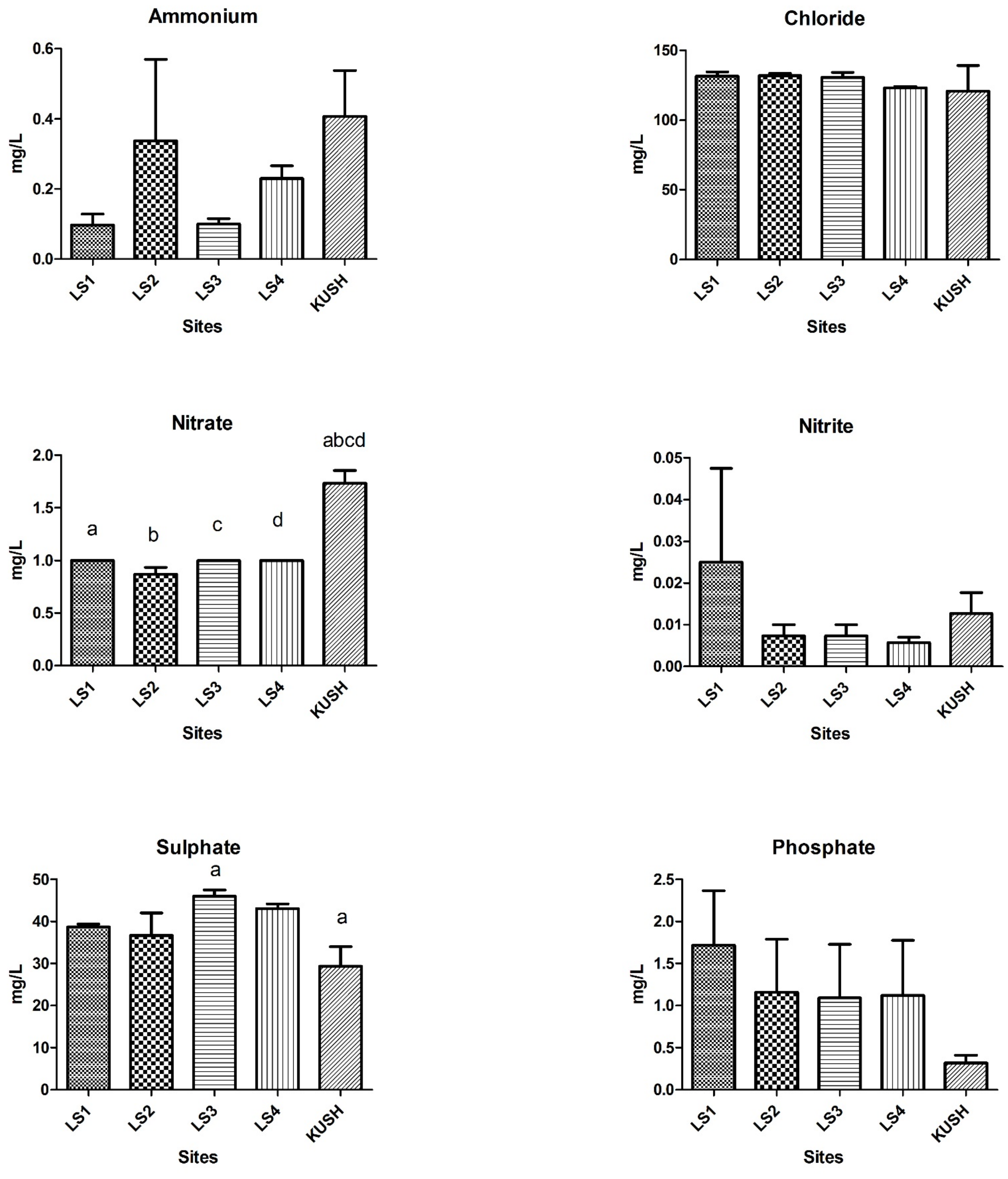

Figure 3.4. Mean concentrations (mg/L) of ammonium, chloride, nitrate, nitrite, sulphate and phosphate present in the water samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. Similar letters indicate significant differences ($p < 0.05$) between those sites.

Aluminium (Al), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), strontium (Sr), cadmium (Cd), mercury (Hg) and lead (Pb) metal concentrations were tested in the water samples from the different lakes in the Kosi Bay system (Figures 3.5 – 3.7). All of the metal concentrations are expressed as milligram per litre (mg/L). There were no significant differences ($p < 0.05$) between the sites for Al, Mn, Ni, Zn, As, Cd and Hg (Figure 3.5, 3.6 and 3.7). There was a significant difference ($p < 0.05$) between L1 and L4 and between L1 and MAL for Cr (Figure 3.5). Both L1 and L2+3C had a significant difference between L4 and MAL for Fe (Figure 3.5). Both L4 and MAL had significant differences between them and L1, L2, L2+3C, L3, L3SE and L3C for Co (Figure 3.5). There was a significant difference between L1 and L3SE, L3C, L4 and MAL for Cu (Figure 3.6). Lake 2 had a significant difference between L4 and MAL for Cu (Figure 3.6). Both L1 and L2+3C had significant differences between them and L4 and MAL for Se and Sr (Figure 3.6). Lake 4 and MAL had significant differences between them and L1, L2, L2+3C, L3E, L3SE and L3C for Pb (Figure 3.7). The metal concentration trends were the same for all metals, with higher concentrations in the first few sites and lower concentrations further upstream in the system.

Aluminium, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Hg and Pb metal concentrations were also tested in the water samples of August 2015, December 2015 and February 2016 for Lake Sibaya and Kushengeza (Figures 3.8 – 3.10).

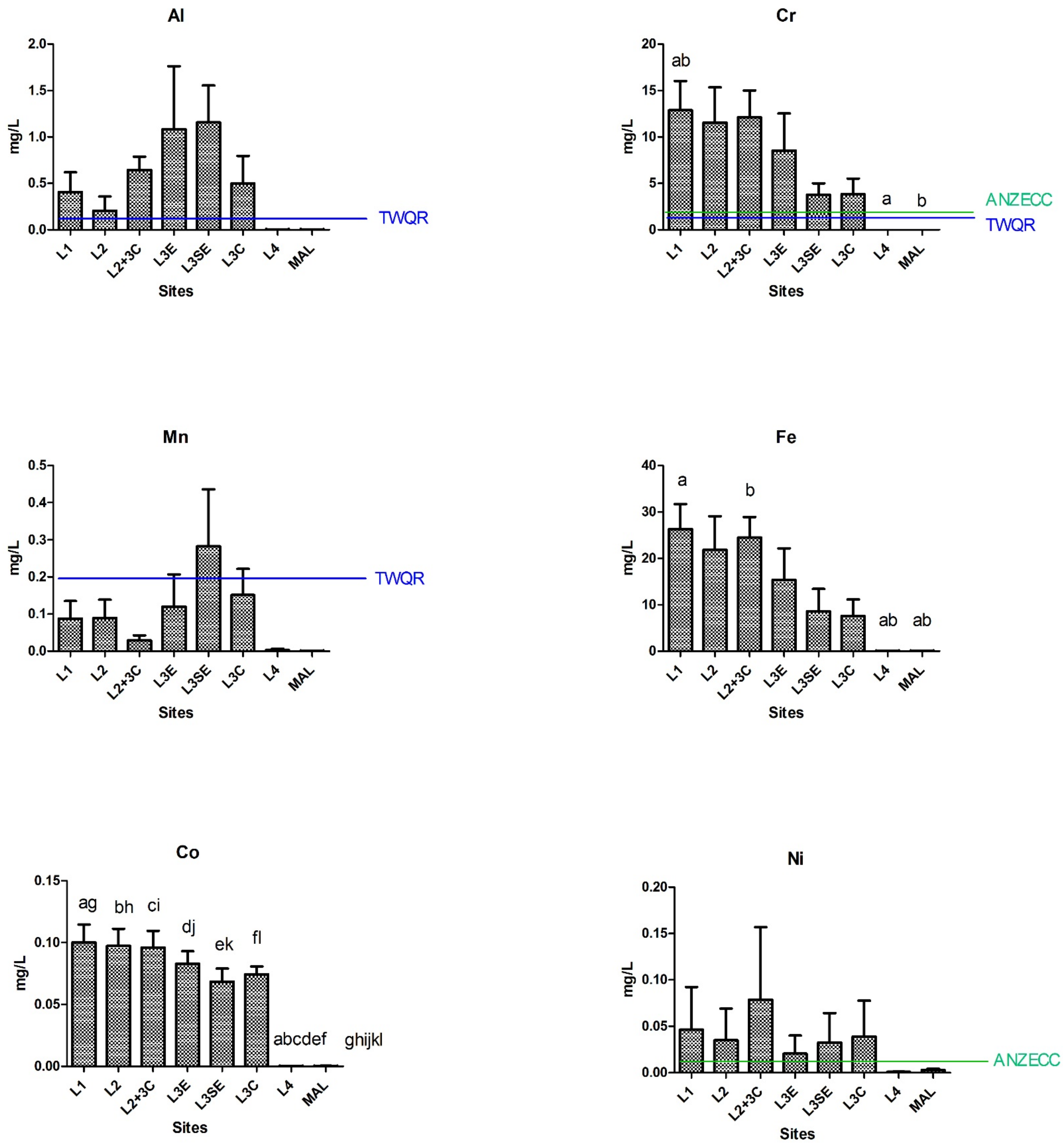

Figure 3.5. Mean metal concentrations (mg/L) of Al, Cr, Mn, Fe, Co and Ni present in the water samples of the different Kosi Bay lakes with Lake 4 and Malangeni for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The green line indicates the Australian and New Zealand environmental and conservation council (ANZECC) guidelines for water quality guidelines. Similar letters indicate significant differences ($p < 0.05$) between those sites for a specific metal.

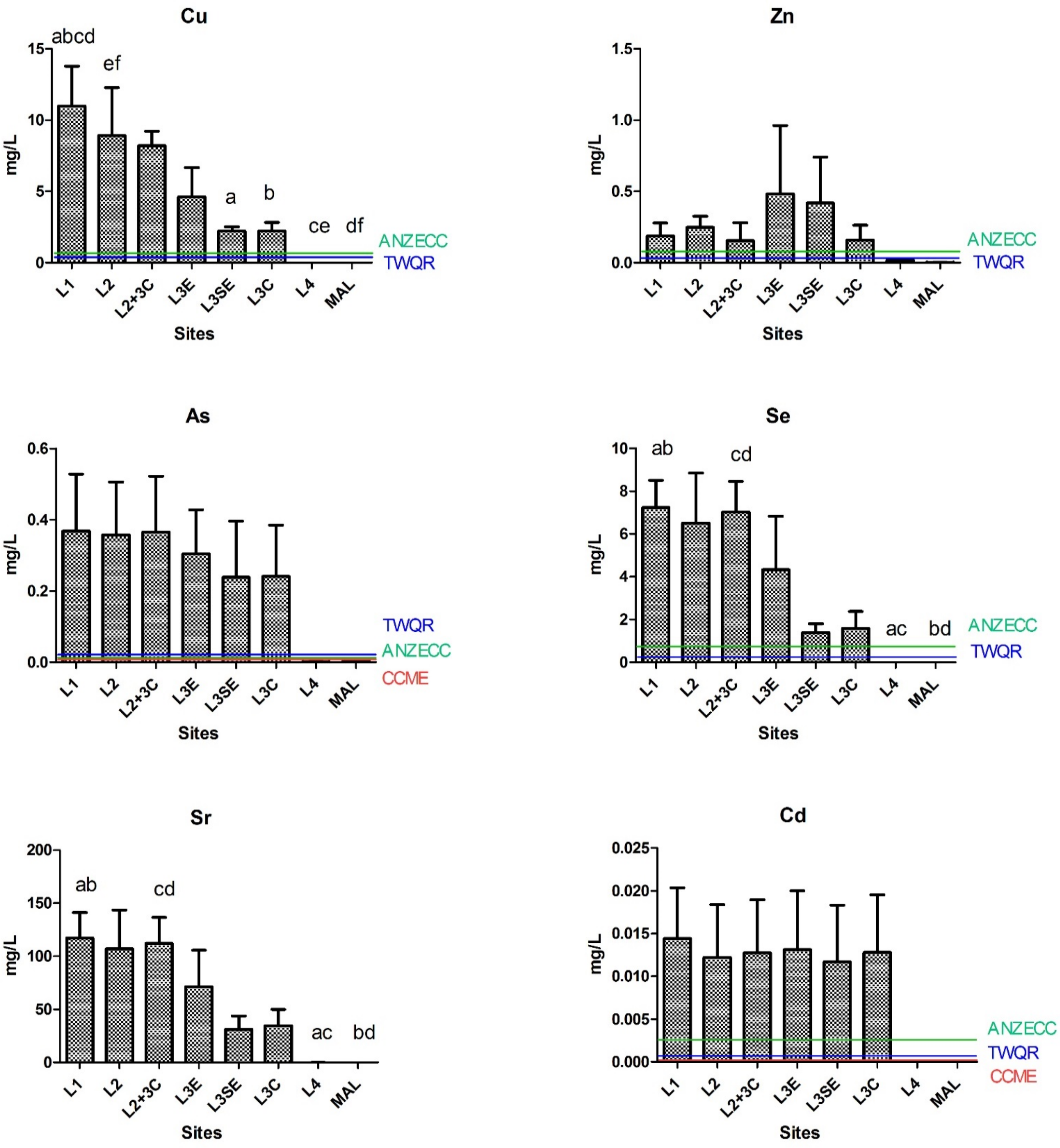

Figure 3.6. Mean metal concentrations (mg/L) of Cu, Zn, As, Se, Sr and Cd present in the water samples of the different Kosi Bay lakes with Lake 4 and Malangeni for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The green line indicates the Australian and New Zealand environmental and conservation council (ANZECC) guidelines for water quality guidelines. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Similar letters indicate significant differences ($p < 0.05$) between those sites for a specific metal.

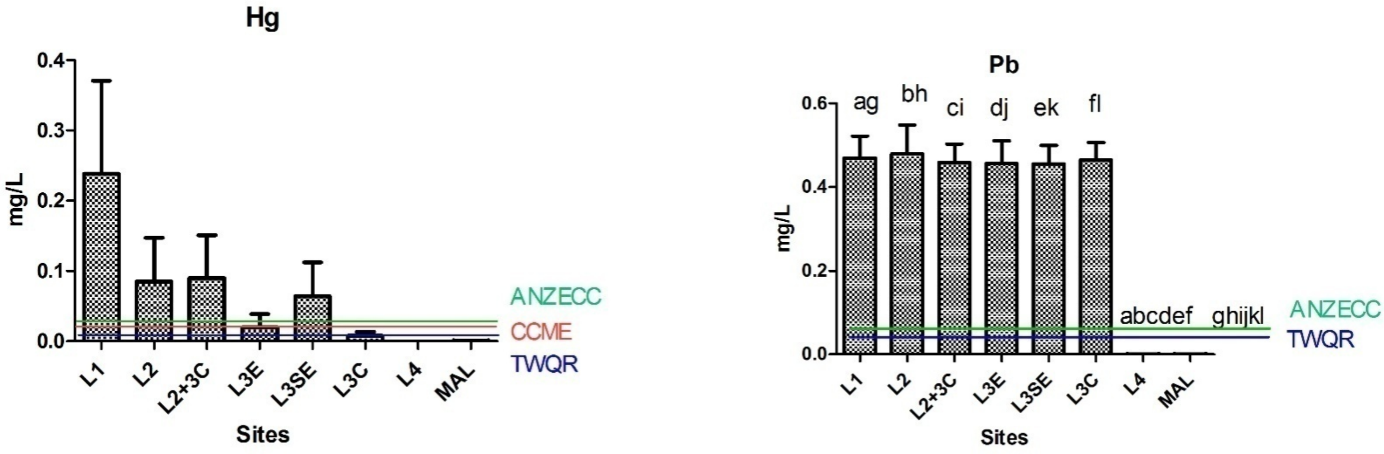

Figure 3.7. Mean metal concentrations (mg/L) of Hg and Pb present in the water samples of the different Kosi Bay lakes with Lake 4 and Malangeni for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The green line indicates the Australian and New Zealand environmental and conservation council (ANZECC) guidelines for water quality guidelines. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Similar letters indicate significant differences ($p < 0.05$) between those sites for a specific metal.

There were significant differences ($p < 0.05$) between Kushengeza and all of the Lake Sibaya sites for Al, Mn, Fe, Co and Pb (Figure 3.8 and 3.10).

Metal concentrations in the water samples of Kosi Bay were also compared to Kushengeza and Lake Sibaya for any significant differences ($p < 0.05$). There was a significant difference between L1, L2 and L2+3C to all of the Lake Sibaya sites and Kushengeza for Cr, Fe, Cu, Se and Sr. There was also a significant difference between L1, L2, L2+3C, L3E and L3SE to all of the Lake Sibaya sites and Kushengeza for Co and Pb

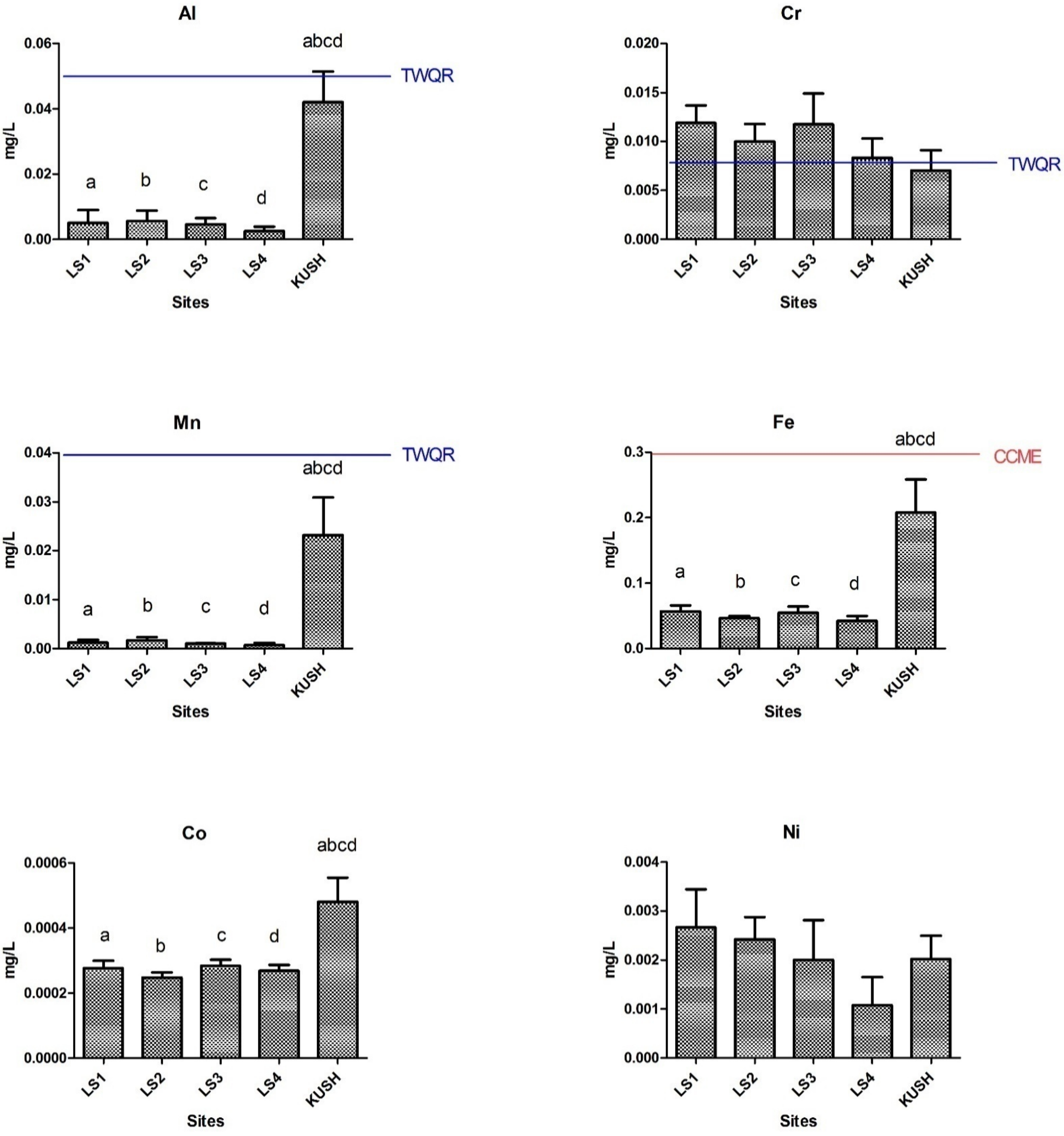

Figure 3.8. Mean metal concentrations (mg/L) of Al, Cr, Mn, Fe, Co and Ni present in the water samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Similar letters indicate significant differences ($p < 0.05$) between those sites for a specific metal.

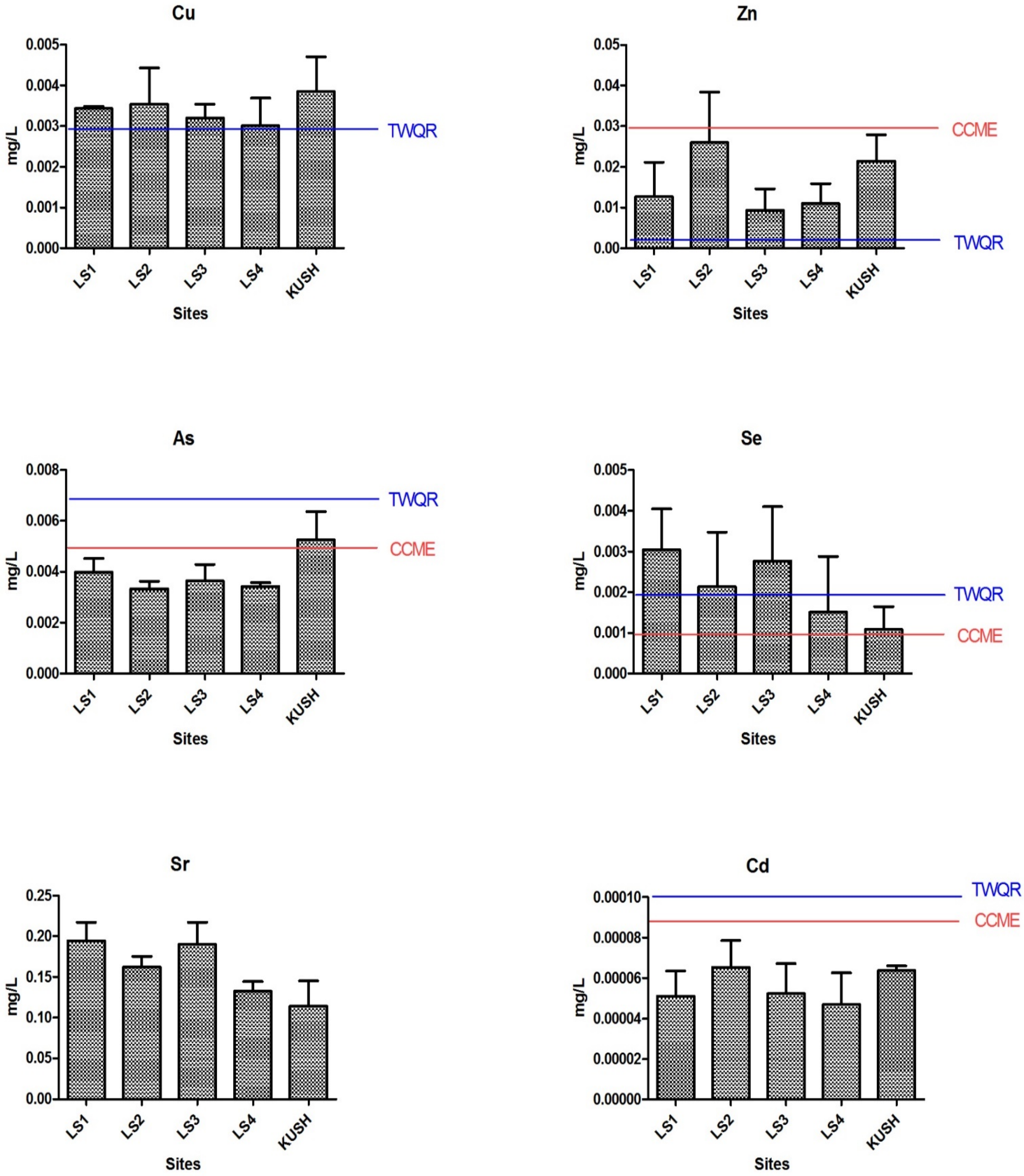

Figure 3.9. Mean metal concentrations (mg/L) of Cu, Zn, As, Se, Sr and Cd present in the water samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life.

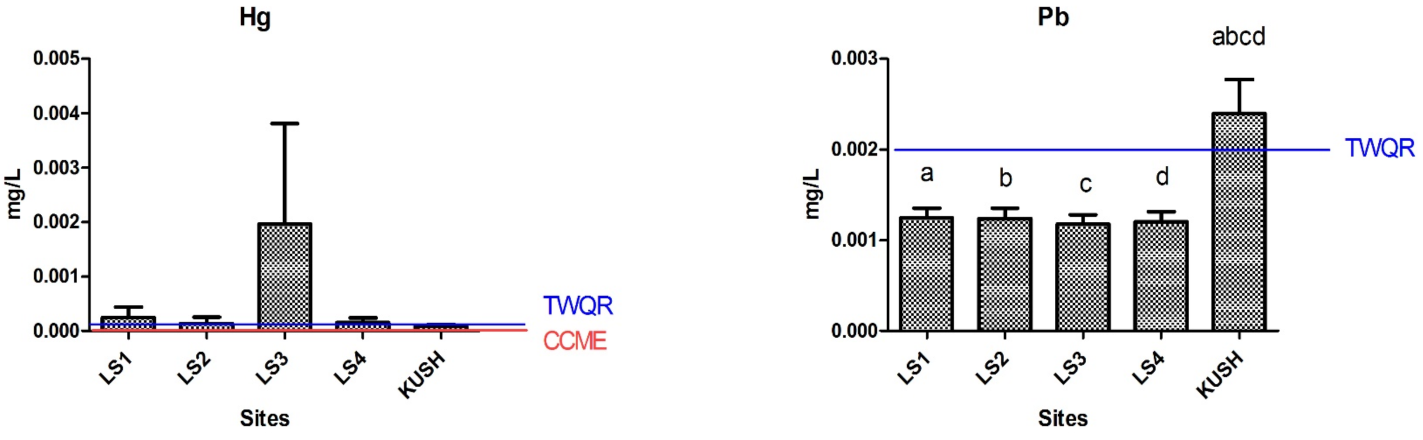

Figure 3.10. Mean metal concentrations (mg/L) of Hg and Pb present in the water samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The blue line indicates the target water quilty range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Similar letters indicate significant differences ($p < 0.05$) between those sites for a specific metal.

3.3.2 Sediment quality

Aluminium, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Hg and Pb were tested in the sediment samples from the Kosi Bay system of August 2015, December 2015 and February 2016 (Figures 3.11 – 3.13). The ANOVA analysis showed no significant differences ($p < 0.05$) between the sites and surveys of specific metals, except for Sr (Figure 3.12).Strontium had a significant difference between the MS site and all the other sites except for the M site (Figure 3.12). Strontium also had a significant difference between the M site and all the other sites except for the MS and L4 site.

The MS and M sites had higher concentrations than most of the other sites (Figures 3.12 and 3.13). Aluminum, Cr and Mn had higher concentrations in the estuary mouth and in L4 than the rest of the sites. Iron and Co concentrations in L4 were higher than the rest of the sites (Figure 3.11). Nickel and As had higher concentrations in the estuary mouth and in L4 than the rest of the sites (Figures 3.11 and 3.12) Selenium had a higher concentration level in L4 than the rest of the sites (Figure 3.12). Copper concentrations were higher in the MS site than the rest of the sites (Figure 3.12). Cadmium concentrations were higher in M site and in L4 (Figure 3.12). Strontium concentrations were higher in the MS, M and L4 (Figure 3.12). Lead concentrations were higher in the L3E site (Figure 3.13). Mercury concentrations were higher in L3SE site than the rest of the sites (Figure 3.13).

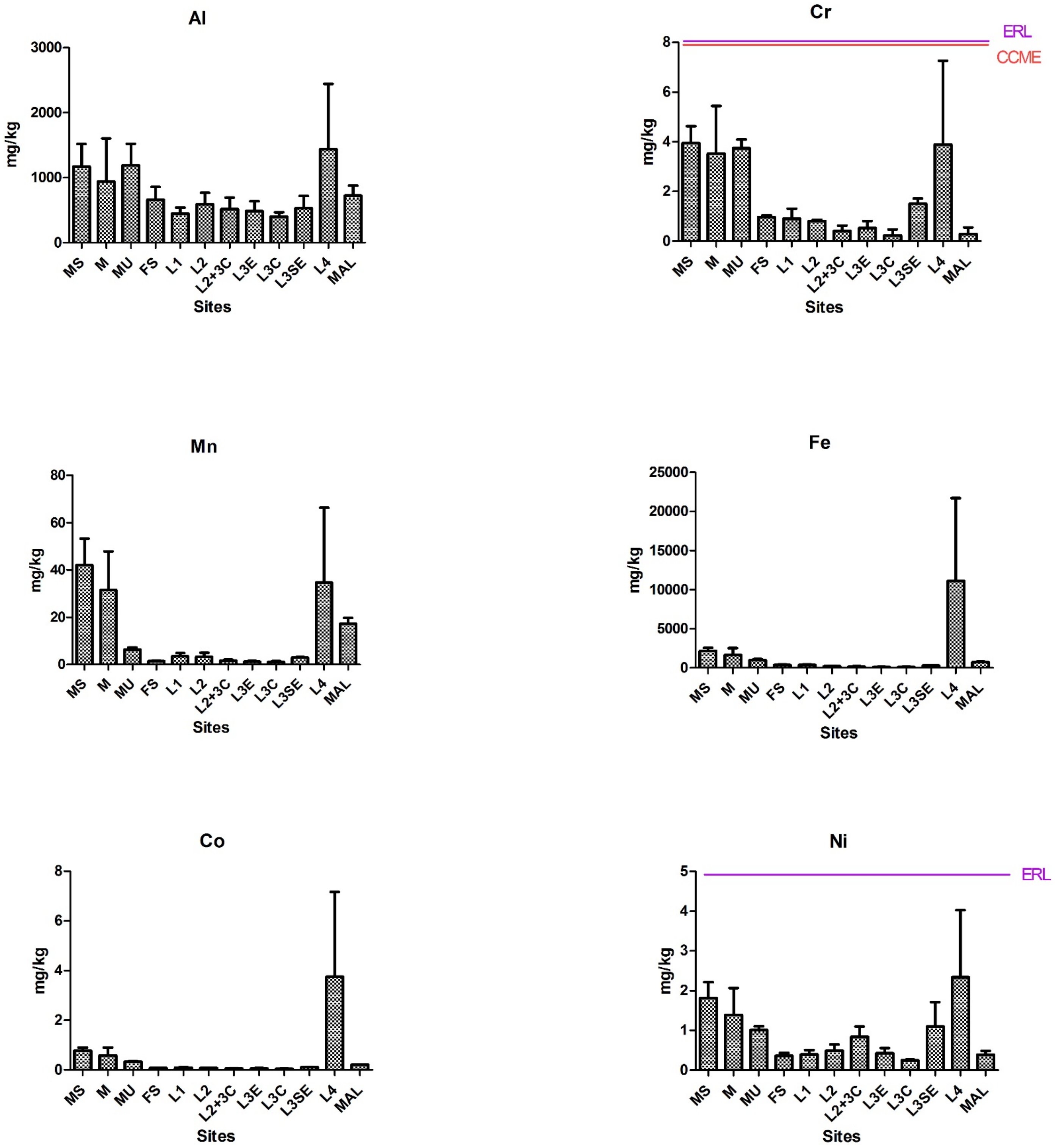

Figure 3.11. Mean metal concentrations (mg/kg) of Al, Cr, Mn, Fe, Co and Ni present in the sediment samples for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. The purple line indicates a range of metal guideline concentrations to assess possible adverse biological effects within the ranges of chemical concentration in marine and estuarine sediments; effects range – low (ERL).

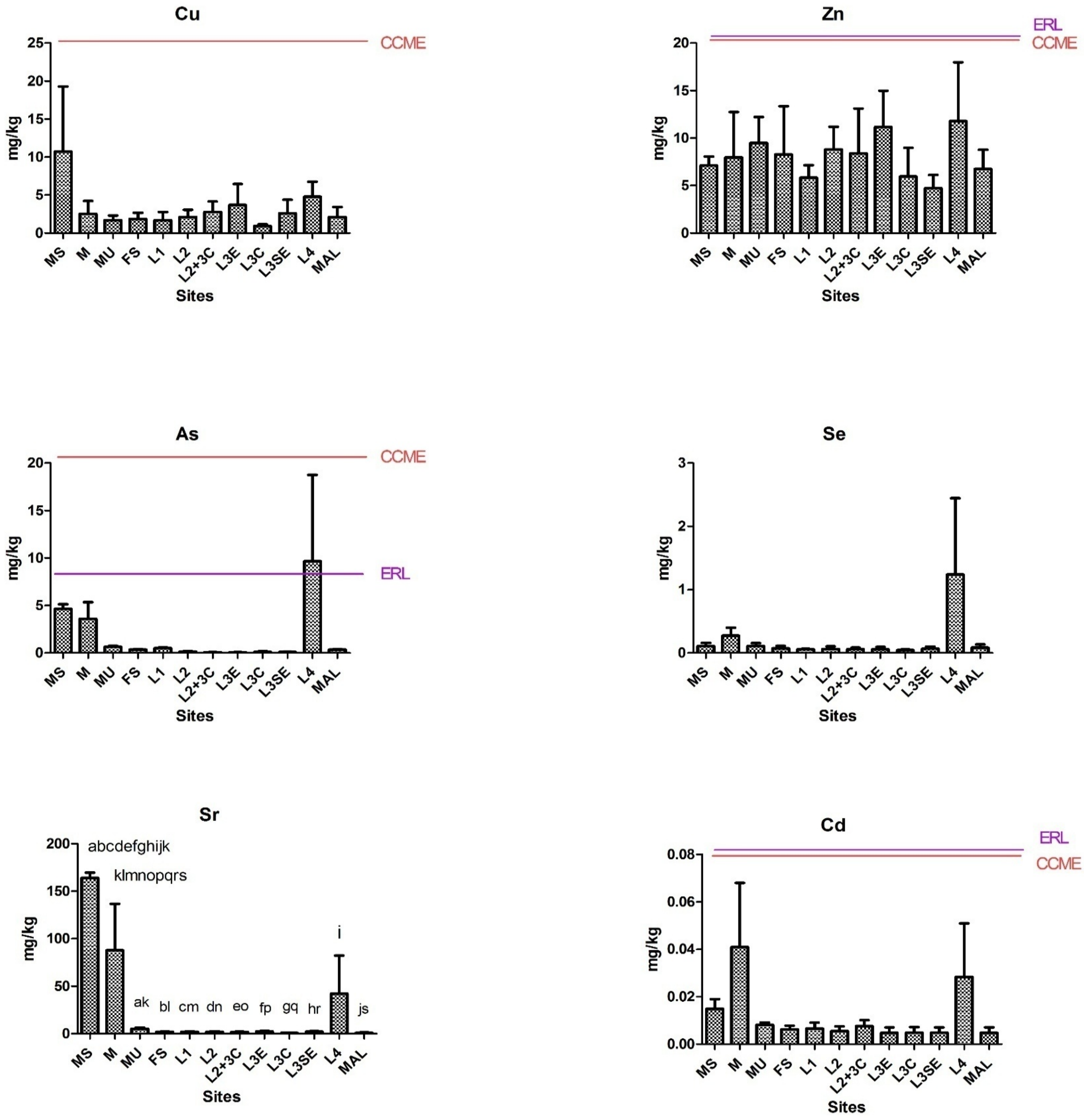

Figure 3.12. Mean metal concentrations (mg/kg) of Cu, Zn, As, Se, Sr and Cd present in the sediment samples for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. The purple line indicates a range of metal guideline concentrations to assess possible adverse biological effects within the ranges of chemical concentration in marine and estuarine sediments; effects range – low (ERL). Similar letters indicate significant differences ($p < 0.05$) between those sites for specific metal.

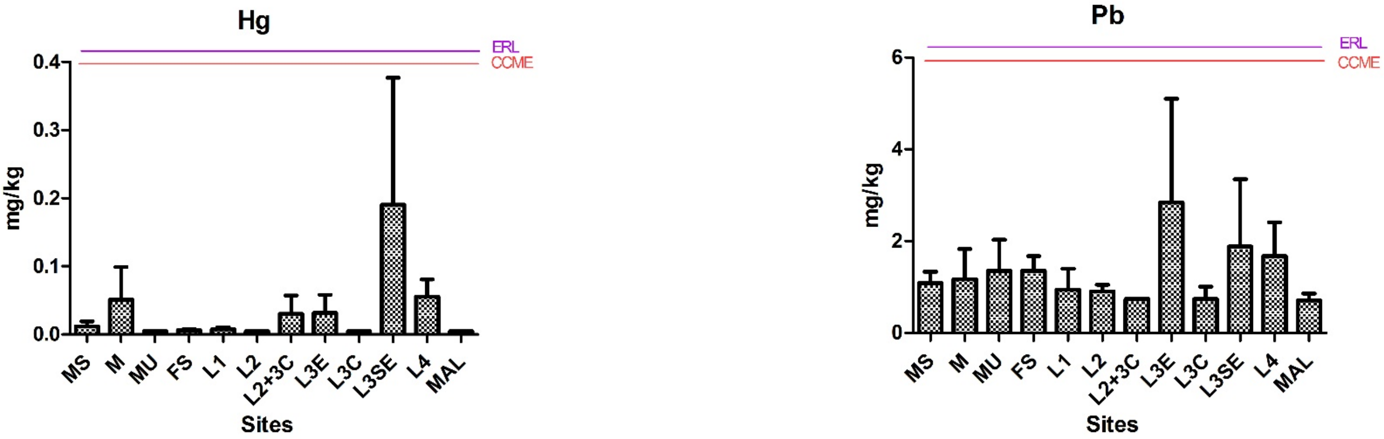

Figure 3.13. Mean metal concentrations (mg/kg) of Hg and Pb present in the sediment samples for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. The purple line indicates a range of metal guideline concentrations to assess possible adverse biological effects within the ranges of chemical concentration in marine and estuarine sediments; effects range – low (ERL).

Aluminium, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Hg and Pb concentrations were also determined in the sediment samples from Lake Sibaya and Kushengeza (Figures 3.14 – 3.16). There was a significant difference ($p < 0.05$) between LS2 and between LS1, LS3, LS4 and KUSH for Cr (Figure 3.14). Iron had the same trend of significant differences as Cr ($p < 0.05$) (Figure 3.14). Cobalt had significant differences between LS1 and LS2, LS2 and LS3, LS2 and KUSH (Figure 3.14). Arsenic had the same significant difference as Co but also had a significant difference between LS2 and LS4 (Figure 3.15). Strontium had the same significant differences as As (Figure 3.15). There were no significant differences between the sites for Al, Mn, Ni, Cu, Zn, Se, Cd, Hg and Pb.

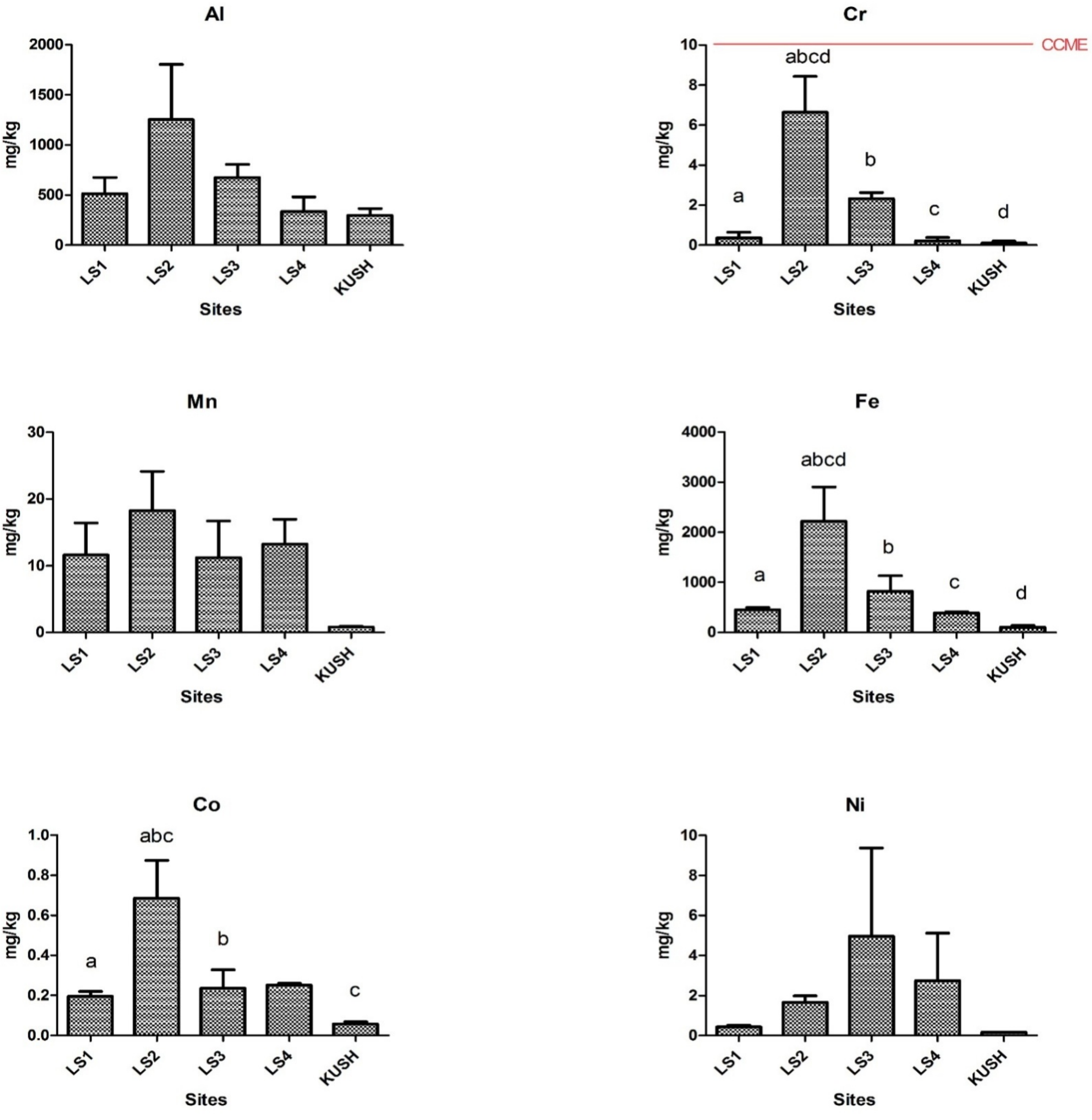

Figure 3.14. Mean metal concentrations (mg/kg) of Al, Cr, Mn, Fe, Co and Ni present in the sediment samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Similar letters indicate significant differences ($p < 0.05$) between those sites for specific metal.

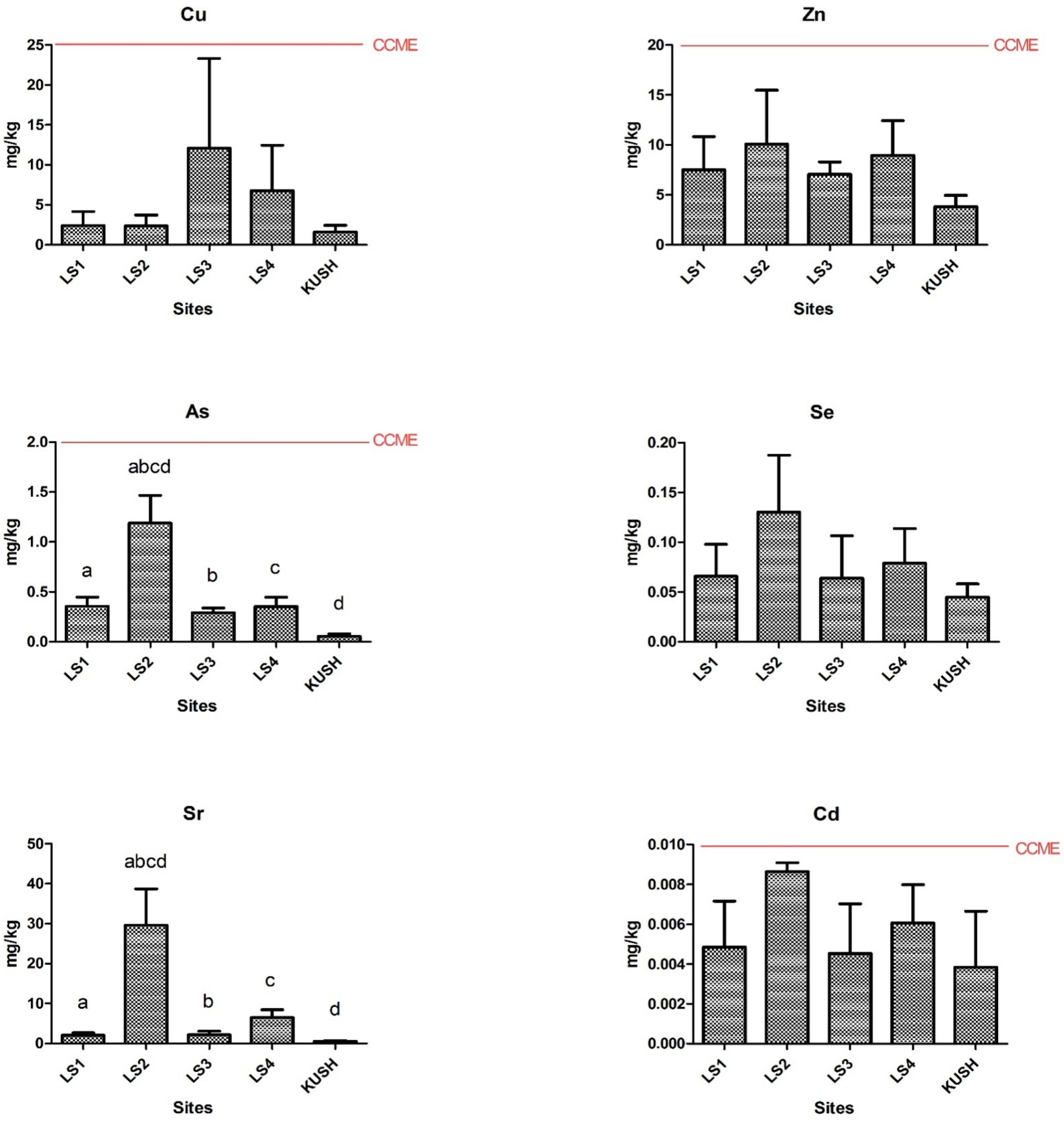

Figure 3.15. Mean metal concentrations (mg/kg) of Cu, Zn, As, Se, Sr and Cd present in the sediment samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life. Letters indicate significant differences ($p < 0.05$) between the sites for specific metal.

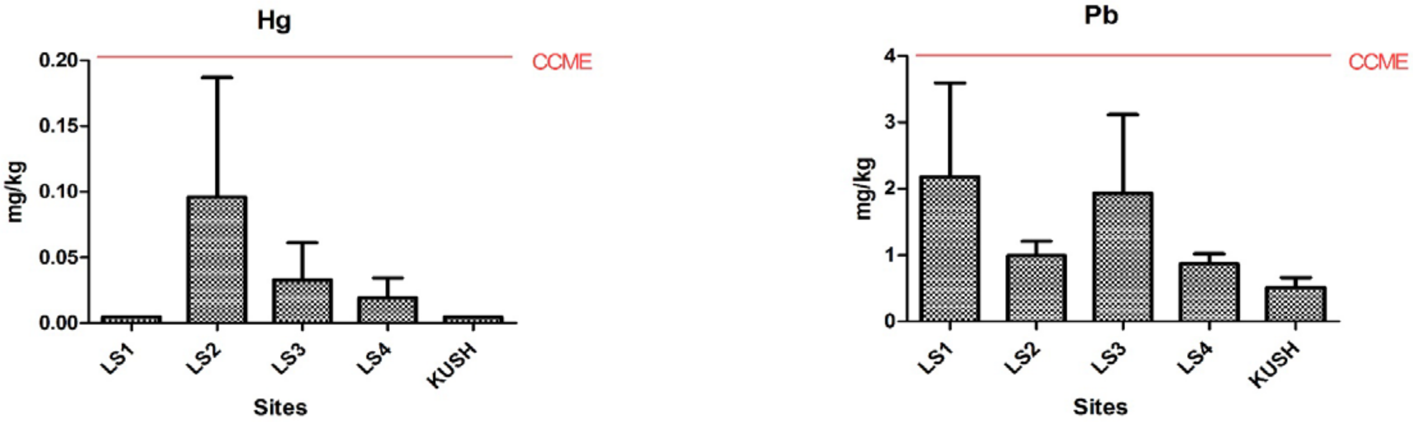

Figure 3.16. Mean metal concentrations (mg/kg) of Hg and Pb present in the sediment samples of Lake Sibaya and Kushengeza for August 2015, December 2015 and February 2016 with standard error of the mean showing seasonal variations. The red line indicates the Canadian council of ministers of the environment (CCME) quality guidelines for the protection of aquatic life.

The grain size distribution (%) from the sediment samples from each site of Kosi Bay during the three surveys is provided in Tables 3.2 – 3.4. The sites had a predominantly medium grain size (500 µm – 212 µm). In comparing the results from all three surveys, a similar trend of decreasing grain size further up into the system from the estuary mouth could be observed. A decrease in grain size is present from the estuary mouth to higher up the system to L4, where finer sediment (212 µm – 53 µm) is the dominant grain size (Table 3.2). Similar trends from the August 2015 survey were seen in grain size for the December 2015 and February 2016 surveys.

Table 3.1. Grain size distribution (%) of sediment samples with classification in the Kosi Bay system taken during the first survey in August 2015. MS – Mouth sea, M - Mouth, MU – Mouth upper, FS – Fisherman's spot, L1 – Lake 1, L2+L3C - Channel linking Lake 2 and 3, L2 – Lake 2, L3E – Lake 3 entrance, L3C – Lake 3 campsite, L4 – Lake 4, L3SE - South east of Lake 3 and MAL - Malangeni.

Classification	August 2015 Survey							
Grain size (µm)	**MS**	**M**	**MU**	**FS**	**L1**	**L2**	**L2+L3C**	**L3E**
> 4000	0	0	0	0	0	0	0	0
2000-4000	0	0	0	0	0	0	0	0
500-2000	28.34	16.56	16.8	8.05	3.71	1.3	3.59	1.34
212-500	63.34	71.51	49.6	62.45	65.75	35.45	52.49	57.74
53-212	6.58	8.62	32.71	28.87	29.8	62.12	43.42	40.37
< 53	0	0	0	0	0	0	0	0.29

Classification				
Grain size (µm)	**L3C**	**L3SE**	**L4**	**MAL**
> 4000	0	0	0	0
2000-4000	0	0	0	0
500-2000	14	0.53	4.74	0.47
212-500	83.35	43.8	66.74	61.89
53-212	1.61	53.46	28.4	37.26
< 53	0	0	0.03	0

Table 3.2. Grain size distribution (%) of sediment samples in the Kosi Bay system taken during the second survey in December 2015. MS – Mouth sea, M - Mouth, MU – Mouth upper, FS – Fisherman's spot, L1 – Lake 1, L2+L3C - Channel linking Lake 2 and 3, L2 – Lake 2, L3E – Lake 3 entrance, L3C – Lake 3 campsite, L4 – Lake 4, L3SE - South east of Lake 3 and MAL - Malangeni.

Classification	December 2015 Survey							
Grain size (µm)	**MS**	**M**	**MU**	**FS**	**L1**	**L2**	**L2+3C**	**L3E**
> 4000	0	0	0	0	0	0	0	0
2000-4000	0.64	0.44	0	0	0	0	0	0
500-2000	51.86	38.09	21.16	5.19	5.36	4.11	2.6	1.87
212-500	43.91	59	51.62	56.57	67.01	51.91	48.38	54.77
53-212	2.66	1.56	26.52	36.9	26.88	43.43	47.66	42.52
< 53	0	0	0.09	0	0	0	0	0

Classification				
Grain size (µm)	**L3C**	**L3SE**	**L4**	**MAL**
> 4000	0	0	0	0
2000-4000	0	0	0	0
500-2000	0.16	0.31	2.66	0.2
212-500	69.12	42.22	72.79	35.27
53-212	30.37	55.93	23.78	63.52
< 53	0	0	0.09	0.07

Table 3.3. Grain size distribution (%) of sediment samples in the Kosi Bay system taken during the third survey in February 2016. MS – Mouth sea, M - Mouth, MU – Mouth upper, FS – Fisherman's spot, L1 – Lake 1, L2+L3C - Channel linking Lake 2 and 3, L2 – Lake 2, L3E – Lake 3 entrance, L3C – Lake 3 campsite, L4 – Lake 4, L3SE - South east of Lake 3 and MAL - Malangeni.

Classification	February 2016 Survey											
Grain size (µm)	**MS**	**M**	**MU**	**FS**	**L1**	**L2**	**L2+3C**	**L3E**	**L3C**	**L3SE**	**L4**	**MAL**
> 4000	0	0	0	0	0	0	0	0	0	0	0	0
2000-4000	0	0.18	0	0	0	0	0	0	0	0	0	0
500-2000	53.70	47.37	15.74	11.75	6.62	10.55	5.97	1.14	3.11	12.31	6.65	0.23
212-500	44.58	50.23	40.39	67.66	72.99	57.63	62.44	49.36	81.98	73.01	72.30	51.98
53-212	1.15	1.62	43.13	19.93	19.56	31.29	30.74	49.03	7.24	13.93	20.03	47.01
< 53	0	0	0.13	0.05	0.06	0.12	0.08	0	0	0	0.10	0

Similar trends of Lake Sibaya and Kushengeza from the August 2015 survey were seen in grain size for the December 2015 and February 2016 surveys with finer sediment (212 µm – 53 µm) being the more dominant grain size (Table 3.5).

Table 3.4. Grain size distribution (%) of sediment samples with classification in Lake Sibaya (LS 1 – 4) and Kushengeza (KUSH) system taken during the August 2015, December 2015 and February 2016.

Classification	August 2015 Survey				
Grain size (µm)	LS1	LS2	LS3	LS4	KUSH
> 4000	0	0	0	0	0
2000-4000	0	0	0	0	0
500-2000	1.31	0.91	1.26	3.29	17
212-500	31.63	66.23	82.41	59.21	72.86
53-212	64.5	31.67	15.77	33.95	9.61
< 53	0	0	0	0	0
Classification	December 2015 Survey				
Grain size (µm)	LS1	LS2	LS3	LS4	KUSH
> 4000	0	0	0	0	0
2000-4000	0	0	0	0	0
500-2000	1.64	3.51	1.64	3.14	1.18
212-500	33.98	58.41	66.56	62.55	43.02
53-212	63.13	37.36	31.15	32.64	54.99
< 53	0	0	0	0	0
Classification	February 2016 Survey				
Grain size (µm)	LS1	LS2	LS3	LS4	KUSH
> 4000	0	0	0	0	0
2000-4000	0	0	0	0	0
500-2000	1.75	0.8	0.65	3.97	1.35
212-500	34.42	65.69	39.14	59.98	52.49
53-212	63.4	32.92	59.52	35.13	45.45
< 53	0.07	0	0.1	0.11	0

3.4 Discussion

3.4.1 Water quality

3.4.1.1 Nutrients

Kosi Bay

Nutrient contribution to any system is fixed and relatively constant to a particular catchment except when new developments are present within the area (Dallas and Day, 2004). Developments could potentially change the nutrient input of a system. Other influences on a system include climatic factors such as, rainfall, runoff and catchment characteristics such as land form and geology (Dallas and Day, 2004). Sewage treatment works, intensive animal enterprises and industry are anthropogenic point source types of nutrients. Non-point source types are agricultural runoff, urban runoff and atmospheric deposition. Agricultural and urban activities are major sources of phosphorus and nitrogen to an aquatic ecosystem (Dallas and Day, 2004). Phosphorus and nitrogen accelerate eutrophication which is a widespread problem in rivers, lakes, estuaries and coastal waters (Kočić *et al*., 2008). It is important to observe nutrient conditions in an aquatic system, due to the fact that these conditions can affect the water and status of the overall biota in the aquatic environment (Kočić *et al*., 2008).

Ammonium

Ammonium is generally associated with sewage and industrial effluents and occurs in the un-ionised form NH_3 (ammonia) or as ammonium ions (NH_4^+).Ammonium contributes to eutrophication and results from the decomposition of nitrogenous organic matter (Dallas and Day, 2004). Surface waters that are not contaminated with organic wastes have a low ammonia concentration, less than 0.2 mg/L. Nitrates occur when ammonia is microbiologically oxidised. Organic decomposition under anaerobic conditions tends to have high concentrations of ammonium and is normally found in agricultural runoff (DWAF, 1996). According to the study of Ewald (2000), raw untreated sewage has ammonium levels that exceed 10 mg/L. According to South African water quality guidelines of coastal marine waters (1st volume) ammonium rarely exceeds 0.07mg/L in unpolluted coastal waters (DWAF, 1995). Ammonium levels for all of the Kosi Bay sites exceeded these limits except for MAL which had a concentration of 0.07 mg/L (see Appendix B). This higher level can be due to the anoxic nature of the lakes in winter (Kyle and Kwangwanase, 1995) and released into the water from the sediments (Dallas and Day, 2004). Respiratory damage and physiological changes can occur in aquatic organisms that are exposed to high ammonium levels (Dallas and Day, 2004).

Nitrates

Nitrates are ubiquitous in the aquatic environment and associated with breakdown of organic matter and eutrophic conditions (DWAF, 1996). They are also associated with agricultural and urban runoff as well as highly productive systems and growth of aquatic plants. Nitrate and nitrite are the oxyanions of nitrogen and occur together in the environment where interconversion readily occurs (DWAF, 1996). Tropical waters are normally poor in nutrients and higher nutrient concentrations are caused by upwelling (DWAF, 1995). According to the South African water quality guidelines for aquatic ecosystems (freshwater), a concentration of < 0.5 mg/L is considered to be an oligotrophic system, 0.5 – 2.5 mg/L a mesotrophic system, 2.5 – 10 mg/L a eutrophic condition and > 10 mg/L a hypertrophic system. Lake 4 had a mean concentration of 1.7 mg/L and MAL a mean concentration of 2.2 mg/L. Both these sites are considered to be mesotrophic systems and could be due to organic contents present in these areas.

According to the South African water quality guidelines of coastal marine waters (1st volume) (DWAF, 1995), the average concentrations of nitrate levels on the east coast is 0.035 mg/L in Port Edward, 0.047 mg/L in Durban and 0.038 mg/L in Richards Bay area. All of the Kosi Bay sites exceed these levels, with the highest mean level of 9.1 mg/L in the upper region of the mouth of the estuary (MU) (see Appendix B). A nitrate level of > 100 mg/L can be detrimental to marine fish species (DWAF, 1995). Indirect effects could occur if the system would become eutrophic.

Nitrite

Nitrite naturally occurs in fresh and saline waters but industrial production of metals, sewage effluents and agriculture increase the concentrations of nitrite in the aquatic environment and are toxic at certain concentrations (Dallas and Day, 2004). According to the South African water quality guidelines of coastal marine waters (1st volume), the average concentrations of nitrite levels on the west coast is 0.0042 mg/L and 0.028 mg/L on the south coast (DWAF, 1995). There is no data available for the east coast. The MS and M sites were below the values for the west and south coast with 0.0020 mg/L. The MU, FS, L1, L2, L2+3C and MAL sites were in between these concentrations. The L3E, L3C, L3SE and L4 sites exceeded these thresholds (see Appendix B).

Phosphates

Phosphates can be found in the form of dissolved inorganic orthophosphates and can bind to fine and coarse particulate materials (Wepener *et al*., 2006). It is essential to know the role of mechanisms that control the supply of bioavailable phosphates to avoid eutrophication in a system (Dallas and Day, 2004). Phosphates bind to most soils and sediment and can be released from the sediments under high anoxic conditions (Dallas and Day, 2004). Point source pollution such as sewage outlets can be a contributing factor to phosphates in a system. Decaying plant and animal matter can also increase phosphate levels within a system (Dallas and Day, 2004). The South African water quality guidelines state (DWAF, 1995) that the average concentrations of phosphates in sea water is 0.062 mg/L. Phosphate levels on the east coast are 0.019 mg/L in Port Edward, 0.019 mg/L in Durban and 0.024 mg/L in the Richards Bay area. All of the Kosi Bay sites exceed these levels. Higher phosphate levels can cause eutrophication and can lead to turbidity which is important in the Kosi Bay system because it is a clear water system (DWAF, 1995). Kosi Bay has no problem with eutrophication and the phosphate levels have no health effect on the fish species. Higher phosphate levels in the last three (L3SE, L4 and MAL) sites can also be due to more organic contents with vegetation growth (Wepener *et al*., 2006).

Sulphate

Sulphur in water occurs as the sulphate ion and it is an essential component of proteins (Dallas and Day, 2004). Sulphates are not toxic but in excess amounts they form sulphuric acid and can negatively impact on an aquatic system (Dallas and Day, 2004). Sulphate arises from dissolution of mineral sulphates in soil and rock and is soluble in water. There is no data available, according to the South African water quality guidelines of coastal marine waters (1st volume) (DWAF, 1995), for sulphate, but according to DWAF (1996) the concentration of sulphate in sea water is just over 900 mg/L.

Lake Sibaya and Kushengeza

The target water quality range (TWQR) for the South African Water Quality guidelines for aquatic ecosystems were used to get values of ranges for constituents. The constituents looked at in the Lake Sibaya system and at Kushengeza were inorganic nitrogen (nitrate, nitrite, and ammonium) and phosphates. Ammonium limits for Lake Sibaya and Kushengeza were within the natural limits (< 7 mg/L) according to the South African Water Quality guidelines for aquatic ecosystems (DWAF, 1996). Nitrate levels were between 0.5 - 2.5 mg/L for Lake Sibaya and Kushengeza, and according to the South African Water Quality guidelines for aquatic ecosystems are considered to be mesotrophic systems. Nitrite levels for Lake Sibaya and Kushengeza were below 0.5 mg/L and according to the South African Water Quality guidelines for aquatic ecosystems they are considered to be oligotrophic systems. Phosphate levels for Lake Sibaya and Kushengeza were below 5 mg/L and according to the South African Water Quality guidelines for aquatic ecosystems they are considered to be oligotrophic systems.

3.4.1.2 Metal concentrations (water)

Metal concentrations from the water samples of the different lakes within the Kosi Bay system were compared to the Canadian water quality guidelines for the protection of marine aquatic life (CCME, 2016). Guidelines were indicated on water result figures. The metals from the first six sites were compared to the guidelines for As, Cd and Hg. These were the only metals that the Canadian water quality guidelines had for a more marine environment. These six sites sampled exceeded these guidelines (see Appendix C). The metals from L4 and MAL were compared to the Canadian water quality guidelines for the protection of freshwater aquatic life for Zn, Se, Hg, Fe, As and Cd. All of the levels were below the Canadian water quality guidelines except for the Se levels in L4 and Hg levels in MAL (Appendix C). The target water quality range (TWQR) for accepted levels of the South African Water Quality guidelines for aquatic ecosystems (DWAF, 1996) was used for L4 and MAL to get values of ranges for constituents. The metals looked at were Al, As, Cd, Cr, Cu, Pb, Mn, Hg, Se and Zn. The metal concentrations of Al, As, Cd, Pb, and Mn for L4 and MAL were below the South African Water Quality guidelines for aquatic ecosystems (see Appendix C). The metal concentrations of Cr, Cu, Se and Zn for MAL were also below the guidelines; however, Cr, Cu, Se and Zn concentrations at L4 exceeded the guidelines (Figure 3.15 and 3.16) (see Appendix C). The metal concentrations of Hg for L4 were below the guidelines; however, Hg for MAL exceeded the guidelines. If Hg exceed the guidelines for a long period of time, they can cause growth deficiencies, respiratory failure and mortalities in fish (DWAF, 1996). The metal concentrations of Cr, Ni, Cu, Zn, As, Se, Cd, Hg and Pb were also compared to the Australian water quality guidelines for the marine environment (ANZECC, 1994). All of the concentrations from the different lakes within the Kosi Bay system exceeded the guidelines. Although these concentrations exceed the guidelines, the systems are different kinds of systems and are not alike, so there is no threat to the system.

The metal concentrations in the water were also compared to the study of Holbach *et al*. (2012), which was also on the Kosi Bay system. The results from the study of Holbach *et al*. (2012) were lower than the results from this study. The metals compared were Mn, Fe, Ni, Cu, Zn, Sr and Cd. All of these metals were higher in this study compared to the study of Holbach *et al*. (2012). This could be caused by the increase of the local community in the areas and agricultural activities along the system and further up in the river.

Metal concentrations of the Lake Sibaya and Kushengeza coastal lakes were compared to the South African Water Quality guidelines for aquatic ecosystems. The metal concentrations for all the Lake Sibaya sites and KUSH were below the guidelines for As, Cd, Pb and Mn (Appendix C). Kushengeza concentrations for Pb exceeded the guidelines. The metal concentrations for all the Lake Sibaya sites and Kushengeza exceeded the guidelines for Cr, Hg and Zn (see Appendix C). Lake Sibaya sites and Kushengeza were below the guidelines for Al (see Appendix C). The metal concentrations for the Lake Sibaya sites and KUSH exceeded the guidelines for Cu (see Appendix C). Kushengeza was the only site with metal concentrations below the guidelines for Se. These metals can also cause growth deficiencies, respiratory failure and mortalities in fish if the exposures are high for a long period of time (DWAF, 1996).

3.4.2 Sediment quality

3.4.2.1 Metal concentrations (sediment)

The L4 site had the highest sediment metal concentrations for most sites. Figures 3.8 and 3.9 show concentrations of Pb, Hg and Cu to be the only concentrations where L4 had no clear difference to the rest of the sites. An elevated level of trace metals in sediments can give good indications of anthropogenic pollution (Binning and Baird, 2001). Sediments integrate contaminants over time and once metals are discharged into estuarine and coastal waters, it partitions and is incorporated into the sediment (Binning and Baird, 2001). Determination of metals in sediments provides information that helps the assessment of long term risk contamination and management (Pardo *et al*., 1990).

A range of metal guideline concentrations was proposed by Long *et al*. (1995) to assess possible adverse biological effects within the ranges of chemical concentrations in marine and estuarine sediments. The ERL (effects range-low) and ERM (effects range-medium) delineates three concentration ranges for a specific metal. The effects of below ERL values on biota would rarely be observed. Metal concentrations equal to the ERL but below the ERM could occasionally affect the biota. Concentrations equal to or above the ERM will have frequent effects on the biota (Long *et al*., 1995).

The metal concentration values from Kosi Bay were below the ERL guideline except for As in L4 (Figure 3.8) and Hg in L3SE (Figure 3.9). The values were above the ERL guideline meaning that effects could be observed on the biota of the system. These effects from metal accumulation can result in respiratory failures, development deficiencies and organ failures in the fish species. Although these values were higher than the ERL guidelines, it was lower than the values reported in other estuaries (Binning and Baird, 2001; Mzimela *et al*., 2003; Wepener and Vermeulen 2005; Sukdeo *et al*., 2012). For example Al and Fe values of the other studies were higher than the values of Kosi Bay (Table 3.6).

Estuarine water has pronounced gradients and has a high variability of water quality parameters. This is due to the influences of freshwater and seawater and their specific dynamics. The metals gradually lowered further up in the system to more freshwater environments. the final lake and up in the river revealed higher metal concentrations, which could be caused op higher nutrient values in the freshwater from further up in the river system (see Appendix D for values).

Table 3.5. Mean metal concentrations (mg/kg) of aluminium - Al, chromium - Cr, manganese - Mn, iron - Fe, lead - Pb, selenium - Se, copper - Cu, zinc - Zn and strontium - Sr in the sediment of other estuary system related studies.

Mean metal concentrations (mg/kg) in the sediment of other estuary related studies

Sites	**Mean metal concentrations (mg/kg)**								
	Al	**Cr**	**Mn**	**Fe**	**Pb**	**Se**	**Cu**	**Zn**	**Sr**
Swartkops Estuary	/	20.8	82.0	/	44.9	/	6.4	34.8	359.8
Mhlathuze Estuary	29170.2	74.2	200.6	18146.6	18.6	/	9.9	28.0	/
Richards Bay Harbour	31322.4	110.3	411.3	27429.4	/	/	19.2	98.1	/
Mvoti estuary	50.63	0.11	1.37	74.99	/	0.13	/	0.07	16.97

/ – No Data

The metal concentrations were also compared to the Canadian limit for sediments and most concentrations were below the Canadian limits in the marine environment (CCME, 2016). Arsenic and Hg were the only metals that were over sediment quality guidelines (Figure 3.8 and Figure 3.9). These higher concentrations were near the south eastern side of Lake Nhlange and in Lake Amanzimnyama. The higher concentrations can be due to the higher organic content near these sites. Another reason for higher concentrations in that area is the fact that Malangeni River flows into Lake Amanzimnyama, bringing down higher concentrations of nutrients and increasing organic contents. This was observed during the surveys in the Kosi Bay and Malangeni River area.

Metal concentrations of the Lake Sibaya and Kushengeza coastal lakes were compared to the Canadian limit for sediments and most concentrations were below the Canadian sediment quality guidelines. The metals compared to the Canadian limits were As, Cd, Cr, Cu, Pb and Hg. There was no data available for Ni, Se, Mn, Fe, Co and Al from the Canadian limits (see Appendix E for values)

3.4.2.2 Grain sizes

Finer grain sizes tend to be associated with increased metal concentrations (Binning and Baird, 2001). The first site (MS) shows a coarser sand present in comparison to finer sand present further up into the system (Tables 3.2 – 3.4). There were minimal differences between the surveys in terms of grain size in percentage. The only sites that have very coarse sand were the first two sites at the estuary mouth (Table 3.2 – 3.4). The finer grain sizes further up into the system were due to very low energy flow between the lakes and channels connecting the lakes. The grain size distribution provides information on the characteristics of the sediment and is controlled by hydrodynamic energy conditions, where in this case the estuary mouth has a high hydrodynamic energy (Ning *et al*., 2016). The Kosi Bay system varies from a high energy to a very low energy system as one moves up through the system. The higher the energy, the higher proportion of the coarse grain size (Ning *et al*., 2016). Other characteristics that influence grain size are water depth, wind direction and strength (Ning *et al*., 2016). These characteristics influence the hydrodynamics and may lead to different characteristics in grain-size distributions (Ning *et al*., 2016). Taking these characteristics into consideration, the grain sizes in the Kosi Bay system can be seen to change through the system as water depth and higher energy alter the grain sizes.

Finer grain sizes are associated with higher metal concentrations. The aim at this part of the project was to determine if different grain sizes of a certain area could possibly cause higher or lower metal concentrations within fishes of that specific area. The metal concentrations varied at different sites but according to the grain size classification Figure 3.2, the sediment at the L2+L3C site where the fish were caught was classified as medium sand. The metal concentrations were not high at this site.

Sediment in Lake Sibaya and Kushengeza had shown a predominantly finer grain size than sediment from the Kosi Bay system (Table 3.5). The grain sizes have similar trends between each survey and slight differences between the surveys in terms of percentage grain size were noted. The only clear differences noticed were the very fine sand in the third survey (Table 3.15). The grain sizes of LS1, LS3 and LS4 had a grain size of < 53 μm (classified as mud).

3.4 Conclusion

Water quality and sediment quality was compared between the different sites and surveys. This was done to determine any potential impact that the fish community in the Kosi Bay system are exposed to.

Nutrient values of each nutrient tested differed and had different trends throughout the system. There were high nutrient values at the L4 site; due to the freshwater nature of this particular site as well as the more abundant aquatic vegetation. Higher nutrient values could possibly be due to the Malangeni River bringing down organic contents. Lake Sibaya nutrient values had a similar trend between the sites with Kushengeza having relatively higher values than Lake Sibaya.

The water results indicated that, in general the metal concentrations of the Kosi Bay system were higher at the mouth and the lower part of the estuary. Further upstream metal concentrations were lower and L4 and MAL had significantly lower concentrations than all the other sites. The metal concentrations of Lake Sibaya and Kushengeza showed similar trends to that of Kosi Bay. Kushengeza had higher concentrations than the Lake Sibaya sites.

Kosi Bay had coarser sand at the mouth and in the estuary but had smaller grain sizes further upstream. The L2+L3C site had a grain size classification of medium sand and had low metal concentrations. The trends for all the surveys were the same with coarser sand in the estuary to finer sand further up in the system. The grain sizes for Lake Sibaya had the same trends for all the surveys and sites. Lake Sibaya consisted mostly of medium to fine sand.

The sediment results indicated that Kosi Bay sites had similar trends in the concentrations levels. Lake 4 site had high concentrations for each metal tested. The sediment results indicated that Lake Sibaya sites had higher metal concentrations than Kushengeza especially LS2 and LS3.

Most results were below the guidelines used except for the metal concentrations in the water of the different lakes. Almost all of the sites water exceeded the various metal concentration guidelines.

4. Fish Health Assessment Index Protocol on the selected fish species (*Oreochromis mossambicus*, *Rhabdosargus sarba and Terapon jarbua*) of the Kosi Bay system.

4.1 Introduction

Ecological information of a certain ecosystem can be expensive to obtain and it often requires extensive research to understand and to utilise it correctly in management systems (Harrison and Whitfield, 2006). A method to evaluate the fundamentals of an ecosystem (without complexities) is to use environmental indicators (Harrison and Whitfield, 2006). Biotic indicators, such as fishes, can be inexpensively examined to determine the effects environmental factors have on them (Harrison and Whitfield, 2006).

The health of organisms can provide an indication to the overall health of the ecological system they inhabit (Adams *et al*., 1993). Fish are found in most aquatic ecosystems on all different trophic levels in an aquatic food pyramid and have proved to be good indicators of the health of a system (Adams *et al*., 1993). Due to their ability to integrate the effects of biotic and abiotic variables present in the system, fish are good environmental indicators and allow secondary chronic symptoms to be shown through the food chain (Adams *et al*., 1993). During their life cycle fish are exposed to numerous stressors including fluctuating temperatures, sediment loads, dissolved oxygen concentrations and limited food availability which can cause physiological stress and this could impair their health (Adams *et al*., 1993). Other impacts on fish are anthropogenic stressors such as agricultural activities, drainage of pesticides, herbicides and fertilisers (DWA, 2009). These stressors influence the growth and reproduction as fish will use more energy to deal with the stress, diverting energy away from critical functions (Adams *et al*., 1993). Fish can also develop disease and reduce their capacity to tolerate additional stressors (Adams *et al*., 1993).

Numerous studies and indices have been used on fish to determine the current ecosystem health – by using the fish community in estuaries. Some fish community indices used in estuaries include: estuarine fish community index (EFCI), estuarine fish health index (FHI), estuarine biotic integrity index (EBI) and estuarine fish recruitment index (FRI).

The EFCI, developed by Harrison and Whitfield (2004) is an index that combines 14 metrics (or measures) which represent four fish community attributes: species diversity and composition, nursery function, species abundance and trophic integrity into a single measure of estuarine health (Whitfield and Elliot, 2002). The EFCI was used on 190 South African estuaries, one being Kosi Bay (Whitfield and Elliot, 2002).

The FHI, developed by Harrison *et al*. (2000), focuses on the qualitative and quantitative comparisons in a ‘reference’ fish community (Whitfield and Elliot, 2002). The number of fish species in an estuary is compared to the average number for the group to which it belonged. The number of taxa examined is rated according to whether the taxa exceeds, approximates or is below the average of its reference group. A species assemblage of an estuary can be compared with a reference assemblage of each estuary type based on the most frequently captured taxa (Whitfield and Elliot, 2002).

The EBI, developed by Deegan *et al*. (1997), is a useful fish indicator of estuarine ecosystem status and is used to reflect the relationship between anthropogenic effects in the ecosystem and the status of higher trophic levels (Whitfield and Elliot, 2002). Eight metrics were followed: total number of species, fish abundance, number of nursery species and resident species, number of estuarine spawning species, dominance, proportion of benthic-associated species and abnormal or diseased fish. The EBI found that habitat degradation is associated with the individual metrics and overall index.

The FRI was developed by Quin *et al*. (1999) in an attempt to use ichthyological information to assess changes in habitat integrity, especially the availability and suitability of marine migrant fishes within nursery areas (Whitfield and Elliot, 2002). The FRI is a management directed index and is based on three key information sets: 1) importance and significance of marine fish species in an estuarine environment and whether it is endemic to South Africa or not; 2) timing of immigration period of a particular species; 3) known environmental requirements of recruitment by juvenile marine fish into South African estuaries.

Previous fish related studies conducted within the Kosi Bay system mainly focused on the diversity of fish species. Blaber (1978) established the important characteristics and seasonal variation of the fish of each part of the system where Kyle and Robertson (1997) determined fish diversity and movements of the fish within the Kosi Bay system. (See Chapter 2 for more references on fish studies in the Kosi Bay system.

No detailed heath assessments have been done on the fish of the Kosi Bay system. The other indices focussed on fish diversities. The aim for this part of the project was to determine the health of the selected fishes following the Fish Health Assessment Index (FHAI) protocol. This protocol is a necropsy method to determine the overall health of any fish species and is important because the fish are consumed in the system by the local community. The FHAI protocol used was proposed by Adams *et al.* (1993), modified from Goede and Barton (1990).

4.2 Material and methods

4.2.1 Sampling protocol

Fish sampling was carried out on three surveys during August 2015, December 2015 and February 2016 to determine the seasonal variation in fish health in the Kosi Bay system. Fish from the selected sites were collected using the following methods:

- Seine netting: seine nets were pulled through the water (5 to 10 drags) in slow shallow habitats to sample fish amongst the vegetation.
- Fyke nets: fyke nets (20 mm mesh size) were placed in the channel (L2+3C) between Lake Makhawulani and Lake Nhlange in shallow riparian areas in the morning for 2 – 3 hours.
- Rod and reel: rod and reel was used during the surveys as it was an easy, inexpensive method that could target the selected fish species.

A permit (OP 238/2016 and A15 0073844) was issued by the iSimangaliso Wetland Park Authority and the Ezemvelo KZN wildlife authorities for catching the specific fish needed for this project. Ethical approval for the research were also received from the North-West University.

4.2.3 Selection of target species

Three fish species were selected for determining the fish health at Kosi Bay, namely, *Oreochromis mossambicus*, *Rhabdosargus sarba* and *Terapon jarbua.* These fish species were selected due to the fact that they are an important food source for the local community of Kosi Bay and for recreational fishing. Most of the fish caught by the local people are *R. sarba* with the use of traditional fish traps. *Oreochromis mossambicus* are caught by the local communities, but mostly with traditional spears along the reeds throughout the system. *Terapon jarbua* are also caught with spears. However, recently the local community has had access to more modern materials to upgrade their traps; this resulted in catches including even smaller fish species and juveniles of most species. All of these fish species can also be caught by rod and reel by the local communities and by recreational fisherman, although these fishermen mostly target *R. sarba* and *Pomadasys commersonni* (commonly known as the spotted grunter).

4.2.2 Necropsy procedure

Sampled fish were transferred into insulated Coleman cooler boxes and taken to the field laboratory where the FHAI procedure took place. Each fish was weighed using a BBADAM (PGW 75e) balance. The total length (TL), standard length (SL) and fork length (FL) of the fishes were measured. The condition factor (CF) of the fishes was calculated using the measurements of each fish. The CF was calculated as $CF = 100000 * W/L^3$, where W = body weight (g) and L = fork length (cm) (Zimmerli *et al*., 2007).

After the measurements were completed the internal and external examinations were done following the necropsy method (Figure 4.1) by Goede and Barton (1990). Fish were externally screened for any abnormalities or parasites. The external variables that were examined were skin, eyes, fins, opercula, hindgut, gills and mouth (Figure 4.1). Following external examination, fish were humanely sacrificed by being stunned with a blow to the head and then severing the spinal cord. The fish were then dissected to determine the presence of any internal abnormalities that might be present, followed by removal and examination of the organs (spleen, liver and gonads). The rest of the intestines were also screened for any parasites. Fat percentages were also noted within the fishes during dissection. The fat percentages were determined by following the FHAI procedure.

The FHAI provides a health profile based on the percentage of anomalies observed in the tissues and organs of the fish (Adams *et al*., 1993). This necropsy method, developed by Goede and Barton (1990), can be grouped into the follow categories: 1) Three blood parameters (hematocrit, leukocrit and plasma protein) length, weight and CF; 2) percentage of fish with normal and abnormal eyes, gills, pseudobranches (if present), spleens, kidneys and liver; 3) Index values of damaged skin, fins, thymus, hindgut inflammation, fat deposits and bile colour. Blood parameters were not included in this study due to the small sizes of the fish collected.

The removed organs were weighed to determine the gutted mass, CF and the organosomatic indices. Goede and Barton (1990) used organosomatic indices for stress related studies. These indices are ratios of organ weight to body weight. Organosomatic indices are used to detect gross changes in the health and condition of the fish and provides a database for detecting condition trends in the health and condition of fish over time The organosomatic index (OSI) was calculated with the following formula: OSI= Total organ weight (g) / total body weight (g) x 100 (Kharoubi *et al*., 2008). The heptosomatic (HSI) (liver), gonadsomatic (GSI) (gonads) and the splenosomatic indexes (SSI) (spleen) were calculated with the OSI formula.

To determine the fish health of the selected fishes sampled, values were assigned for each organ if there were abnormalities present. With these values FHAI was calculated. A zero was assigned if the organ had no abnormalities, a 10 if there were mild abnormalities, 20 if moderate abnormalities and 30 if there were severe abnormalities present (Adams *et al*., 1993).

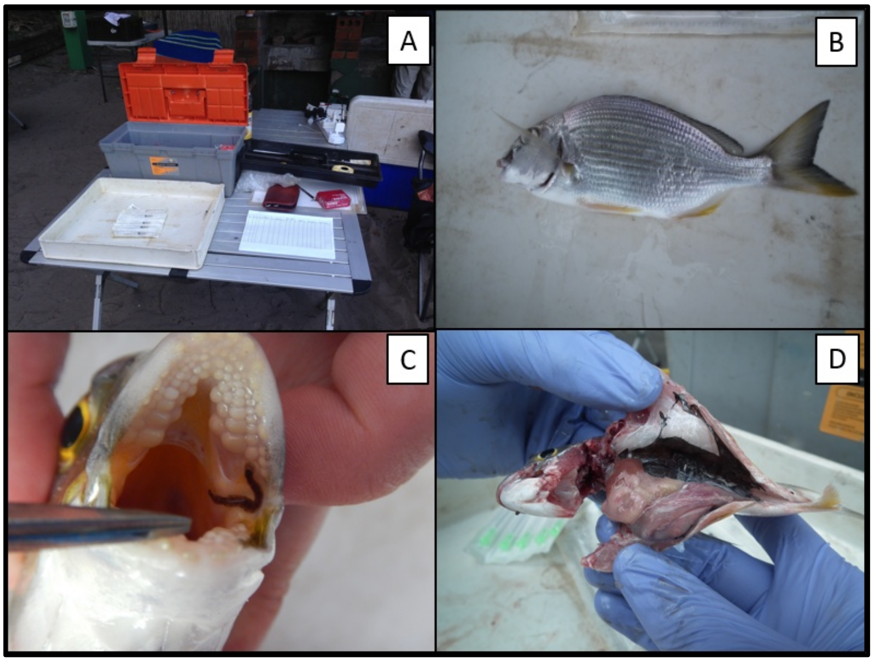

Figure 4.1. Necropsy procedure. (A): station set up for necropsy procedure. (B): External examination done on the fish. (C): Parasite noted during external examination. (D): internal examination completed to see if any organ abnormalities were present.

4.2.3 Statistical analysis

Significant differences between the fish (FHAI scores and somatic indices) were determined using the Tukey posthoc analysis with a value of $p < 0.05$ (Gerber *et al*. 2015). One-way analysis of variance (ANOVA) was performed with the aid of GraphPad Prism 5.

4.3 Results

4.3.1 FHAI and gross organ indices

A total of 55 fishes were sampled during the August 2015, December 2015 and February 2016 surveys. Eight fishes were sampled during August 2015, which consisted of two species, *R. sarba* and *T. jarbua* (Table 4.1).

Table 4.1. Mean (± SD) mass, total length (TL), condition factor (CF) and gutted condition factor (GCF) values for *Oreochromis mosssambicus*, *Rhabdosargus sarba* and *Terapon jarbua* of August 2015, December 2015 and February 2016 surveys.

Survey	Fish sampled	Mass	TL	CF	GCF
Aug-15	*Terapon jarbua* n = 5	22.36 ± 4.74	116.4 ± 7.44	1.73 ± 0.26	1.7 ± 0.25
	Rhabdosargus sarba n = 3	59.3 ± 24.16	148.33 ± 23.63	2.26 ± 0.13	2.23 ± 0.13
Dec-15	*Rhabdosargus sarba* n = 10	30.6 ± 6.03	121.6 ± 8.34	2.8 ± 0.38	2.5 ± 0.67
	Oreochromis mossambicus n = 10	98.33 ± 41.4	177.5 ± 25.86	2.79 ± 0.76	2.76 ± 0.39
Feb-16	*Rhabdosargus sarba* n = 12	105.9 ± 90.37	178.33 ± 50.74	2.12 ± 0.21	2.6 ± 0.25
	Oreochromis mossambicus n = 15	169.16 ± 21.01	219.27± 12.35	2.88 ± 0.32	1.81 ± 0.53

Rhabdosargus sarba (n = 3) had 66.7% external parasites (Monogenea, *Ergasilus* sp., Copepoda, Hirudinea and Nematoda) present (Table 4.2). The rest of the external and internal observations were normal with no obvious abnormality. *Terapon jarbua* (n = 5) only had abnormalities of the liver colour with 80% of the fish showing a slight discolouration in the liver tissue (Table 4.2). All other internal variables of both fish species of the August 2015 survey had no abnormalities.

Twenty fish were sampled during December 2015 which consisted of two species, *R. sarba* and *O. mossambicus.* External abnormalities identified on *R. sarba* (n = 10) included 10% skin abrasion and 30% slight liver discolouration (Table 4.2). External parasites (10%) were also present on *R. sarba* parasites (Copepoda, *Ergasilus* sp., Monogenea and Nematoda). The remainder of the variables for *R. sarba* were normal with no abnormalities detected. External and internal abnormalities identified on *O. mossambicus* included fin erosion (20%), slight discolouration of the liver (40%) and 50% relatively discolouration of the liver (Table 4.2). *Oreochromis mossambicus* also included 10% of mottled liver but had no other major abnormalities; and no parasites were present on *O. mossambicus* (Table 4.2).

Twenty - seven fish were sampled during February 2016. These fish species were *R. sarba* and *O. mossambicus*. External abnormalities identified on *R. sarba* (n = 12) included skin abrasion (33.3%), fin erosions (16.7%) and liver discoloration (8.3%) (Table 4.3). The rest of the variables had no abnormalities and only a few parasites (8.3%) were present on *R. sarba* (Copepoda, *Ergasilus* sp., Monogenea and Nematoda) (Table 4.3). *Oreochromis mossambicus* (n = 15) included skin abrasions (33.3%) and fin erosions (26.7%) (Table 4.2). A total of 26.7% of *O. mossambicus* had slight discolouration in the liver, where 46.7% was fairly discoloured and 13.3% had fatty livers (Table 4.2). The rest of the variables had no abnormalities and no parasites were present on *O. mossambicus.*

Table 4.2. Abnormalities (%) present in *Oreochromis mosssambicus*, *Rhabdosargus sarba* and *Terapon jarbua* of August 2015, December 2015 and February 2016 surveys.

	August 2015 Survey			
	External parasites	Liver abnormality	Skin abrasion	Fin erosion
Terapon jarbua (n = 5)	/	80%	/	/
Rhabdosargus sarba (n = 3)	66.7%	/	/	/
	December 2015 Survey			
	External parasites	Liver abnormality	Skin abrasion	Fin erosion
Rhabdosargus sarba (n = 10)	10%	30%	10%	/
Oreochromis mossambicus (n = 10)	/	40% / 50% / 10%	20%	/
	February 2016 Survey			
	External parasites	Liver abnormality	Skin abrasion	Fin erosion
Rhabdosargus sarba (n = 12)	8.3%	8.3%	33.3%	16.7%
Oreochromis mossambicus (n = 15)	/	26.7% / 13.3%	33.3%	26.7%

The fishes from the August 2015 survey were all juveniles. It was difficult to determine the sex of the fishes because of their immature gonads. The *R. sarba* from the December 2015 survey were also all juveniles. Three of the ten *O. mossambicus* sampled in December 2015 were females and the other seven males. Twelve *R. sarba* were sampled in the February 2016 survey where four were females and the other eight males. Fifteen *O. mossambicus* were sampled in the February 2016 survey with two being females and the rest males.

The FHAI results showed no significant difference ($p < 0.05$) between the surveys of August 2015, December 2015 and February 2016. In Figure 4.2 the average FHAI score for all fish species on all three surveys had a score lower than 30. There were no significant differences ($p < 0.05$) between the CF, GCF, SSI, HSI and GSI values of *R. sarba* and *O. mossambicus* of August 2015, December 2015 and February 2016 (Table 4.3). *Terapon jarbua* was only sampled on the first survey, therefore could not be compared between surveys. The values that are significantly different ($p < 0.05$) from each other were *T. jarbua* from August 2015 and *O. mossambicus* in the February 2016 survey (Figure 4.2).

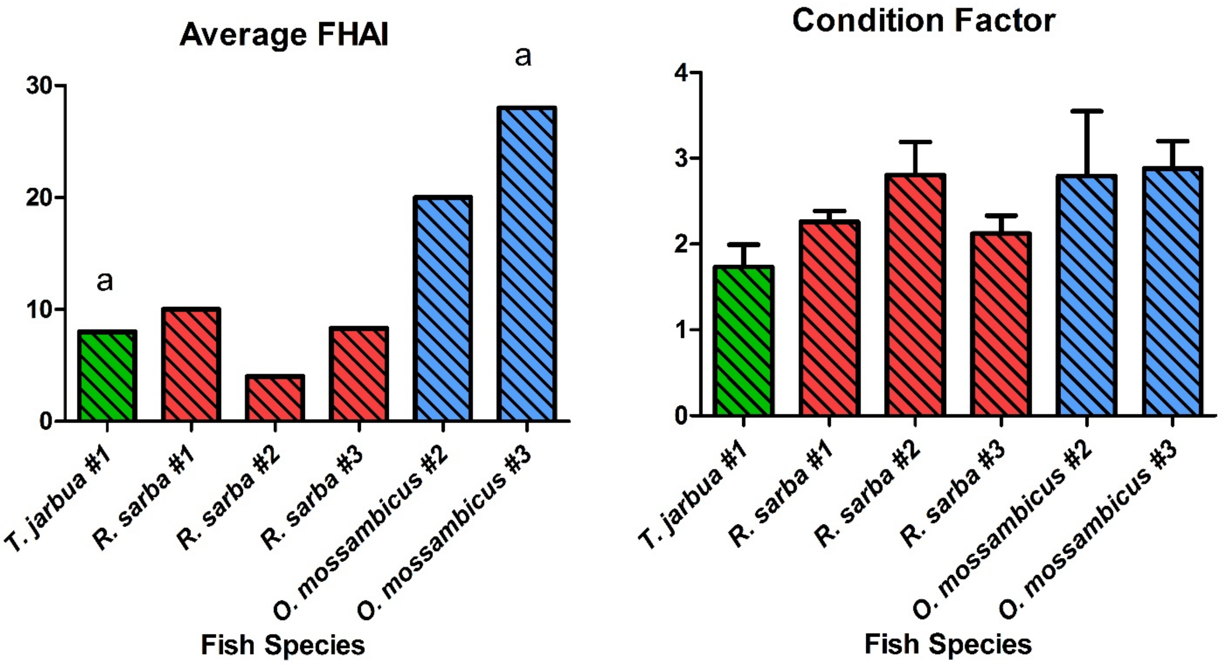

Figure 4.2 Mean Fish Health Assessment Index (FHAI) (a) score and condition factor (CF) (b) for the fish species sampled in August 2015 (#1), December 2015 (#2) and February 2016 (#3). Letters indicate significant differences ($p < 0.05$) between fish species and surveys.

Table 4.3. Mean (± SD) hepatosomatic index (HSI), splenosomatic index (SSI), GSI and health assessment index (HAI) values for *Oreochromis mosssambicus*, *Rhabdosargus sarba* and *Terapon jarbua* from August 2015, December 2015 and February 2016 surveys.

Survey	Fish sampled	HSI (%)	SSI (%)	GSI (%)	Average HAI	Total HAI
Aug-15	*Terapon jarbua* n = 5	1.18 ± 0.28	0.21 ± 0.16	0.17 ± 0.17	30	90
	Rhabdosargus sarba n = 3	0.66 ± 0.15	0.08 ± 0.08	0.04 ± 0.03	10	20
Dec-15	*Rhabdosargus sarba* n = 10	1.38 ± 0.58	0.12 ± 0.05	0.12 ± 0.07	11	110
	Oreochromis mossambicus n = 10	1.03 ± 0.22	0.07 ± 0.03	0.45 ± 1.03	32	320
Feb-16	*Rhabdosargus sarba* n = 12	0.89± 0.34	0.03 ± 0.02	0.07 ± 0.05	25.83	310
	Oreochromis mossambicus n = 15	1.07± 0.33	0.04 ± 0.02	0.29 ± 0.32	32.67	490

The fat present in the fishes of all three surveys were approximate intestinal fat. There were no fat present in the August 2015 survey of *T. jarbua and R. sarba* (Table 4.4). In the December 2015 survey, *R. sarba* had a fat percentage of 0%, were nine of the

O. mossambicus fish had a fat percentage of < 50% (Table 4.4). Both *R. sarba* and *O. mossambicus* of the February 2016 survey showed present fat percentages. Four *R. sarba* fish had a fat percentage of > 50% and seven a fat percentage of < 50%. Eleven *O. mossambicus* fish had a fat percentage of < 50% and one a fat percentage of > 50% (Table 4.4).

Table 4.4. Fat percentages (%) present in *Oreochromis mosssambicus*, *Rhabdosargus sarba* and *Terapon jarbua* of August 2015, December 2015 and February 2016 surveys.

	August 2015 Survey
	Fat percentage
Terapon jarbua (n = 5)	0%
Rhabdosargus sarba (n = 3)	0%
	December 2015 Survey
	Fat percentage
Rhabdosargus sarba (n = 10)	0%
Oreochromis mossambicus(n = 10)	(n = 9) < 50%
	February 2016 Survey
	Fat percentage
Rhabdosargus sarba (n = 12)	(n = 4) > 50% and (n = 7) < 50%
Oreochromis mossambicus (n = 15)	(n = 11) < 50% and (n = 1) > 50%

4.4 Discussion

A variety of approaches have been used to determine the effects that stress has on the health of fish and these approaches have been used on many aquatic systems that experience environmental stressors (Adams *et al*., 1993). The more commonly used approaches for assessing the health of fish are age and growth analysis, CF, various condition or organosomatic indices, measures of biochemical, as well as physiological and pathological condition (Adams *et al*., 1993). Most of these types of health measurements cannot be rapidly and inexpensively applied because these measurements require a lot of time, specialist training and laboratory analysis (Adams *et al*., 1993).

The main purpose of the method developed by Goede and Barton (1990) is to detect gross changes in the health of fish populations early enough for remedial actions to be taken (Adams *et al*., 1993). The health assessment index is a quantitative index that allows statistical comparisons of fish health from data sets and is a quick and simple procedure as well as inexpensive, to rapidly assess the health of the fish in an aquatic ecosystem (Adams *et al*., 1993).

No fish were caught using the seine or fyke nets. The possible reason for this is that the water is too clear making the nets visible, causing the fish to avoid the nets. From all the different methods used during all the surveys, rod and reel was the most effective method for catching the fish needed for the project. The fish were mostly caught along the reeds and in the channel that connects the lakes to each other.

A score of above one for CF is considered as a healthy relationship between the weight and length of a fish (Adams *et al*., 1993). All the fish collected during each survey had a score of above one and indicated that they were all in a good condition (Figure 4.2). The CF values for *O. Mossambicus* (2.79 and 2.88) from Kosi Bay had higher CF values indicating that these fishes were in a better condition (Table 4.1). The CF, which is the relationship between the mass and length of the fish, can be seen as the general well-being, physiological condition and fitness of a fish (Bolger and Connolly, 1989). Based on these length-weight relationships it is believed that a heavier fish will be in a better condition but this can be influenced by factors like temperature fluctuations, water degradation, food availability and seasonal changes (McHugh, 2012). Condition factor can also be interpreted as depletion of energy reserves and is used in many fishery studies because weight and length are routinely measured (Anderson and Neumann, 1996). A higher fat percentage could indicate a higher CF value in the fishes where they store more energy for spawning. The fishes caught had low fat percentages and higher muscle growth. The CF can change with physiological development, sexual maturity and variation within species due to geographic location.

A study by Marchand *et al.* (2012) on Roodeplaat Dam, focused on the gills, liver, ovaries, testes, kidney and the heart of *O. mossambicus.* The Roodeplaat Dam is a hyper-trophic impoundment which is impacted by the effluent of sewage treatment plants near Pretoria, South Africa. *Oreochromis mossambicus* was investigated because of the water quality analysis that showed that selected variables such as pH and nutrients were above recommended levels (Marchand *et al.,* 2012). There were a number of alterations to these organs of the *O. mossambicus* in the study of Marchand *et al*. (2012), with the most being on the liver. The CF was also calculated and had a mean (± SD) value of 1.68 ± 0.12. Although the Roodeplaat Dam is impacted and had alterations on the organs, the *O. mossambicus* had a high CF, indicating a healthy condition. All of the *O. mossambicus* from the Kosi Bay system had higher CF values with minimal alterations to the organs and were potentially in a healthier condition (Table 4.3).

A study by Van Dyk (2014) also investigated the liver of *O. mossambicus* in the Roodeplaat Dam by carrying out a fish health assessment. The microscopic investigations presented a cholangioma, which is a neoplastic growth on the liver. The CF of *O. mossambicus* from this study had a mean (± SD) value of 1.7 ± 0.1 and the liver of *O. mossambicus* showed no macroscopic liver abnormalities. These mentioned studies were the only studies where the

CF of *O. mossambicus* was reported. No other study on CF and the health of *O. mossambicus* was reported in the Kosi Bay system before.

Rhabdosargus sarba is a very common species that occurs in the Kosi Bay system. The catch of *R. sarba* in fish traps has drastically increased from a mean of 4.7% in 1981 to 34.7% in 1995 and has remained an important component of trap catches (Kyle, 2013). With the use of new materials by the local communities the catch of fishes will increase because of the smaller size distribution. The possible reason for the increase in the number of *R. sarba* juveniles entering the Kosi Bay system is due to the closure of the Lake St Lucia estuary. Migrating up into a system is part of the important life cycle for juvenile *R. sarba* and thus Kosi Bay has become an important nursing ground for the juvenile *R. sarba* (James *et al*., 2001).

A study by Richardson *et al*. (2011) investigated *Rhabdosargus holubi* in three temporarily open/closed estuaries along the east coast of South Africa. The EFCI was used to determine the community level health status of the fish from each of the estuaries. The estuaries investigated were East Kleinmonde, Old Woman's and Mtana. Developments occurred along the banks and catchment of East Kleinmonde and Old Woman's (Richardson *et al*., 2011). These were mainly housing developments, agriculture and a golf course in the lower and middle reaches of Old Woman's catchment. Mtana is mostly undeveloped with low cattle grazing along the banks and catchment (Richardson *et al*., 2011). The EFCI was a successful and useful indicator in the study of Richardson *et al*. (2011) for the overall environmental conditions of these three estuaries. East Kleinmonde had a score rating of 48, Old Woman's a score rating of 48 and Mtana a score rating of 44, where 40 - 44 is indicated as a moderate condition and 46 - 62 a good condition score (Richardson *et al*., 2011). All the CF scores for *Rhabdosargus holubi* form these estuaries were above one, indicating that the fish species was in a healthy condition. All of the CF scores from the *Rhabdosargus* family (*Rhabdosargus sarba)* from Kosi Bay were also above one which indicated that the fish species were in a healthy condition (Figure 4.2). *Rhabdosargus sarba* is an important fish species for the local community of Kosi Bay in terms of food and is also part of the KwaZulu-Natal recreational shore-fishery. Thus, the loss and reduction of this fish species will impact this important fishery.

A good HAI result was expected due to the pristine Kosi Bay system which falls within the iSimangaliso Wetland Park. The water quality results showed that most of the metals tested within the different lakes exceeded the guidelines that they were compared to (See Chapter 3). Although these guidelines were exceeded, there was no ecological pressure or any clear anthropogenic impact noted throughout the system. The only possible impact on the fish species of the system is the local people's fish traps and increasing population closer to the lakes. These fish traps have more direct effects on the fish diversity but might not have direct physiological effects on the fish. The low HAI scores were due to minimal organ abnormalities and minimal parasites present on the fish species sampled.

The other health assessment indices mentioned earlier all focus on species diversities and nursery functions of migrating fish found in estuaries. These indices might give an overall indication and broad overview of the health of the estuary regarding fish diversity, but not a clear individual fish based health investigation like the FHAI procedure followed in this study.

4.5 Conclusion

Fish Health Assessment Index protocol was used to detect any health abnormalities present in *T. jarbua*, *R. sarba* and *O. mossambicus* of the Kosi Bay system. The fish were sampled in August 2015, December 2015 and February 2016. Other factors such as CF and organosomatic indices were also investigated to determine the condition and health of the fishes. The results from the fish species sampled on all the surveys indicated that the overall health of the selected fish species within the Kosi Bay system is in a good condition. There were minimal abnormalities present on the fishes and the CF of all the fishes sampled had a healthy CF value. The abnormalities that were found on the fishes had no potential impact to the health of the specific fish.

The results from this chapter can be used as a baseline for health of selected fish within the system. Investigating the overall health of selected fish species (*Oreochromis mossambicus*, *Terapon jarbua* and *Rhabdosargus sarba*) gave a good indication that the system is in a healthy condition.

5. Metal bioaccumulations, metallothionein inductions and human consumption hazard of selected fish species in Kosi Bay

5.1 Introduction

The increased use of metals in industry and agriculture has led to environmental pollution in ecosystems (Zhou, 2008). Accumulation of metals in ecosystems have been receiving considerable attention due to their toxicity and potential for bioaccumulation in aquatic species (Pourang, 1995). Some metals are essential for the normal metabolism of organisms while others are nonessential and have no biological role in the organisms (Van Dyk *et al.,* 2007). Zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) at the right concentrations are essential metals for metabolic activity. However, low levels of cadmium (Cd), mercury (Hg), chromium (Cr) and lead (Pb) are potentially toxic to aquatic organisms (Peerzada *et al*., 1990).

There are two major concerns with metals; firstly, the imminent health hazard to humans, especially metals mentioned like cadmium (Cd), lead (Pb) and mercury (Hg) are some of the metals that have effects on human health (Pourang, 1995). Secondly, the aquatic environment that is being threatened due to biological deterioration caused by metal pollution and in the process results in a potential threat to the ecological equilibrium (Pourang, 1995).

Metal contamination in aquatic ecosystems tend to accumulate in the sediments through adsorption and precipitation processes (Coetzee *et al*., 2002). Fish accumulate these metals via the oral route ingesting water and absorbing it through the gills and skin, although body surface uptake does not play a dominant role (Pourang, 1995). Generally the metals accumulated by the fish are stored in the liver, kidney and muscle tissue of the fish (Manahan, 1989). These metals in the organs are generally present in the oxidised form or are chemically bonded with proteins, such as enzymes or other biological tissues. They create strong bonds which increase bioaccumulation of metals and reduce metal excretion (Manahan, 1989). The effects of metal pollution can already be seen on cellular or tissue levels before significant changes can be observed in the fish in regard to behaviour and external appearances (Van Dyk *et al*., 2007). Other effects that metals have on fish, especially lead under high- toxicity levels, can be drowning, caused by respiratory failure due to the excessive amount of mucus production (Kaya and Akbulut, 2015).

Biomarkers are used in the assessment of environmental risks, posed by contaminants and can be defined as a biochemical, physiological, cellular or behavioural alteration using tissue or fluids from organisms to analyse exposure from chemical contaminants (Erk *et al*., 2008). Biomarkers are proactive tools for the detection of pollutants before damage is done to the ecosystem (Pheiffer *et al*., 2015). Using biomarkers gives information on biological effects to pollutants rather than a mere quantification of their environmental levels (Wepener and Vermeulen, 2005). Metallothionein (MT) is considered as an affective biomarker assay to provide an integrated biomarker response assessment of a system (Wepener and Vermeulen, 2005).

Fish are an important source of protein to humans and are consumed regularly (Gilbert and Avenant-Oldewage, 2014), especially in the Kosi Bay system. Ingestion of fish is a route which humans can be exposed to trace metals and using fish tissues for the monitoring of trace metal levels is therefore of great importance (Yilmaz, 2003).

The aim of this part of the project was to determine the metal exposure of fish within the Kosi Bay system and the associated risk for human consumption. This was achieved using metal concentrations in fish muscle tissue, an exposure biomarker in the form of metallothionein (MT) protein induction, and calculation of bioconcentration factors (BCF), between the environment and the muscle tissues from the selected fish species. The dissected fish liver and muscle tissue were used to determine the metal concentrations, metallothionein (MT) protein induction and the BCF. The BCF was done in order to ascertain the bioaccumulation exposures between the environment (water and sediment) and biota (McGeer *et al*., 2003). Consumption hazard assessments were also done to determine if fish eaten by the local community could pose a possible contamination threat (Gilbert and Avenant-Oldewage, 2014).

5.2 Material and methods

5.2.1 Sampling protocol

Fish were collected during three different surveys in August 2015, December 2015 and February 2016 from various sites in the Kosi Bay system (Chapter 2). The fish were collected by rod and reel and were dissected to obtain the liver and muscles tissue. The muscle tissue was collected by dissecting a piece of muscle (4 cm x 4 cm) from each fish to determine metal concentrations and the BCF. The muscle tissue were placed in 15 ml tubes and stored in liquid nitrogen. Likewise, the liver from each fish was obtained to analyse the MT protein. The liver was placed in an Eppendorf tube and preserved in Hendrikson's buffer. The Hendrikson's buffer was prepared by adding Tris-hydrocloride (0.2422 g), b-Mercapto-ethanol (0.03487 ml), EDTA (0.0186 g) and BSA (0.02 g) together, and made up to 50 ml with distilled water. After the distilled water was added, 50 ml of glycerol was added to make up a total of 100 ml of. The Hendrikson's buffer keeps the tissue from deteriorating and preserves the proteins within the tissue. After the buffer was added, the tube was marked and placed into liquid nitrogen for transport back to the laboratory for further analysis.

5.2.2 Laboratory analysis

5.2.2.1 Metal concentrations in muscle tissues

In the laboratory, the muscle tissue samples were defrosted and oven dried for four days at 60 °C. With the use of the Milestone Microwave Digestion system the tissues were digested following a method from Gilbert and Avenant-Oldewage (2014). Tissue samples were weighed to 0.2 g and added to the Teflon digestion vessel. Seven ml of Suprapur 65 % nitric acid was added to the vessel and left for 10 min for digestion to start. After the 10 min waiting period, the vessels were closed and placed into the microwave. The digestion run was set at 40 min with a maximum temperature of 200 °C. A certified reference material (CRM) of fish proteins was used as a reference. The samples were transferred into 50 ml volumetric flasks and made up to 50 ml with 1% nitric acid. Before the ICP - MS reading, the samples were filtered with a 0.45 µm cellulose nitrate filter. Filtered samples were analysed for metal concentrations using an ICP - MS (Agilent 7500ce). The metals analysed were Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Hg and Pb. These metals were tested to determine the current level of metal accumulation in the muscle tissue of the fish collected.

5.2.2.2 Metallothioneins (MT)

The samples from the surveys were stored in a –80 °C freezer until the analyses could be completed. The liver samples were allowed to thaw before the analysis and the samples were kept on ice during the entire procedure. The Viarengo *et al*. (1997) procedure was used for the MT analysis. The protein content was determined with the Bradford (1976) method for further calculations of the MT concentrations (Viarengo *et al*., 1997).

The liver samples (0.1 g) were homogenised in a tube with 500 µl MT homogenising buffer that consisted of 0.02 M Tris-HCL buffer, pH 8.6, 0.5 M sucrose, 0.006 mM leupeptine, 0.5 mM PMSF (phenylmethylsulphonylfluoride) and 0.01% b-mercaptoethanol. The homogenate was centrifuged at 30000 rpm for 20 min at 4 °C. After the centrifuge run, 500 µl ethanol (96%) and 40 µl chloroform were added followed by another centrifuge run for 10 min at 7000 rpm (4 °C). The samples were then added with three volumes (1.5 ml) of cold ethanol (96%) and incubated at - 20 °C for 1 hour. Thereafter, the pellet was washed twice with the washing buffer (87% ethanol + 1% chloroform + 12% homogenising buffer) and then left on tissue paper to dry. After the pellet was washed and dried, the pellet was resuspended in NaCl and Tris-EDTA. Following a 15 min incubation period at room temperature, a total of 210 µl DTNB (5,5'Dithio-bis(2-Nitrobenzoic acid)) for the blank, standards, and samples was prepared. This was added to the microtitre plate which was read at 412 nm with a BioTek absorbance microplate reader (ELx800) (Figure 5.1).

5.2.2.3 Protein assay

Protein assay was modified from the Bradford (1976) procedure. A total of 5 µl Milli-Q water, 5 µl BSA for the standards and 5 µl homogenate of the liver sample was prepared for the microtitre plate. Thereafter, 245 µl Bradford reagent was added to the blanks, standards and the samples. After these reagents were added, the plate was allowed to incubate for 5 min. The microplate was then read at 595 nm on a BioTek absorbance microplate reader (ELx800) (Figure 5.1). The values obtained from the protein assay were used for further calculations of the MT results.

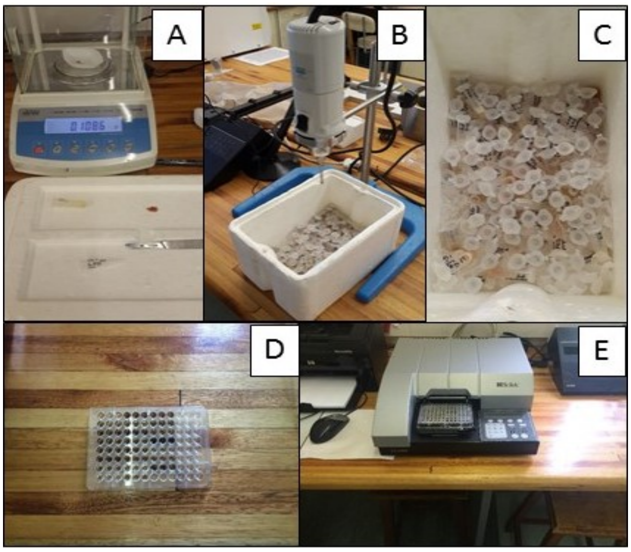

Figure 5.1. MT and Protein (PT) assay procedure. (A): Liver sample (0.1 g) weighed for MT and PT procedure. (B): Sample homogenised and placed inside Eppendorf tube and filled with homogenising buffer for MT's, and Milli-Q water for protein samples (C): Samples after centrifuge and incubation. (D): Microtitre plate prepared and left for incubation period. (E): Microtitre plate analysed with a BioTek absorbance microplate reader (ELx800).

5.2.3 Statistical analysis

Using GraphPad Prism 5, a one-way analysis of variance (ANOVA) was performed on the metal and MT results. Significant differences were determined using the Tukey posthoc analysis with a value of $p < 0.05$.

5.2.4. Bioconcentration factor

Bioconcentration factors were calculated according to the method of Abel (1988) between fish organs and the environment (water and sediment) (Gilbert and Avenant-Oldewage, 2014). Alhashemi *et al*. (2012) used a very similar method and this was also used as a reference for the BCF calculations.

The BCF was calculated with the following formulas:

$$BCFw = \frac{C_{(Fish\ organ)}}{C_{(Water)}}$$

$$BCFs = \frac{C_{(Fish\ organ)}}{C_{(Sediment)}}$$

where: the C (fish organ) and C (sediment) is in µg/g dry weight and C (water) is in µg/L.

5.2.5. Human health risk assessment

Exposures to metal contaminants were calculated by using the average daily doses (ADD) formula (Heath *et al.,* 2004):

$$ADD = \frac{C_M \times IR_M \times ED}{BM \times ED}$$

where: Cm = average concentration of pollutant in food substance (mg/kg), IRm = average intake rate (mg/kg) (the average intake per day according to FAO (2010) is 0.02 kg/d); ED = exposure duration (365 days) and BM = body mass (66.5 kg). The average body mass of an adult is 66.5 kg according to Puoane *et al.* (2002) and this was used for the calculations of the current study.

After calculating the ADD, the hazard index (HI) was calculated (Heath *et al.,* 2004):

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

where: ADD = average daily dose and RfD = mg/kg – per day. The RfD is the total allowable (mg/kg) intake of a specific metal per day.

5.3 Results

5.3.1 Metal concentrations (muscle)

Aluminium, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Hg and Pb were tested in the muscle tissues of *T. jarbua, R. sarba* and *O. mossambicus.* The results presented here are summarised for the August 2015, December 2015 and February 2016 surveys. The concentrations for each metal for each survey for each fish species are presented in Appendix E. Muscle tissues were compared to the CRM as a reference source. The CRM was within an allowable recovery percentage (between 80% - 110%).

The highest concentration of Al was reported from *O. mossambicus* in Dec 2015 and was significantly higher than the other samples (Figure 5.2). Manganese concentrations were the highest for *R. sarba* in Dec 2015 (Figure 5.2). In the case of Fe, the highest value was *T. jarbua* in Aug 2015 and was significantly higher than all the other values (Figure 5.2*). Oreochromis mossambicus* cobalt concentrations were the highest in Dec 2015 and significantly higher than *R. sarba* in Feb 2016. The highest concentration of Ni was seen in *R. sarba* in Aug 2015 and was significantly higher than all the other samples (Figure 5.2). The Cu concentration was the highest in *R. sarba* in Feb 2016 and was significantly higher than the other fish sampled (Figure 5.3). In the case of Zn, the highest value was seen for *T. jarbua* in Aug 2015 and was significantly higher than the other samples (Figure 5.3).

The highest reported As concentration was from *R. sarba* in Feb 2016 and was significantly higher than the other samples (Figure 5.3). There were significant differences ($p < 0.05$) between *T. jarbua* Aug 2015 and *O. mossambicus* Dec 2015 and Feb 2016 for Se (Figure 5.3). There were also significant differences between all of the *R. sarba* and *O. mossambicus* for Se (Figure 5.3). The only significant difference for Hg was between *R. sarba* in Feb 2016 and *O. mossambicus* in Dec 2015 (Figure 5.4). There were no significant differences between the Pb concentrations (Figure 5.4), although the *O. mossambicus* from the Dec 2015 was found to have the lowest concentrations.

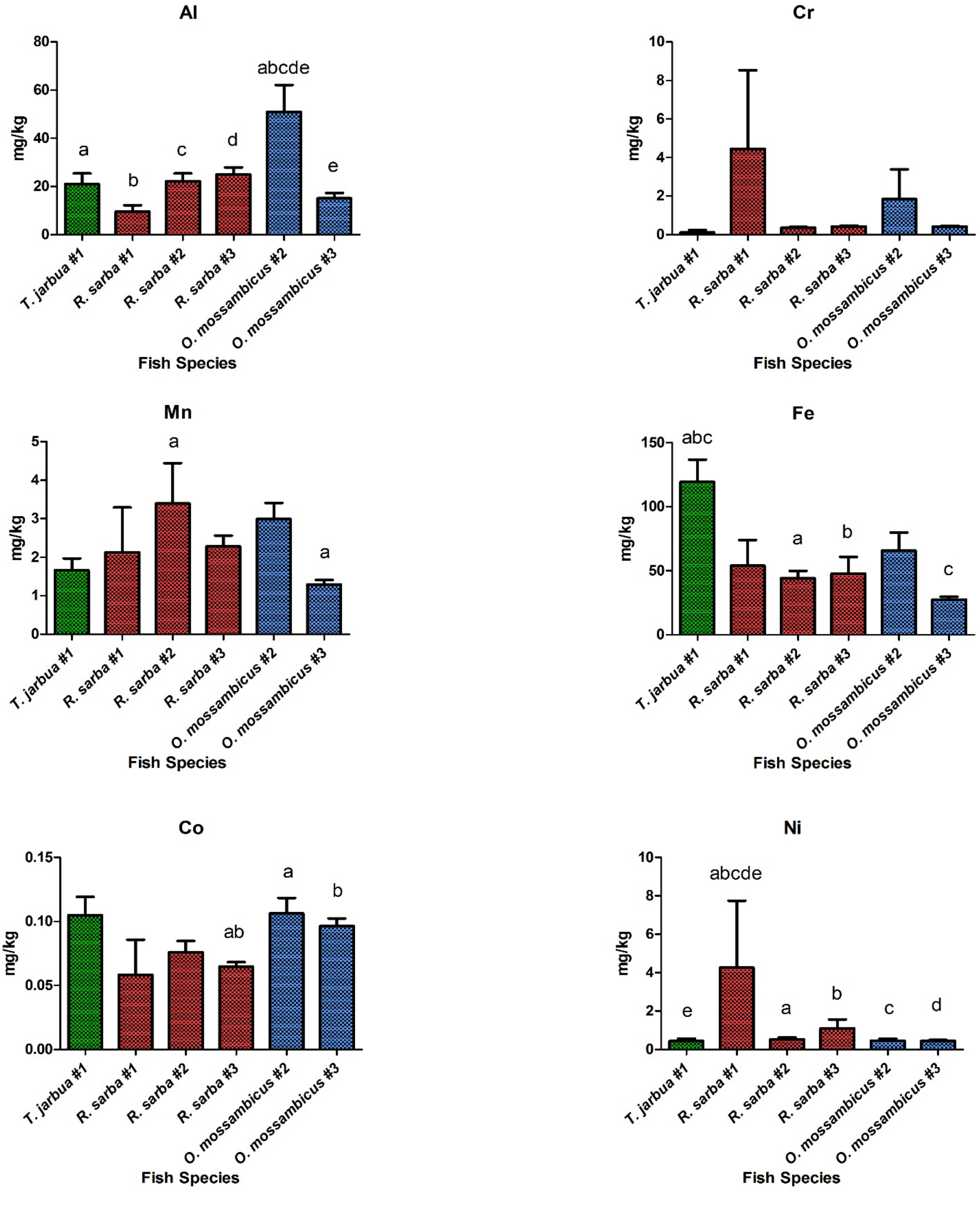

Figure 5.2. Mean metal concentrations (mg/kg) of Al, Cr, Mn, Fe, Co and Ni present in the tissue samples of *Terapon jarbua*, *Rhabdosargus sarba* and *Oreochromis mossambicus* for August 2015 (#1), December 2015 (#2) and February 2016 (#3), with standard error of the mean (SEM) showing seasonal variations. Letters indicate significant differences ($p < 0.05$) between fish species for specific metals.

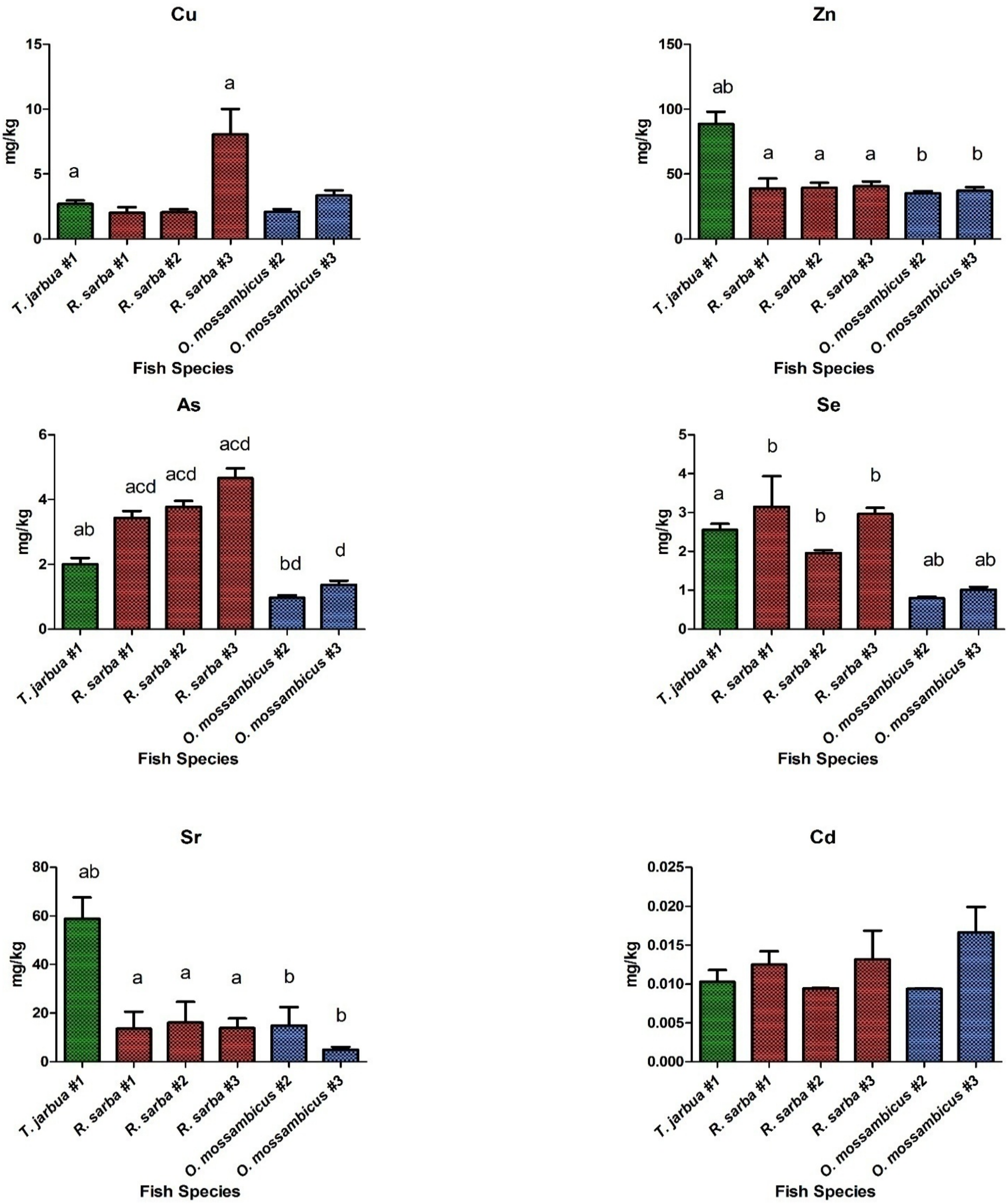

Figure 5.3. Mean metal concentrations (mg/kg) of Cu, Zn, As, Se, Sr and Cd present in the tissue samples of *Terapon jarbua*, *Rhabdosargus sarba* and *Oreochromis mossambicus* for August 2015 (#1), December 2015 (#2) and February 2016 (#3), with standard error of the mean (SEM) showing seasonal variations. Letters indicate significant differences ($p < 0.05$) between fish species for specific metals.

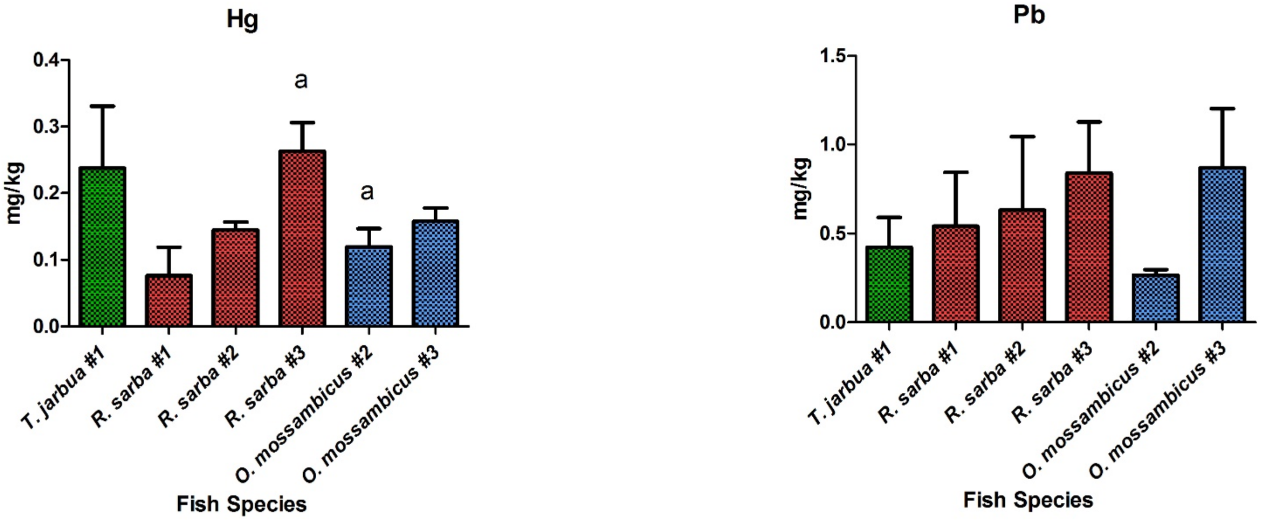

Figure 5.4. Mean metal concentrations (mg/kg) of Hg and Pb present in the tissue samples of *Terapon jarbua*, *Rhabdosargus sarba* and *Oreochromis mossambicus* for August 2015 (#1), December 2015 (#2) and February 2016 (#3), with standard error of the mean (SEM) showing seasonal variations. Letters indicate significant differences ($p < 0.05$) between fish species for specific metals.

5.3.2 Metallothioneins (MT)

The MT results (Figure 5.5) showed that the highest value was *R. sarba* in August 2015 and was higher than *T. jarbua* from 2015, *R. sarba* from 2016 as well as *O. mossambicus* in 2015 and 2016. *Rhabdosargus sarba* in Aug 2015 also had a higher concentration and was significantly higher than *T. jarbua* in Aug 2015, *R. sarba* Feb 2016 as well as *O. mossambicus* Dec 2015 and Feb 2016.

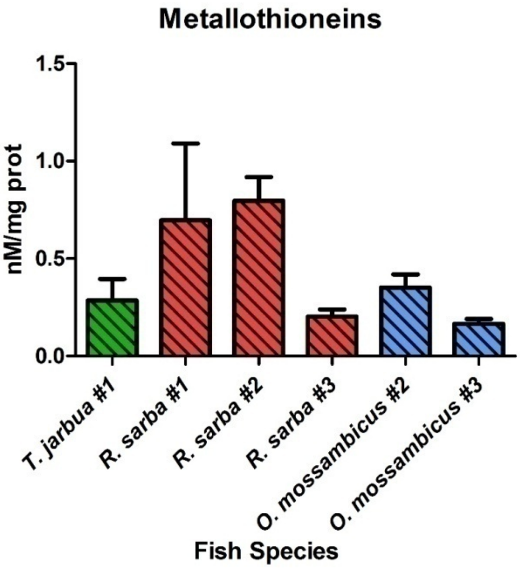

Figure 5.5. Metallothioneins in the liver tissues of *Terapon jarbua*, *Rhabdosargus sarba* and *Oreochromis mossambicus* for August 2015 (#1), December 2015 (#2) and February 2016 (#3), with standard error of the mean (SEM) showing seasonal variations.

5.3.3 Bioconcentration factors (BCF)

Mean metal concentrations recorded for water and sediment from the lake 2+3 channel (L2+L3C) site (Chapter 3) for BCF comparison is indicated in Table 5.1. The reason for only including the L2+L3C site in the BCF calculations was because this was the site where most of the fish were caught.

Bioconcentration factors for metals in the water, compared with the muscle tissue of *T. jarbua, R. sarba* and *O. mossambicus,* indicated that Al, Mn, Co, Ni, Zn, As, Cd, Hg and Pb levels had higher concentrations in the tissue compared to the water. Chromium, Fe, Cu, Se and Sr levels had higher concentrations in the water than was found in the muscle tissue of all three fish species (Table 5.2).

The BCF values for *T. jarbua*, when compared to the sediment indicated that Al, Cr, Mn, Fe, Ni, Cu and Pb levels were higher in the sediment. Cobalt, Zn, As, Se, Sr and Hg levels had higher concentrations in the fish muscle tissues (Table 5.2).

The BCF values for *R. sarba*, when compared to the sediment indicated that Al, Mn, Fe, Cu and Zn levels were higher in the sediment. Chromium, Co, Ni, As, Se, Sr, Cd, Hg and Pb levels had higher concentrations in the fish muscle tissues (Table 5.2).

The BCF values for *O. mossambicus*, when compared to the sediment indicated that Al, Mn, Fe, Ni, Cu and Zn levels were higher in the sediment. Chromium, Co, Cu As, Se, Sr, Cd, Hg and Pb levels had higher concentrations in the fish muscle tissues (Table 5.2).

Table 5.1. Mean metal concentrations recorded for water and sediment samples from the lake 2+3 channel (L2+L3C) site from the Kosi Bay system (for BCF comparison) in August 2015, December 2015 and February 2016.

Metal	Water (mg/L)	Sediment (mg/kg)
Al	0.64	519.4
Cr	12.08	0.41
Mn	0.03	1.73
Fe	24.47	164.2
Co	0.1	0.07
Ni	0.08	0.84
Cu	8.2	2.79
Zn	0.16	8.37
As	0.37	0.05
Se	7.01	0.06
Sr	112.1	1.56
Cd	0.01	0.007
Hg	0.09	0.03
Pb	0.46	0.74

Table 5.2. Mean BCF values between median trace element concentrations in water and sediment compared to *Terapon jarbua, Rhabdosargus sarba* and *Oreochromis mossambicus* organ (muscle) levels from the Kosi Bay system. These fish were collected from the 2+3 channel (L2+L3C) site in August 2015, December 2015 and February 2016.

Metal	Water			Sediment		
	T. jarbua	*R. sarba*	*O. mossambicus*	*T. jarbua*	*R. sarba*	*O. mossambicus*
Al	32.73	29.38	51.3	0.04	0.04	0.06
Cr	0.01	0.14	0.09	0.3	4.27	2.79
Mn	57.55	90.01	74.2	0.96	1.5	1.24
Fe	4.88	1.99	1.9	0.73	0.3	0.28
Co	1.09	0.69	1.05	1.61	1.02	1.55
Ni	5.62	24.92	5.7	0.53	2.33	0.53
Cu	0.33	0.49	0.33	0.96	1.45	0.97
Zn	569.26	253.65	231.05	10.6	4.72	4.3
As	5.5	10.83	3.2	37.54	73.93	21.87
Se	0.36	0.38	0.13	42.55	44.83	15.13
Sr	0.52	0.13	0.09	37.69	9.28	6.39
Cd	0.81	0.92	1.02	1.34	1.53	1.7
Hg	2.64	1.79	1.54	7.89	5.36	4.6
Pb	0.92	1.46	1.24	0.57	0.9	0.77

5.3.4 Human health risk assessment

The hazard index (HI) calculations were categorised using the hazard quotient method guidelines by Lemly (1996) (Table 5.2). A HI value smaller than 0.1 indicates that there is no potential hazard. A value between 0.1 and 1 indicates a low hazard. A value between 1.1 and 10 indicates a moderate hazard and a value higher than 10 indicates a high hazard.

Table 5.3. Hazard quotient method guidelines (Lemly, 1996).

Smaller than 0.1 = No hazard
Between 0.1 and 1 = Low hazard
Between 1.1 and 10 = Moderate hazard
Higher than 10 = High hazard

The majority of the values had a no to low hazard rating according to the Lemly (1996) hazard quotient guidelines (Tables 5.3 – 5.7). Even though the majority of the values were low hazard, there were values that indicated there is a moderate hazard for metal exposures. Looking at the August 2015 survey, one of the Cr values for *R. sarba* was above 1.1, indicating a moderate hazard (Table 5.3). All of the fish species for As were also above 1.1 and one *T. jarbua* fish was above 1.1 for Hg (Table 5.3).

Table 5.4. Metal hazard index values of *Terapon jarbua* and *Rhabdosargus sarba* for human by the local community in the August 2015 survey. Green = no hazard, yellow = low hazard, orange = moderate hazard, red = high hazard.

Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
T. jarbua 1	0.00052	0.0039	0.00061	0.0041	0.22	0.10	2.55	0.14	0.0039	0.14
T. jarbua 2	0.00053	0.0018	0.00028	0.0049	0.14	0.061	1.66	0.15	0.0017	1.73
T. jarbua 3	0.00051	0.0032	0.00068	0.0087	0.13	0.12	1.54	0.16	0.0028	0.76
T. jarbua 4	0.00049	0.0059	0.00045	0.0026	0.18	0.08	2.069	0.13	0.0027	0.67
T. jarbua 5	0.059	0.0032	0.00061	0.013	0.15	0.085	2.25	0.19	0.0044	0.27
R. sarba 1	0.00052	0.0011	0.00013	0.0039	0.098	0.026	3.49	0.095	0.0027	0.48
R. sarba 2	0.074	0.0032	0.00018	0.02	0.17	0.038	3.045	0.23	0.0044	0.13
R. sarba 3	1.26	0.0094	0.00057	0.17	0.09	0.053	3.78	0.24	0.0041	0.076

Most of the Cu and Hg values for both *O. mossambicus* and *R. sarba* for December 2015 survey were between 0.1 and 1, and *R. sarba* had values between 0.1 and 1 for Se, indicating a low hazard (Tables 5.4 and 5.5). There was one *O. mossambicus* which had a moderate hazard risk for Cr (Table 5.4). *Oreochromis mossambicus* and *R. sarba* had low or moderate hazard values for As. *Oreochromis mossambicus* had a few values that indicated a moderate hazards but most of the values were between 0.1 and 1, indicating a low hazard (Table 5.4). The As values for *R. sarba* were all above 1.1 indicating a moderate hazard (Table 5.5).

Table 5.5. Metal hazard index values of *Oreochromis mossambicus* for human consumption by the local community in December 2015 survey. Green = no hazard, yellow = low hazard, orange = moderate hazard, red = high hazard.

Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
O. mossambicus 1	0.05	0.01	0.0009	0.02	0.18	0.028	0.63	0.04	0.0028	0.98
O. mossambicus 2	0.031	0.0039	0.0006	0.0054	0.13	0.029	1.48	0.042	0.0029	0.61
O. mossambicus 3	0.039	0.011	0.0008	0.0075	0.17	0.045	0.86	0.042	0.0028	0.47
O. mossambicus 4	1.56	0.0057	0.0006	0.011	0.12	0.037	0.79	0.062	0.0028	0.28
O. mossambicus 5	0.047	0.0093	0.0005	0.0054	0.098	0.038	0.78	0.043	0.0028	0.26
O. mossambicus 6	0.024	0.0080	0.0005	0.0040	0.15	0.033	1.15	0.049	0.0028	0.22
O. mossambicus 7	0.023	0.0043	0.0003	0.0042	0.16	0.04	1.059	0.047	0.0029	0.19
O. mossambicus 8	0.03	0.0041	0.0004	0.0043	0.083	0.029	1.067	0.056	0.0029	0.18
O. mossambicus 9	0.031	0.0038	0.0003	0.0035	0.082	0.037	0.91	0.056	0.0028	0.19
O. mossambicus 10	0.021	0.0044	0.0004	0.004	0.085	0.035	1.019	0.042	0.0028	0.2

Table 5.6. Metal hazard index values of *Rhabdosargus sarba* for human consumption by the local community in the December 2015 survey. Green = no hazard, yellow = low hazard, orange = moderate hazard and red = high hazard.

Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
R. sarba 1	0.042	0.027	0.0007	0.0068	0.13	0.070	3.59	0.12	0.0028	0.36
R. sarba 2	0.066	0.0061	0.0005	0.019	0.15	0.043	3.41	0.12	0.0029	0.41
R. sarba 3	0.035	0.0036	0.0003	0.008	0.088	0.040	3.37	0.139	0.0028	0.47
R. sarba 4	0.033	0.0064	0.0004	0.015	0.13	0.039	3.41	0.103	0.0028	0.33
R. sarba 5	0.049	0.0054	0.0003	0.0059	0.094	0.045	3.95	0.119	0.0028	0.56
R. sarba 6	0.021	0.0048	0.0003	0.0053	0.082	0.023	4.52	0.129	0.0028	0.38
R. sarba 7	0.021	0.0043	0.0003	0.0045	0.095	0.031	3.87	0.126	0.0028	0.31
R. sarba 8	0.039	0.0042	0.0003	0.0050	0.092	0.041	2.84	0.099	0.0029	0.39
R. sarba 9	0.021	0.0041	0.0003	0.0043	0.14	0.031	4.34	0.13	0.0028	0.43
R. sarba 10	0.037	0.0066	0.0004	0.0053	0.23	0.033	4.57	0.091	0.0029	0.7

The Cu and Hg values for both *O. mossambicus* and *R. sarba* for February 2016 were between 0.1 and 1 indicating low hazard risks but some values for Cu and Hg for *R. sarba* were above 1.1 indicating a moderate hazard (Table 5.6 and 5.7). The Se values for *R. sarba* were all between 0.1 and 1 indicating a low hazard (Table 5.7). The majority of the As values were above 1.1 for both *O. mossambicus* and *R. sarba* indicating a moderate hazard (Table 5.6 and 5.7).

Table 5.7. Metal hazard index values hazard of *Oreochromis mossambicus* for human consumption by the local community in the February 2016 survey. Green = no hazard, yellow = low hazard, orange = moderate hazard and red = high.

Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
O. mossambicus 1	0.042	0.0023	0.0005	0.0058	0.19	0.029	0.94	0.072	0.015	0.96
O. mossambicus 2	0.048	0.0032	0.0006	0.0057	0.19	0.045	1.06	0.059	0.012	0.6
O. mossambicus 3	0.037	0.0031	0.0004	0.0061	0.19	0.045	1.33	0.071	0.0048	0.42
O. mossambicus 4	0.054	0.0034	0.0005	0.0063	0.21	0.048	1.55	0.082	0.0043	0.5
O. mossambicus 5	0.037	0.0021	0.0006	0.0060	0.24	0.034	1.08	0.081	0.0081	0.49
O. mossambicus 6	0.046	0.0037	0.0005	0.0071	0.19	0.04	2.16	0.076	0.0050	0.43
O. mossambicus 7	0.047	0.0004	0.0002	0.0005	0.098	0.0076	0.36	0.018	0.0014	0.07
O. mossambicus 8	0.053	0.0041	0.0006	0.0051	0.17	0.05	1.53	0.078	0.0053	0.88
O. mossambicus 9	0.032	0.0023	0.0004	0.0052	0.13	0.026	2.11	0.058	0.0028	0.58
O. mossambicus 10	0.037	0.0017	0.0004	0.0068	0.12	0.037	1.53	0.059	0.0027	0.42
O. mossambicus 11	0.039	0.0024	0.0004	0.017	0.29	0.053	1.25	0.048	0.0028	0.37
O. mossambicus 12	0.047	0.0040	0.0006	0.01	0.48	0.038	1.24	0.054	0.0028	0.6
O. mossambicus 13	0.032	0.0024	0.0006	0.0074	0.18	0.034	0.84	0.05	0.0028	0.28
O. mossambicus 14	0.047	0.0034	0.0004	0.0064	0.14	0.032	1.75	0.058	0.0029	0.24
O. mossambicus 15	0.058	0.0032	0.0004	0.0045	0.19	0.037	1.85	0.055	0.0028	0.27

Table 5.8. Metal hazard index values of *Rhabdosargus sarba* for human consumption by the local community in the February 2016 survey. Green = no hazard, yellow = low hazard, orange = moderate hazard and red = high hazard.

Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
R. sarba 1	0.048	0.0066	0.0004	0.022	1.24	0.0478	4.78	0.21	0.0024	0.5
R. sarba 2	0.053	0.0041	0.0003	0.0088	0.6	0.031	3.37	0.14	0.0045	0.66
R. sarba 3	0.031	0.0037	0.0004	0.092	1.37	0.044	4.64	0.18	0.0009	0.82
R. sarba 4	0.068	0.0045	0.0004	0.0084	0.41	0.023	6.51	0.19	0.0024	1.7
R. sarba 5	0.047	0.0014	0.0003	0.012	0.15	0.031	5.46	0.15	0.0028	1.61
R. sarba 6	0.026	0.0026	0.0002	0.0073	0.48	0.044	2.7	0.13	0.0040	0.54
R. sarba 7	0.038	0.0030	0.0003	0.013	0.34	0.024	5.94	0.2	0.0017	0.86
R. sarba 8	0.054	0.0082	0.0004	0.0068	0.31	0.051	5.06	0.22	0.015	1.04
R. sarba 9	0.033	0.0054	0.0003	0.0097	0.19	0.028	3.92	0.2	0.0028	0.47
R. sarba 10	0.046	0.0051	0.0003	0.0051	0.21	0.056	4.41	0.18	0.0027	0.57
R. sarba 11	0.049	0.0080	0.0004	0.0080	0.3	0.059	4.98	0.22	0.0014	0.38
R. sarba 12	0.032	0.0062	0.0003	0.0057	0.2	0.048	4.26	0.13	0.0066	0.34

Table 5.9. Pooled metal hazard index values of *Rhabdosargus sarba, Terapon jarbua and Oreochromis mossambicus* for human consumption by the local community of all the collection periods (August 2015, December 2015 and February 2016). Green = no hazard, yellow = low hazard, orange = moderate hazard and red = high hazard

Collection period	Fish Species	Cr	Mn	Co	Ni	Cu	Zn	As	Se	Cd	Hg
August 2015	*T.jarbua*	0.01	0.004	0.0005	0.007	0.16	0.09	2.01	0.15	0.003	0.71
August 2015	*R.sarba*	0.44	0.005	0.0003	0.064	0.12	0.04	3.44	0.19	0.004	0.23
December 2015	*O.mossambicus*	0.19	0.006	0.0005	0.007	0.13	0.04	0.97	0.05	0.003	0.36
December 2015	*R.sarba*	0.04	0.007	0.0004	0.008	0.12	0.04	3.79	0.12	0.003	0.43
February 2016	*O.mossambicus*	0.04	0.003	0.0005	0.007	0.2	0.04	1.37	0.06	0.005	0.47
February 2016	*R.sarba*	0.04	0.005	0.0003	0.017	0.48	0.04	4.67	0.18	0.004	0.79

5.4 Discussion

Anthropogenic processes continuously increase the amount of metals within an ecosystem, especially in an aquatic system (Agah *et al*., 2009). Several potentially toxicants are bio-accumulated and biomagnified through the food chain (Agah *et al*., 2009). This can affect human health when consuming fish that have been contaminated with these metals (Agah *et al*., 2009). The accumulation of metals within the aquatic environment has been receiving increased attention in recent years (Agah *et al*., 2009). Some trace metals are essential in small amounts for growth and development for example Cr, Co, Cu, Mn and Zn. Other metals such as As, Cd, Pb are potentially toxic in higher doses; especially Pb which is the most toxic and potentially carcinogenic to humans (Agah *et al*., 2009).

Investigating the metal concentrations in the tissues of the fish will provide an indication of the metals that are present in the system. Additionally, it will indicate if the levels of these metals pose any potential health risks to the fish community and possibly the local people of the area after consuming the fish. The results obtained from these investigations are important to understand the current situation of the fish within the system and what future potential problems local people will encounter when consuming the fishes.

However, there are currently limited industrial activities near the Kosi Bay system and there is minimal land use (agricultural and industrial areas). The land use present is primarily for agriculture. As mentioned in Chapter 1, more fertilisers and chemicals could potentially be introduced into the catchment from these activities. The metals present in the system are mostly natural. The non-natural metals present are potentially introduced via the Malangeni (Sihadla) River into Lake Amanzimnyama. Other possible routes for metal introductions is the pollutants that leak from the engines of the speedboats that use the Kosi Bay system for tours and recreational fishing. These metals and organic substances are Pb, Cd, Hg, gasoline and oil-based products (Asplund, 2000).

5.4.1 Metal concentrations in the tissue samples

Terapon jarbua metal concentration were high for some metals (Fe, Co, Zn, Sr and Hg) due to the fact that this fish species feeds on small crabs and shrimps off the sediment floor (Van der Elst, 1993). The sediment that the fish ingest while feeding on small crabs and shrimps causes higher metal concentrations. The concentrations in *R. sarba* for most of the metals (Cr, Mn, Cu, As, Se, Hg and Pb) also had high concentrations due to the same feeding habitat and diet as *T. jarbua*. These levels were higher due to the feeding of algae, bivalves and the amphipod *Grandidierella lignorum* from the sediment floor which the fish species ingest sediment while feeding (Blaber, 1984). *Terapon jarbua* and *R. sarba* were therefore directly in contact with the sediment and digests the sediment while feeding on small organisms on the sediment floor. Mentioned earlier was the fact that *T. jarbua* is located all along the Indian Ocean and ecotoxicology studies is available from the Philippines and India but with no studies from South Africa.

The assessment of metals of fish in Manila Bay, Philippines was conducted by Su *et al*. (2009). Manila Bay is a popular tourist attraction and is known for its recreational activities. It also serves as a habitat to a number of terrestrial and aquatic organisms (Su *et al*., 2009). A coastal lagoon (Las Pinas-Paranaque) in Manila Bay was declared a critical habitat and important wetland were numerous endemic wildlife species, migratory birds and mangroves are found (Su *et al*., 2009). It is a relatively pristine environment although there were signs of direct air pollution, local point sources and oil combustion in the area. One of fish species that was sampled in Las Pinas-Paranaque lagoon was *T. jarbua* (Su *et al*., 2009). Chromium, Cd and Pb were tested in the muscle tissues of *T. jarbua*. The mean metal concentrations (mg/kg) in the muscle for *T. jarbua* were: Cr (0.86 mg/kg), Cd (0.093 mg/kg) and Pb (0.841 mg/kg). The mean concentration results for *T. jarbua* of Kosi Bay (Table 5.1) were below the concentrations of the coastal lagoon in Manila Bay.

Because of the protected and pristine area of Manila Bay (Su *et al*., 2009), the concentration levels that they have found in the *T. jarbua* would be seen as a low concentration. The Kosi Bay concentrations levels were even lower than the concentrations levels of Manila Bay, indicating that Kosi Bay is potentially relatively free from metal pollution at this point in time.

Another study from fish along the southeast coast of India was conducted by Thiyagarajan *et al*. (2012). Cadmium, Cr, Fe, Pb, Cu, Mn, Zn and Hg concentrations were tested within the muscle tissues of five species, one of them being *T. jarbua*. Fast development of industries, urbanization and human population is common on the coastal environments which increases the prevalence of metals. Mean metal concentrations (mg/kg) for *T. jarbua* along the southeast coast of India study were: Cd (0.01 mg/kg), Cr (0.13 mg/kg), Cu (1.49 mg/kg), Fe (1.27 mg/kg) Mn (0.16 mg/kg), Pb (0.14 mg/kg), Zn (2.23 mg/kg) and Hg (0.11 mg/kg). The mean concentration results for *T. jarbua* of Kosi Bay (Table 5.1) were higher than the concentrations of the southeast coast of India except for Cr.

The higher concentrations of *T. jarbua* in the Kosi Bay system compared to the southeast coast of India, does not necessarily indicate that the system is in a bad condition. The Fish health assessment index (FHAI) showed that even though the concentrations were higher in the Kosi Bay system for *T. jarbua*, the overall heath and condition of *T. jarbua* were healthy and in a good condition.

The assessment of metals in fish near a major primary treatment sewage plant outfall was conducted by Gibbs and Miskiewicz (1995). The study was off Sydney's largest cliff sewage outfall sewage treatment plant. The catchment in the area contains many industrial facilities causing high proportions of industrial wastes. Chromium, Cu, Zn, As, Se, Cd, Pb and Hg were tested in the muscle tissues of *R. sarba*. The mean concentrations (mg/kg) were: Cr (0.08 mg/kg), Cu (0.17 mg/kg), Zn (6.75 mg/kg), As (6.00 mg/kg), Se (0.42 mg/kg), Cd (0.002 mg/kg), Pb (0.02 mg/kg) and Hg (0.57 mg/kg). The mean concentration results from the August 2015, December 2015 and February 2016 surveys for *R. sarba* of Kosi Bay (Table 5.1) were higher than the concentrations of the sewage outfall in Sydney except for As and Hg.

The reason for higher concentration levels of *R. sarba* in the Kosi Bay system than the Sydney sewage treatment plant could also be due to the feeding on crustaceans and algae off of the sediment floor as *T. jarbua*. Another reason could be the treatment plant minimizing metals and releasing clean water. The FHAI protocol also confirmed that the health and condition of *R. sarba* is in a healthy and good condition.

Oreochromis mossambicus metal concentrations mostly had the same trend for the last two surveys in December 2015 and February 2016. There was no clear difference in the concentration levels for this fish species except for Al and Pb were the levels had a clear difference between the last two surveys. Although these last two surveys had different concentration levels; there was no statistical differences ($p < 0.05$) between the surveys.

The metal results of *O. mossambicus* were compared to the metal results of the lower Olifants River (Coetzee *et al*., 2002). The Olifants River is contaminated by increasing afforestation, mining, power generation, irrigation, domestic and industrial activities (Coetzee *et al*., 2002). The Olifants ecosystem has been increasingly contaminated by pollutants such as metals (Kotze *et al*., 1999) that has biological effects on fish. The Olifants River has been regarded as one of South Africa's most polluted river systems and is influenced by human activities such as mining and agriculture (Gerber *et al*., 2015). The lower Olifants River in the Kruger National Park is not a pristine system and was compared to the Kosi Bay system to see if Kosi Bay is as affected as the Olifants River.

The study from Robinson and Avenant-Oldewage (1997) sampled tissue of *O. mossambicus* from two sites in the lower Olifants River. The first site at the Mamba weir and the second at Balule. Chromium, Cu, Fe and Mn were tested in the muscle tissues of *O. mossambicus*. The mean metal concentrations were: Cr (98.09 mg/kg), Cu (12.18 mg/kg), Fe (360.2 mg/kg) and Mn (9.28 mg/kg) for Mamba weir and Cr (84.14 mg/kg), Cu (8.09 mg/kg), Fe (1759.34 mg/kg) and Mn (16.80 mg/kg) for Balule. The mean concentration results from the December 2015 and February 2016 surveys for *O. mossambicus* of Kosi Bay (Table 5.1) were lower than the concentrations of the lower Olifants River.

According to the study of Yap *et al*. (2015), *O. mossambicus* were collected from a contaminated pond in Seri Serdang and an uncontaminated pond from Universiti Putra Malaysia (UPM). Muscle tissues were collected and were analysed for the concentrations of Cd, Cu, Fe, Ni, Pb and Zn (Yap *et al*., 2015) Seri Serdang receives human-induced sources of metal pollution such as domestic wastes, runoff from vehicle emissions and atmospheric deposition (Yap *et al*., 2015). The mean metal concentrations (mg/kg) for Seri Serdang were: Cd (0.34 mg/kg), Cu (2.08 mg/kg), Fe (36.12 mg/kg), Ni (2.83 mg/kg), Pb (1.93 mg/kg) and Zn (25.60 mg/kg). The mean concentration results from the December 2015 and February 2016 surveys for *O. mossambicus* of Kosi Bay (Table 5.1) were higher than the concentrations of the contaminated pond in Seri Serdang except for Cd, Ni and Pb.

Although some concentration levels of *O. mossambicus* from the Kosi Bay system were higher than the compared studies, it does not mean that the Kosi Bay system is not in a pristine condition. Natural factors and factors like different feeding habits or chemicals leaking from recreational fishing motorboats in the channels can cause higher concentration levels in the tissues. The FHAI protocol also confirmed that *O. mossambicus* is healthy and in a good condition.

5.4.2 Bioconcentration factors (BCF)

The bioaccumulation of essential and nonessential metals can be found in all biota of any aquatic system. The BCF results confirmed that there were metal concentrations tested in *T. jarbua*, *R. sarba* and *O. mossambicus* that were higher in the tissues than the concentrations in the water and sediment (Table 5.2). The majority of the values were under one (BCF < 1) and some concentrations were just over one (BCF > 1) (Table 5.2). According to McGeer *et al.* (2003) sediment functions act as a natural 'sink' within aquatic ecosystems and thus should have higher metal concentration values in the sediment than in the tissues of the fishes tested. Although the sediment values were below the guidelines described in Chapter 3, the results were still higher in the tissues of the fishes. The high levels of Al, Zn and Mn (Table 5.2) can relate to high content of suspended clay particles in the water (Gilbert and Avenant-Oldewage, 2014).

The BCF values can also be seen as the determination of partitioning between fish and the environment (McGeer *et al*., 2003). The dietary habitats of the different fish species can cause different BCF values between water and sediment, thus accumulate via ingestion of different food types and diffusion across the gills (Gilbert and Avenant-Oldewage, 2014).

5.4.3 Metallothioneins

Metallothioneins occur naturally in tissues of organisms and play a significant role in the homeostasis of essential metals but it also binds to nonessential metals. This occurs due to the stimulation of MT's synthesis (Atli and Canli, 2008). Metallothioneins has cysteine-rich (20 - 30%) metal binding proteins with a low molecular weight whose neosynthesis has a response to metals present in organisms (Viarengo *et al*., 1997). Metals can take part in the essential life functions in the tissues of the organism but can also influence toxic actions, or be detoxified by binding to the MT protein (Olsson *et al*., 1998). Metallothioneins detoxifies metals through binding the free ions making them less available for interaction with the sensitive biomolecules (Olsson *et al*., 1998). These metals translocate over the MT protein plasma membrane were the cell reacts to these metals and can only be removed from the body by excretion (Olsson *et al*., 1998).

The liver of *T. jarbua*, *R. sarba* and *O. mossambicus* were used for the determination of the MT's to see what metal exposure the fish might have been exposed to. The reason for the use of the liver organ is due to the fact that the liver is the main detoxification organ that is responsible for the metabolism and the excretion of toxic substances (Van Dyk *et al.,* 2007). Histological changes might occur in the liver due to the exposure of these metals and can therefore serve as a good indication (Van Dyk *et al.,* 2007).

Exposure to metals stimulates the increase of MT's within the tissues of fish species. In this study it shows the MT results of the different fish species have increased due to metal exposures. Looking at the MT results from Figure 5.5 it shows that *R. sarba* from the August 2015 and December 2015 surveys had higher concentrations. Both natural and non-natural metals were found in the tissues of the selected fish species. Although some of these metals that were higher in the Kosi Bay system, no interferences or clear anthropogenic impacts are present in the system.

5.4.4 Human health risk assessment (hazard indices)

The human health risk assessment was performed to determine if the selected metals would have any potential hazardous effects on the local community that consumed these fish species. Looking at the results in Table 5.3 – 5.7 it showed that the metal that had the highest health hazard was As. There was a similar trend of values between the surveys for As, except for the survey in February 2016 which had higher hazard values for As compared to the previous surveys. The values of Cu, Se and Hg have mostly all low hazards for each survey. According to South African water quality guideline of coastal marine waters (1st volume), As can be introduced into a system through weathering and transport of sediment (DWAF, 1995). Other anthropogenic sources of As can be due to waste from manufacturing of herbicides and insecticides (DWAF, 1995). Organic As is naturally present in groundwater and is widely distributed throughout the environment in the air, water and land. Arsenic in its inorganic form is toxic and poses health risks. Arsenic can cause skin, bladder, liver and lung cancer and chronic exposures to As can lead to arsenicosis. Arsenicosis includes peripheral vascular disease, skin lesions and Blackfoot disease (Sun *et al*., 2014).

The pooled data (Figure 5.8) shows what fish species can pose a risk after consumption by the local community. Most of the values presented in Table 5.8 show an average of a no to low hazard index value, where As was the only metal that had moderate hazard risk. The fish mostly caught and consumed by the local community is *O. mossambicus*. *Oreochromis mossambicus* collected during December 2015 had mostly no hazard values but the values that had a low hazard were close to not having a hazard risk according to Lemly's Hazard quotient method guidelines. Values for As were higher and posed a low to moderate risk. The method has its limitations because it is based on the FAO and not on local populations; for example the amount of fish being consumed and the weight (kg) of the local community consuming the fish.

5.5 Conclusion

The health of *Terapon jarbua, R. sarba* and *O. mossambicus* were assessed for three surveys during 2015 / 2016 in the Kosi Bay system (Chapter 4). The fish species where caught in the channel between Lake Mpungwini and Lake Nhlange with a rod and reel. These fish species were then investigated and tested back in the laboratory for any potential health risks and metal exposures (This chapter). The fish species were used to test for any metal concentrations in the tissues that might affect human health when consuming the fishes.

The methods were: determine metal concentrations, BCF, MT's and human health risk assessments. The metal results from this study were compared to other studies that had similar objectives to detect metal concentrations within the fish tissues of selected species. Some results discussed before showed that there were concentration values that were higher than the compared studies. Although the Kosi Bay system is a relatively pristine system, there were still higher metal concentration values. The BCF values indicated that the majority of the values were under one (BCF < 1) and some concentrations were just over one (BCF > 1). The metals that had high concentrations could be related to the suspended clay particles in the water and the different feeding habits of the selected fishes.

Metallothioneins tested within the liver of the selected fishes showed that *R. sarba* from the August 2015 and December 2015 surveys had the highest concentrations of MT levels due to metal exposures. However, further studies would be needed to confirm this. No other MT studies were available in the selected fish from estuaries to serve as a reliable comparison.

The human health risk assessment indicated that As was the metal that showed the highest hazard in all surveys of 2015 and 2016. The consumption of the selected fish species poses a hazard for As poisoning and future studies should look at the source of As in this system. Future studies on As entering the system from the marine environment should also be investigated further.

6. General Conclusion and recommendations

In South Africa water is a scarce natural resource that needs to be protected, conserved and managed. Humans rely on water to meet their basic human needs, for agricultural activities, animal watering and recreational use (NWA, 1998). Another important factor, that was especially relevant to this study, is the protection of aquatic ecosystems.

Wetlands are aquatic ecosystems that play an important role within landscapes including biodiversity and natural productivity functions (Matthews, 1993). Purification, water storage and retention of pollutants are more key ecosystem services that wetlands offer within the aquatic environment. The loss of wetlands can cause economic pressure and may lead to a decline in reliable good quality water (Ramsar, 2013). Wetlands are habitats for various plant and animal life, especially fish that use wetlands to breed and as a nursery (Meynecke *et al.*, 2008).

Wetlands are protected under the Ramsar Convention and in South Africa there are 23 sites recognized as wetlands of international importance. Kosi Bay and Lake Sibaya are two of these 23 sites and formed the main study sites of the present project. Kosi Bay and Lake Sibaya are coastal lake systems along the east coast of South Africa within the iSimangaliso Wetland Park. Because these systems are classified as Ramsar sites it is important to ensure and encourage ongoing protection of these unique ecosystems.

Kosi Bay has many fish species that use the system for migration and spawning purposes. Some fish species such as *Rhabdosargus sarba* spawns in the ocean. The juveniles swim up into the estuary and stay within the protected estuary environment until they mature and return to the ocean. *Terapon jarbua* is found in a wide distribution in the estuarine environment where they feed and reproduce. They are mostly found in brackish waters and use estuaries as nursing areas. *Oreochromis mossambicus* also inhabit the Kosi Bay system along the reed banks of the channels that connect the lakes. *Oreochromis mossambicus* are freshwater maternal mouth-brooders and spawn in the summer months where temperatures are suitable and enough food is available. These fish species are an important resource for the local community surrounding Kosi Bay in terms of food value. The local community uses traditional spears to catch fish between the reeds and use fish traps throughout the estuary to catch fish that migrate between the ocean, the estuary and various lakes. Recreational fishing is also an important feature of this system while the lakes are big tourism attraction.

Fish reflect environmental conditions in aquatic environments. If the environment deteriorates the fish will be expected to reflect these deteriorating conditions. Using fish as a biomonitoring tool is useful to detect any environmental disturbances or impacts. Therefore, the aim of the project was to assess the health of *O. mossambicus, R. sarba* and *T. jarbua* of the Kosi Bay system to determine if metal concentrations in fish tissue pose a risk to human health following consumption.

The hypothesis of the study was: Due to limited anthropogenic impacts in the Kosi Bay system metal levels in water and sediment will be lower than the DWS proposed water quality levels and therefore fish from this system will be healthy and pose no risk to human health following consumption. The objectives set out to determine if the hypothesis was accepted or rejected were:

- To determine the current metal concentrations in the water and sediment of the Kosi Bay and Lake Sibaya systems.
- Sample and determine the health of the selected fishes following the fish health assessment index (FHAI) protocol.
- To determine the current metal concentrations in the muscle tissues from the selected fishes of the Kosi Bay system.
- To determine metallothionein levels in the liver tissue of the selected fishes of the Kosi Bay system.
- To determine the bioconcentration factor between the muscle tissue of the selected fishes and the environment (water and sediment).
- To determine human consumption hazard of the selected fish species.

Water quality and sediment quality was investigated in Kosi Bay and in Lake Sibaya to determine any potential impact exposure to the fish community. Metal concentrations in the water samples mostly exceeded the international quality guideline thresholds used in this project. Water results indicated that the metal concentrations were higher at the mouth and the lower part of the estuary. The different lakes of the Kosi Bay system exceeded the guidelines for metal concentrations in the water and Lake Amanzimnyama showed the highest metal concentrations for the sediment. Metal concentrations in the water samples from the Lake Sibaya system were mostly below the guidelines with some sites that exceeded the guidelines for certain metals (Cr, Cu, Zn, Se and Hg). Metal concentrations in the sediment samples from the Lake Sibaya system were all below the guidelines.

The health of the fish species targeted for the project were investigated with the use of the Fish Health Assessment Index (FHAI) developed by Goede and Barton (1990) for any health abnormalities. The condition factor (CF) of all the fish species were calculated following an external and internal examination of the fishes. All the fishes sampled had a CF score above one, indicating that the general health of all the fish species was good. There were also no severe signs of abnormalities during the external and internal examinations, which also indicated that the fish species were in a healthy condition.

Metal concentrations, metallothioneins (MT), bioconcentration factors (BCF) and a human consumption risk assessment were also determined. Water and sediment quality results from the L2+3C site were used to analyse the BCF. Metal concentrations in the tissues of *R. sarba* were higher than the values of the other fish species. Bioconcentration factors indicated that metal concentrations were higher in the tissues of the fishes than in the environment (sediment and water). *Rhabdosargus sarba* showed higher MT values than the other fishes which indicated a higher metal exposure in this fish species. There was low to no hazards from most of the metals for the local community consuming the fishes except for arsenic that had a moderate hazard.

Combining all of the factors investigated, the results showed that there were minimal abnormalities present in the selected fishes of Kosi Bay and metal concentrations did not have an effect on these fish species, even though most of the levels exceeded the water quality guidelines, the hypothesis for this study was accepted as there were no effects on the health of the fish species investigated.

6.1 Recommendations

- Studies on the metal concentrations within the tissues of *Callianassa kraussi,* or more commonly known as the sand prawn should be done as these sand prawns are found in burrows in the sediment and are of economic and ecological importance in the Kosi Bay system. Investigating these sand prawns could provide additional information to the metal concentrations in the sediment of the system, because of the constant contact these prawns have with the sediment. These sand prawns are also an important link in the food web of certain fish species.
- Other fish species such as *Pomadasys commersonnii* (spotted grunter) and *Caranx ignobilis* (giant trevally) should also be studied as they also use the Kosi Bay system for breeding and nursing and are both targeted by recreational fishers. Using other fish species can give more information on the health of the fish species within the system.
- Detailed investigation into other toxins, such as DDT, that might pose a health risk to the fish species of Kosi Bay should also be undertaken.

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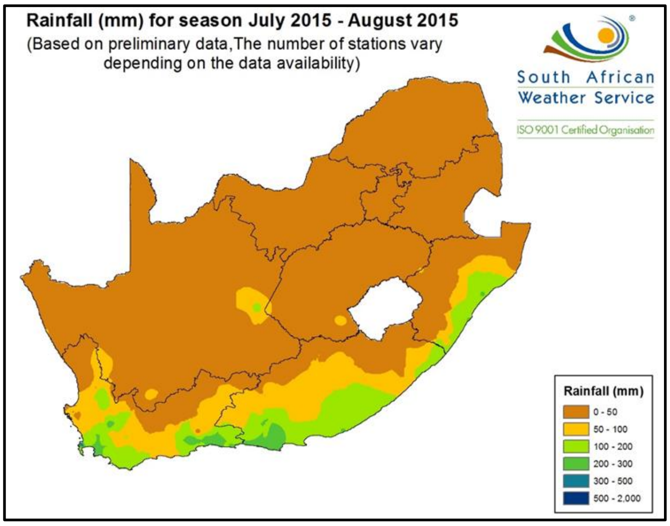
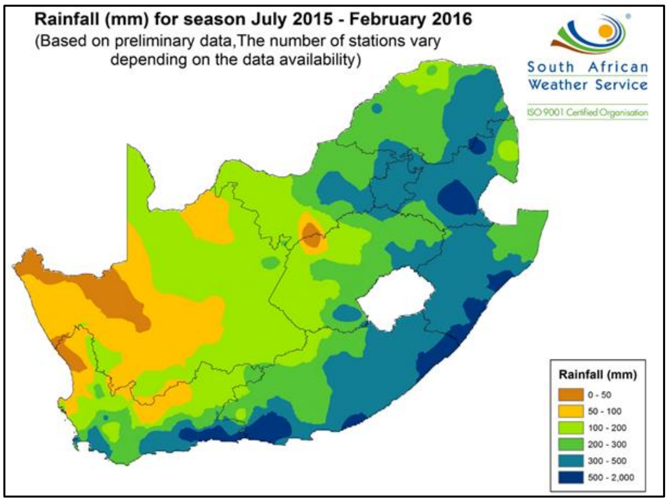
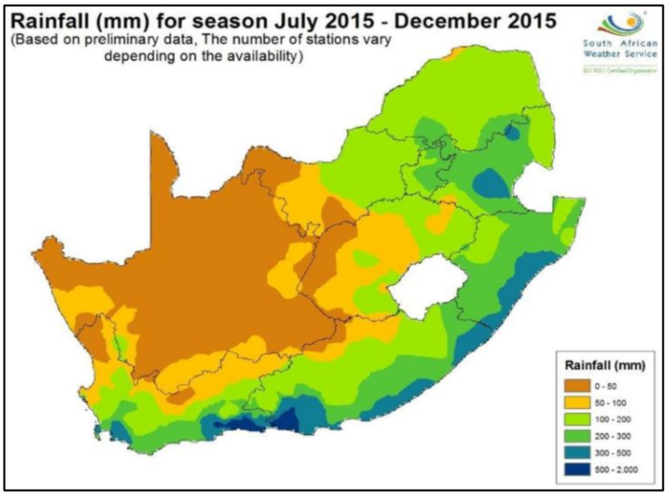
Appendix A







Obtained from SAWS, 2016

Appendix B

Mean nutrient concentrations (mg/L) for Kos Bay with SD (Mean ± SD) for August 2015, December 2015 and February 2016.

Nutrients	**Mouth (Sea)**	**Mouth**	**Mouth Upper**	**Fisherman's Spot**	**Lake 1**	**Lake 2**	**Lake 2+3 (Channel)**	**Lake 3 (Entrance)**	**Campsite (Lake 3)**	**Lake 3 (South East)**	**Lake 4**	**Malangeni River**
Ammonium	0.07 ± 0.03	0.11 ± 0.01	0.08 ± 0.01	0.14 ± 0.02	0.1 ± 0.01	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.11	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.08	0.07 ± 0.02
Chloride	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	250 ± 0.0	181.67 ± 35.7	35.33 ± 6.03
Nitrate	1.23 ± 0.61	8.23 ± 12.18	9.07 ± 13.80	1.1 ± 0.0	3.47 ± 4.37	0.9 ± 0.53	2.57 ± 0.15	3 ± 0.61	2.53 ± 0.06	1.63 ± 0.47	1.73 ± 0.51	2.2 ± 1.32
Nitrite	0.002 ± 0.0	0.002 ± 0.0	0.004 ± 0.0021	0.004 ± 0.00058	0.003 ± 0.0017	0.003 ± 0.001	0.003 ± 0.0012	0.005 ± 0.0053	0.006 ± 0.0055	0.055 ± 0.044	0.051 ± 0.061	0.003 ± 0.0012
Sulphate	300 ± 0.0	300 ± 0.0	300 ± 0.0	300 ± 0.0	300 ± 0.0	300 ± 0.0	246.67 ± 55.63	258.67 ± 71.59	241 ± 102.2	169.33 ± 57.57	31.67 ± 3.79	23.33 ± 1.53
Phosphate	0.15 ± 0.03	0.13 ± 0.02	0.13 ± 0.02	0.18 ± 0.01	0.11 ± 0.07	0.12 ± 0.07	0.17 ± 0.02	0.16 ± 0.05	0.17 ± 0.03	0.69 ± 0.04	0.45 ± 0.34	0.21 ± 0.13

Mean nutrient concentrations (mg/L) for Lake Sibaya and Kushengeza with SD (Mean ± SD) for August 2015, December 2015 and February 2016.

Nutrients	**Lake Sibaya 1**	**Lake Sibaya 2**	**Lake Sibaya 3**	**Lake Sibaya 4**	**Kushengeza**
Ammonium	0.1 ± 0.06	0.34 ± 0.40	0.1 ± 0.03	0.23 ± 0.06	0.41 ± 0.23
Chloride	131.7 ± 4.93	132 ± 2.65	130.7 ± 6.35	123.3 ± 1.53	120.67 ± 32.35
Nitrate	1.0 ± 0.0	0.87 ± 0.12	1.0 ± 0.0	1.0 ± 0.0	1.73 ± 0.21
Nitrite	0.03 ± 0.04	0.007 ± 0.005	0.007 ± 0.005	0.006 ± 0.002	0.013 ± 0.009
Sulphate	38.67 ± 1.16	36.67 ± 9.24	46 ± 2.65	43 ± 2.0	29.33 ± 8.08
Phosphate	1.72 ± 1.12	1.16 ± 1.09	1.09 ± 1.11	1.12 ± 1.14	0.32 ± 0.16

Appendix C

Mean metal concentrations (mg/L) with SD in the water for August 2015, December 2015 and February 2016 of the Kosi Bay system (Mean ± SD).

Mean metal concentrations (mg/L) with SD in the water for August 2015, December 2015 and February 2016 of the Kosi Bay lakes with Malangeni River (Mean ± SD)

Sites	**Mean metal concentrations (mg/L)**													
	Al	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
L1	0.41 ± 0.37	12.86 ± 5.45	0.09 ± 0.08	26.27 ± 9.37	0.10 ± 0.03	0.05 ± 0.08	10.99 ± 4.85	0.19 ± 0.16	0.37 ± 0.28	7.24 ± 2.19	117.1 ± 41.83	0.014 ± 0.010	0.24 ± 0.23	0.47 ± 0.09
L2	0.21 ± 0.26	11.53 ± 6.60	0.09 ± 0.08	21.86 ± 12.49	0.10 ± 0.02	0.03 ± 0.06	8.93 ± 5.84	0.25 ± 0.14	0.36 ± 0.26	6.51 ± 4.1	107.1 ± 63.04	0.012± 0.011	0.09 ± 0.11	0.48 ± 0.12
L2+3C	0.64 ± 0.25	12.08 ± 5.05	0.03 ± 0.02	24.47 ± 7.69	0.10 ± 0.02	0.08 ± 0.14	8.21 ± 1.75	0.16 ± 0.22	0.37 ± 0.27	7.02 ± 2.5	112.1 ± 42.43	0.013 ± 0.011	0.09 ± 0.11	0.46 ± 0.08
L3E	1.08 ± 1.18	8.53 ± 6.93	0.12 ± 0.15	15.34 ± 11.88	0.08 ± 0.02	0.02 ± 0.03	4.62 ± 3.54	0.48 ± 0.83	0.30 ± 0.21	4.34 ± 4.32	71.43 ± 59.24	0.013 ± 0.012	0.02 ± 0.03	0.46 ± 0.09
L3SE	1.16 ± 0.69	3.77 ± 2.16	0.28 ± 0.27	8.66 ± 8.15	0.07 ± 0.02	0.03 ± 0.05	2.22 ± 0.52	0.42 ± 0.56	0.24 ± 0.27	1.38 ± 0.73	31.23 ± 22.07	0.012 ± 0.012	0.06 ± 0.08	0.46 ± 0.08
L3C	0.50 ± 0.51	3.84 ± 2.91	0.15 ± 0.12	7.59 ± 6.18	0.07 ± 0.01	0.04 ± 0.07	2.22 ± 1.07	0.16 ± 0.18	0.24 ± 0.25	1.59 ± 1.37	34.52 ± 26.63	0.013 ± 0.012	0.008 ± 0.009	0.46 ± 0.07
L4	0.003 ± 0.003	0.01 ± 0.007	0.004 ± 0.004	0.06 ± 0.03	0.0003 ± 0.0008	0.0008 ± 0.0007	0.007 ± 0.004	0.02 ± 0.02	0.001 ± 0.0005	0.005 ± 0.004	0.16 ± 0.08	0.00003 ± 0.00002	0.00002 ± 0.0	0.001 ± 0.0003
MAL	0.002 ± 0.0006	0.006 ± 0.004	0.0007 ± 0.0006	0.09 ± 0.04	0.0003 ± 0.0001	0.003 ± 0.003	0.001 ± 0.0007	0.002 ± 0.004	0.002 ± 0.0009	0.001 ± 0.001	0.10 ± 0.07	0.00004 ± 0.00003	0.0004 ± 0.0007	0.001 ± 0.002

Mean metal concentrations (mg/L) with SD in the water for August 2015, December 2015 and February 2016 of Lake Sibaya and Kushengeza (Mean ± SD).

Mean metal concentrations (mg/L) and SD in the water for August 2015, December 2015 and February 2016 of Lake Sibaya and Kushengeza (Mean ± SD)

Sites	**Mean metal concentrations (mg/L)**													
	Al	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
Lake Sibaya 1	0.005 ± 0.007	0.012 ± 0.003	0.001 ± 0.0009	0.06 ± 0.02	0.0003 ± 0.00004	0.003 ± 0.001	0.003 ± 0.0001	0.01 ± 0.015	0.004 ± 0.0009	0.003 ± 0.002	0.19 ± 0.04	0.00005 ± 0.00002	0.0002 ± 0.0003	0.001 ± 0.0002
Lake Sibaya 2	0.006 ± 0.006	0.010 ± 0.003	0.002 ± 0.001	0.05 ± 0.005	0.0002 ± 0.00003	0.002 ± 0.0008	0.004 ± 0.002	0.03 ± 0.02	0.003 ± 0.0005	0.002 ± 0.002	0.16 ± 0.02	0.00007 ± 0.00002	0.0001 ± 0.0002	0.001 ± 0.0002
Lake Sibaya 3	0.005 ± 0.004	0.012 ± 0.005	0.001 ± 0.0002	0.05 ± 0.02	0.0003 ± 0.00003	0.002 ± 0.001	0.003 ± 0.0006	0.009 ± 0.009	0.004 ± 0.001	0.003 ± 0.002	0.19 ± 0.05	0.00005 ± 0.00003	0.002 ± 0.003	0.001 ± 0.0002
Lake Sibaya 4	0.003 ± 0.002	0.008 ± 0.003	0.007 ± 0.0008	0.04 ± 0.013	0.0003 ± 0.00003	0.001 ± 0.001	0.003 ± 0.001	0.011 ± 0.009	0.003 0.0003	0.002 ± 0.002	0.13 ± 0.02	0.00005 ± 0.00003	0.0002 ± 0.0001	0.001 ± 0.0002
Kushengeza	0.042 ± 0.02	0.007 ± 0.004	0.023 ± 0.01	0.21 ± 0.09	0.0005 ± 0.0001	0.002 ± 0.0008	0.004 ± 0.001	0.02 ± 0.011	0.005 ± 0.002	0.001 ± 0.001	0.11 ± 0.05	0.00006 ± 0.00004	0.00008 ± 0.00006	0.002 ± 0.0007

Appendix D

Mean metal concentrations (mg/kg) with SD in the sediment for August 2015, December 2015 and February 2016 of the Kosi Bay system (Mean ± SD).

Mean metal concentrations (mg/kg) with SD in the sediment for August 2015, December 2015 and February 2016 of the Kosi Bay system (Mean ± SD)

Sites	**Mean metal concentrations (mg/kg)**													
	Al	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
Mouth (Sea)	1171.00 ± 604.1	3.94 ± 1.18	42.08 ± 19.28	2167.00 ± 700.4	0.79 ± 0.21	1.81 ± 0.69	10.73 ± 14.81	7.11 ± 1.63	4.64 ± 0.84	0.11 ± 0.09	164.20 ± 9.51	0.015 ± 0.007	0.01 ± 0.01	1.09 ± 0.43
Mouth	941.80 ± 1142	3.52 ± 3.32	31.55 ± 28.25	1646.00 ± 1546	0.59 ± 0.56	1.39 ± 1.17	2.54 ± 2.86	7.97 ± 8.31	3.57 ± 3.08	0.27 ± 0.21	87.66 ± 84.94	0.04 ± 0.05	0.05 ± 0.08	1.17 ± 1.14
Mouth Upper	1189.00 ± 573.2	3.74 ± 0.62	6.39 ± 1.51	1019.00 ± 162.4	0.34 ± 0.03	1.02 ± 0.15	1.67 ± 1.12	9.49 ± 4.76	0.62 ± 0.19	0.11 ± 0.08	5.02 ± 2.19	0.01 ± 0.002	0.00 ± 0.0001	1.36 ± 1.17
Fisherman's Spot	658.40 ± 34.2	0.97 ± 0.138	1.42 ± 0.49	382.70 ± 94.1	0.09 ± 0.006	0.37 ± 0.12	1.84 ± 1.42	8.27 ± 8.77	0.34 ± 0.09	0.07 ± 0.07	1.83 ± 0.55	0.01 ± 0.003	0.01 ± 0.003	1.35 ± 0.55
Lake 1	445.20 ± 162.5	0.91 ± 0.68	3.47 ± 2.43	394.10 ± 81.32	0.09 ± 0.03	0.40 ± 0.19	1.68 ± 1.92	5.81 ± 2.30	0.50 ± 0.14	0.05 ± 0.02	1.95 ± 0.41	0.01 ± 0.005	0.01 ± 0.005	0.94 ± 0.79
Lake 2	589.60 ± 309.3	0.82 ± 0.08	3.37 ± 2.93	219.30 ± 70.27	0.08 ± 0.009	0.49 ± 0.27	2.11 ± 1.68	8.79 ± 4.15	0.13 ± 0.09	0.06 ± 0.07	1.88 ± 0.82	0.01 ± 0.004	0.005 ± 0.0001	0.91 ± 0.25
Lake 2+3 (Channel)	519.40 ± 300.5	0.41 ± 0.37	1.73 ± 0.76	164.20 ± 58.76	0.07 ± 0.004	0.84 ± 0.44	2.79 ± 2.32	8.37 ± 8.23	0.05 ± 0.04	0.06 ± 0.04	1.56 ± 0.95	0.01 ± 0.004	0.03 ± 0.05	0.74 ± 0.007
Lake 3 (Entrance)	485.20 ± 267.6	0.53 ± 0.48	1.27 ± 0.64	108.20 ± 36.61	0.06 ± 0.02	0.43 ± 0.22	3.67 ± 4.82	11.16 ± 6.61	0.07 ± 0.02	0.06 ± 0.07	2.24 ± 1.30	0.005 ± 0.004	0.03 ± 0.05	2.84 ± 3.92
Lake 3 (Campsite)	398.20 ± 118.2	0.24 ± 0.4	1.17 ± 0.46	124.80 ± 29.36	0.05 ± 0.01	0.25 ± 0.04	0.93 ± 0.39	5.95 ± 5.28	0.11 ± 0.12	0.04 ± 0.02	0.85 ± 0.17	0.005 ± 0.004	0.005 ± 0.0002	0.74 ± 0.46
Lake 3 (South East)	528.90 ± 335.1	1.50 ± 0.38	3.03 ± 0.38	295.90 ± 53.63	0.11 ± 0.01	1.10 ± 1.06	2.59 ± 3.07	4.70 ± 2.43	0.12 ± 0.02	0.07 ± 0.05	2.15 ± 1.02	0.005 ± 0.004	0.19 ± 0.32	1.89 ± 2.53
Lake 4	1440.00 ± 1738	3.89 ± 5.84	34.68 ± 54.85	11130.00 ± 18356	3.75 ± 5.9	2.34 ± 2.93	4.75 ± 3.44	11.80 ± 10.74	9.65 ± 15.78	1.24 ± 2.09	42.17 ± 69.47	0.03 ± 0.04	0.06 ± 0.04	1.67 ± 1.27
Malangeni River	724.00 ± 264.4	0.29 ± 0.45	17.27 ± 4.27	739.60 ± 95.5	0.22 ± 0.01	0.39 ± 0.17	2.12 ± 2.26	6.74 ± 3.51	0.34 ± 0.09	0.08 ± 0.09	0.98 ± 0.43	0.005 ± 0.004	0.005 ± 0.0002	0.71 ± 0.25

Appendix E

Mean metal concentrations (mg/kg) with SD in the sediment for August 2015, December 2015 and February 2016 of Lake Sibaya and Kushengeza (Mean ± SD).

Mean metal concentrations (mg/kg) and SD in the sediment for August 2015, December 2015 and February 2016 of Lake Sibaya and Kushengeza (Mean ± SD)

Sites	**Mean metal concentrations (mg/kg)**													
	Al	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**As**	**Pb**	**Se**	**Hg**	**Cu**	**Zn**	**Cd**	**Sr**
Lake Sibaya 1	514.3 ± 278.7	0.35 ± 0.53	11.61 ± 8.33	456 ± 68.76	0.20 ± 0.04	0.4278 ± 0.16	0.36 ± 0.15	2.18 ± 2.45	0.07 ± 0.06	0.005 ± 0.00001	2.407 ± 3.03	7.5 ± 5.79	0.005 ± 0.004	2.02 ± 1.12
Lake Sibaya 2	1254 ± 952	6.64 ± 3.11	18.29 ± 10.18	2218 ± 1187	0.68 ± 0.33	1.666 ± 0.56	1.19 ± 0.48	0.99 ± 0.37	0.13 ± 0.10	0.1 ± 0.16	2.381 ± 2.35	10.1 ± 9.32	0.009 ± 0.0008	29.61 ± 15.78
Lake Sibaya 3	675.3 ± 226.9	2.33 ± 0.52	11.22 ± 9.52	822.4 ± 532.9	0.24 ± 0.16	4.963 ± 7.63	0.29 ± 0.08	1.93 ± 2.06	0.06 ± 0.07	0.03 ± 0.05	12.09 ± 19.46	7.06 ± 2.14	0.005 ± 0.004	2.15 ± 1.62
Lake Sibaya 4	335.1 ± 256.7	0.21 ± 0.29	13.27 ± 6.38	391.3 ± 34.01	0.25 ± 0.01	2.744 ± 4.11	0.35 ± 0.16	0.87 ± 0.25	0.08 ± 0.06	0.02 ± 0.03	6.779 ± 9.87	9 ± 6.02	0.006 ± 0.003	6.49 ± 3.38
Kushengeza	296.40 ± 119.4	0.11 ± 0.17	0.83 ± 0.17	105.80 ± 54.61	0.06 ± 0.019	0.17 ± 0.02	0.05 ± 0.04	0.52 ± 0.25	0.05 ± 0.02	0.005 ± 0.0002	1.62 ± 1.45	3.82 ± 1.98	0.004 ± 0.005	0.57 ± 0.30

Mean metal concentrations (mg/kg) and SD in the muscle tissues for August 2015 (#1), December 2015 (#2) and February 2016 (#3) of *Terapon jarbua*, *Rhabdosargus sarba* and

Mean metal concentrations (mg/kg) and SD in the muscle tissues for August 2015, December 2015 and February 2016 of fishes from Kosi Bay (Mean ± SD)

Species	**Mean metal concentrations (mg/kg)**													
	Al	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Sr**	**Cd**	**Hg**	**Pb**
***T. jarbua* #1**	21.07 ± 9.77	0.12 ± 0.26	1.66 ± 0.69	119.3 ± 39.05	0.11 ± 0.03	0.44 ± 0.28	2.69 ± 0.59	88.75 ± 20.91	2.01 ± 0.42	2.55 ± 0.35	58.76 ± 19.83	0.010 ± 0.003	0.24 ± 0.21	0.42 ± 0.38
***R. sarba* #1**	9.6 ± 4.49	4.45 ± 7.07	2.13 ± 2.02	53.96 ± 34.92	0.06 ± 0.05	4.27 ± 6.03	2.0 ± 0.77	38.77 ± 13.54	3.43 ± 0.37	3.15 ± 1.37	13.49 ± 12.09	0.01 ± 0.003	0.08 ± 0.07	0.54 ± 0.53
***R. sarba* #2**	22.16 ± 10.37	0.36 ± 0.14	3.40 ± 3.31	44.21 ± 17.95	0.08 ± 0.03	0.52 ± 0.33	2.05 ± 0.73	39.30 ± 12.76	3.78 ± 0.56	1.96 ± 0.25	16.12 ± 26.88	0.009 ± 0.0001	0.14 ± 0.04	0.63 ± 1.31
***R. sarba* #3**	24.96 ± 10.08	0.44 ± 0.12	2.28 ± 0.98	47.63 ± 46.23	0.06 ± 0.01	1.10 ± 1.61	8.07 ± 6.74	40.56 ± 12.60	4.66 ± 1.05	2.96 ± 0.55	13.80 ± 14.08	0.01 ± 0.013	0.26 ± 0.15	0.84 ± 1.0
***O. mossambicus* #2**	50.96 ± 35.39	1.86 ± 4.83	3.0 ± 1.31	65.83 ± 44.68	0.11 ± 0.04	0.45 ± 0.33	2.09 ± 0.62	35.07 ± 5.48	0.97 ± 0.24	0.80 ± 0.13	14.90 ± 24.04	0.009 ± 0.0001	0.12 ± 0.09	0.26 ± 0.1
***O. mossambicus* #3**	15.08 ± 8.56	0.44 ± 0.08	1.29 ± 0.45	27.32 ± 9.851	0.10 ± 0.02	0.44 ± 0.23	3.35 ± 1.51	36.98 ± 11.31	1.37 ± 0.49	1.02 ± 0.27	5.03 ± 4.23	0.02 ± 0.01	0.16 ± 0.08	0.87 ± 1.29