**Histopathology-based health assessment of two tilapia species in the Shongweni Dam, and metal accumulation, and human health implications**

By

Smangele Pretty Ncayiyana (215011920)

Submitted in fulfilment of the academic requirements for the degree of

Master of Science (Biological Sciences)

in the

School of Life Sciences

Faculty of Agriculture, Engineering and Sciences

University of KwaZulu-Natal

December 2024

As the candidate's supervisors, we have approved this dissertation for submission.

___[redacted]____ _03/02/2025___

**Supervisor: Dr Jeffrey Lebepe (SMU & UKZN)** **Date**

# Abstract

The Shongweni Dam serves as a repository for pollutants from the upper uMlazi River catchment in KwaZulu-Natal. The uMlazi River catchment is characterized by wastewater treatment plant, agricultural, and industrial activities. Despite the aforementioned anthropogenic activities in the catchment, little is known about their impact on the aquatic biota in the Shongweni Dam and human depending on these biotas for their livelihood. Therefore, this study aims to assess the health of the commonly occurring fish species, *Coptodon rendalli* and *Oreochromis mossambicus* in relation to water quality, metal accumulation and human health risks associated with the consumption of these two species. Sampling was conducted during dry (July – August) and wet seasons (November and December) in 2021. Sampling involved collecting surface water, sediment, and fish samples. The water was analysed for physical properties, nutrients and metal concentrations. Metals were also analysed in sediment to get a comprehensive understanding on their behaviour between the two matrices. Fish were sampled and each individual was weighed and total lengths were measured. Fish were euthanized by severing the spinal cord and opened ventrally. The length and weight measurements were used to calculate the condition factor. Liver and gonads were also weighed and their weights were used to calculate hepatosomatic and gonadosomatic indices, respectively. The general health of fish was assessed using the Health Assessment Index (HAI) protocol. The liver, gill and gonads were cut out, and fixed in 10% buffered neutral formalin for histopathological examinations. A piece of muscle was wrapped with aluminium foil and frozen for metal analyses. Most physical variables were within the guidelines whereas some nutrients; nitrates ($NO_3^-$) and orthophosphates ($PO_4^{3-}$), exceeded the guidelines indicating a eutrophic condition. Metal concentrations such as aluminum (Al), iron (Fe) and lead (Pb) exceeded guidelines whereas all metals exceeded the guidelines in sediment. Moreover, most metals in the water were higher during dry seasons whereas a contrasting trend was observed for sediment. Biometric indices and health assessment index showed no significant difference between species ($p > 0.05$) and seasonal variation was observed for each species ($p > 0.05$). Histopathology revealed regressive changes and circulatory disturbances in gills and livers were prominent and no seasonal variation was observed for both species ($p > 0.05$). However, gonadal lesions varied between species ($p < 0.05$), with pigmented melanomacrophages being the most prevalent. Among organs, gills were most affected, followed by the liver. Muscle exhibited significant concentration of chromium (Cr) and Pb exceeding the permissible limit for safe consumption for both species. Moreover, the non-carcinogenic health risks showed the Target Hazard Quotient (THQ) > 1 for Pb and Cr, with arsenic (As) and antimony (Sb) being on the verge of reaching THQ of 1. Cancer risk (CR) values, exceeding the $10^{-4}$ to $10^{-6}$ range for carcinogens As, Cr, Pb, and cadmium (Cd) indicated unsafe levels for human consumption regardless of season. It is evident that anthro$_{po}$genic activities in the uMlazi River catchment are impacting the water quality and fish are also responding to the water quality deterioration. Moreover, consumption of fish from this dam should be exercised with caution as frequent consumption could result in human health risks.

**Keywords:** uMlazi River, *Coptodon rendalli*, *Oreochromis mossambicus,* fish health, histopathology, lead, chromium

# Preface

The field work detailed in this dissertation was conducted at Shongweni Dam in KwaZulu-Natal between October and December 2021, under the supervision of Dr Jeffrey Lebepe.

The laboratory work presented in this dissertation was conducted at the School of Life Sciences, University of KwaZulu-Natal, Westville Campus, from December 2021 to March 2022.

This dissertation is the original work by the author and has not been submitted in any form for a degree or diploma to any tertiary institution. Any use of the work of others has been duly acknowledged in the text.

As the candidate's supervisor, I have approved this dissertation for submission.

**____[redacted]___** **___03/02/2025_____**

**Supervisor: Dr Jeffrey Lebepe** **Date**

# Declarations

I, ……Smangele Pretty Ncayiyana…… declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This dissertation does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written but the general information attributed to them has been referenced

b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

Signed: [redacted] ……………

# Conference contributions from this dissertation

The Durban Research-Action Partnership Symposium (D'RAP), 2023

Authors: Smangele Ncayiyana, Jeffrey Lebepe

Title: Histopathology-based health assessment of two tilapia species in the Shongweni Dam, metal accumulation, and human health implications.

# Contents list

# List of Figures and Tables

# Abbreviations

DO —Dissolved oxygen

TDS — Total dissolved solids

EC — Electrical conductivity

L — Length

W — Weight

CF — Condition factor

DSW – Durban Solid Wastes

FAO – Food and Agriculture Organization

GSI — Gonadosomatic index

HSI — Hepatosomatic index

HAI — Health assessment index

ICP-OES — Inductive coupled plasma- optic emission spectrometry

BAF — Bioaccumulation factor

FAO — Food and Agriculture Organization

CCME — Canadian Council of Ministers of the Environment

CR — Carcinogenic risk

DWAF — Department of Water Affairs and Forestry

JECFA — Joint FAO/WHO Expert Committee on Food Additives

NMDS — Non-metric multidimensional scaling

THQ — Target hazard quotient

TWQR —Target water quality range

USEPA — United States Environmental Protection Agency

WHO —World Health Organization

WRC – Water Research Commission

# Acknowledgements

I would like to extend my heartfelt thanks and gratitude to the following individuals for their unwavering support throughout the course of this research:

My sincere gratitude to my supervisor, Dr Jeffrey Lebepe, for his continuous guidance and support. I am deeply thankful for his constructive feedback, as well as editorial and technical advice throughout the course of this study. Lastly, for the financial support you provided to complete my academics.

The lab technicians at WaterLab for assistance in generating water and sediment results. As well as Naomi Timmerman/ Rephima Phaswane for assistance with the histology slides.

To my fellow lab colleagues, Sphosake Mdluli and Andile Khuzwayo for your assistance and support in the field work. And to my own mentor, Mr Ashish Misra, thank you for guidance in conducting my laboratory analysis as well as results and the support you have shown throughout this study.

To my friends, Ms Nokulinda Sonjica, Mr Nhlanhla Mwelase and Ms Nonkululeko Ngidi, thank you guys so much for your presence, encouraging words, moral and mental support.

To my mother and siblings, I am forever grateful for your continuous love and support alas not understanding this study. For always being there throughout my academic career.

# Dedication

This dissertation is dedicated to my loving mother, my siblings, and mostly myself.

# Chapter 1

## General introduction

Freshwater ecosystems, though comprising only a fraction (0.01%) of the Earth's water, are among the most vulnerable and least protected natural environments globally (Dudgeon, 2019; Reid et al., 2019). These systems are crucial for human sustenance, and supporting agricultural, industrial, and recreational activities (Apostolaki et al., 2020; Higgins et al., 2021). Nevertheless, freshwater systems have become repositories for pollutants, primarily stemming from anthropogenic activities including agricultural runoff, urbanization, sewage discharges and industrial effluents (Ali et al., 2016; Eker and Kiliç, 2024). Deterioration of water quality in freshwater ecosystems has been a cause for concern, as it adversely affects both human populations and aquatic biodiversity (Akthar et al., 2021).

Anthropogenic activities have accelerated the influx of inorganic pollutants, including nutrients, into aquatic systems, particularly through agricultural, industrial, and urban development (Khatri and Tyagi, 2014). Runoff from these activities, including effluents from industrial discharges, sewer networks, and septic systems, contributes substantial amounts of inorganic and organic matter to the freshwater systems (Bhat and Qayoom, 2021). These stressors have impacted freshwater ecosystems over the years and some of them are now regarded as dead rivers as a result of nutrient enrichment. Nutrient pollution often surpasses the self-purifying capacity of aquatic systems, resulting in eutrophication (Zhong et al., 2022; Akinnawo, 2023).

Among the various pollutants affecting freshwater environments, metals are particularly concerning because of their persistence, bioaccumulation, and toxicological effects on aquatic organisms, especially fish (Moon et al., 2020; Kormoker et al., 2023). While some metals naturally enter freshwater systems, human activities; particularly agriculture, industrial processes, and mining, are dominant contributors to metal increase (El-Bouraie et al. 2010; Aradpour et al., 2020). Metals, such as cadmium (Cd), nickel (Ni), lead (Pb), and chromium (Cr), along with other industrial pollutants, deteriorate water quality and render it unsuitable for aquatic life, irrigation, and human consumption. Metals disrupt vital physiological and reproductive functions in aquatic organisms, leading to population declines and impairing species interactions (Murphy et al., 2018; Rasmussen and Anderson, 2021). For instance, metals such as arsenic (As) and mercury (Hg) are associated with developmental deformities and decreased hatch rates in fish populations (Malaj et al., 2015; Matta et al., 2016). Chronic exposure to metals has been linked to oxidative stress, tissue damage, and impaired reproductive fitness in aquatic species (Authman et al., 2015).

Additionally, bioaccumulation and biomagnification of metals through the food chain pose considerable threats to top predators, including humans, consuming contaminated fish. Potential health risks include cancer, cardiovascular diseases, neurological impairment, and other chronic health issues (Mitra et al., 2022). For instance, Hg exposure is linked to cognitive deficit, especially in young children, highlighting the broader implications of metal contamination in aquatic ecosystems (Moore et al., 2019; Burger and Gochfeld, 2021). The degradation of freshwater systems due to pollutants threatens aquatic biodiversity and humans. Assessing water quality and understanding the trophic status of freshwater ecosystems is vital for predicting changes in aquatic environments and mitigating the effects of pollution (Plew et al., 2018; Vadde et al., 2018).

Numerous biomonitoring approaches have been implemented to evaluate fish and ecosystem health. These include biometric evaluations, age, and growth studies, necropsy-scoring methods, and analyses at biochemical, hematological, immunological, and physiological levels (Adams et al., 1993; Crafford and Avenant-Oldewage, 2009). Moreover, microscopic and histopathological approaches are valuable biomonitoring tools (Van Dyk et al., 2009). Histopathological-based assessment, however, can provide critical insights into how pollutant exposure affects fish health. Research has shown that pollutants can cause significant damage to organs such as the gills, gonads, kidneys, and liver, leading to sublethal effects such as inflammation and necrosis (Khan et al., 2018; Oğuz et al., 2020; El-Naggar et al., 2020). These damages are valuable biomarkers for assessing the impacts of environmental pollution on fish populations (Bibi et al., 2021; Naz et al., 2023). Furthermore, these assessments are vital for evaluating the sustainability of freshwater ecosystems, particularly in this Anthropocene era.

**Problem statement**

South Africa is a semi-arid country where water scarcity and pollution present substantial challenges to freshwater resources, essential for human and ecological health (Wagenaar and

Barnhoorn, 2018; Bakare and Adeyinka, 2022). Most freshwater resources in the country are situated in semi-rural and rural areas, where they are often subject to pollution from fertilizers, pesticides, and untreated sewage, compromising water safety for human consumption (Oberholster and Ashton, 2008; Barnhoorn et al., 2015). Freshwater resources flow in rivers and are primarily stored in impoundments within catchments for human use (van Ginkel, 2011). Unlike open marine systems, these impoundments have limited capacity for pollutant dilution, making them particularly vulnerable to the increase of contaminants, including metals (Dudgeon et al., 2006; Nzeve and Kitur, 2019). When available in elevated concentrations, metals tend to accumulate in aquatic biota and result in deleterious effects in the impoundments. Most impoundments are used as sources of food i.e. fish for human consumption, therefore, increased metal concentrations in impoundments may also result in toxic effects on human consumers. The notion that these contaminants pose significant ecological and health risks, especially through the consumption of contaminated fish has become a cause for concern, particularly in rivers draining catchment that are characterized by serious anthropogenic activities (Jooste et al., 2015; Nibamureke et al., 2016; Lebepe et al., 2020).

The Shongweni Dam, built in 1927, primarily to supply drinking water, is situated in the lower uMlazi River catchment, commonly known for its declining water quality, in KwaZulu-Natal, South Africa. This dam exemplifies an impoundment facing severe water quality issues due to eutrophication and contamination from several human activities in the surrounding area. These human activities include agricultural runoff, industrial effluents, and sewage discharges (Mavundla et al., 2020; DWS, 2022). The dam supports diverse aquatic life, including economically and ecologically important tilapia species (Shah et al., 2019). Tilapia species, particularly *Coptodon rendalli* and *Oreochromis mossambicus*, are central to the local ecosystem, contributing to biodiversity, serving as prey for predators, and providing an essential protein source for local communities. Due to their hardiness and adaptability, tilapia are useful indicators of ecosystem health and water quality (Nwankwo and Akintola, 2019).

Despite the Shongweni Dam serving as a repository for pollutants from the upper uMlazi River catchment, no study has been conducted to explore this pollution effect on the health of fish. Moreover, the dam is surrounded by economically disadvantaged communities that are opting for fish from this dam for their protein supplements, nevertheless, the safety of consuming these fish is not known. Therefore, this study investigates the impact of water pollution on the health of two tilapia species, *Coptodon rendalli* and *Oreochromis mossambicus*, and assesses whether they are safe for human consumption.

**Study aims**

To assess the health of *Coptodon rendalli* and *Oreochromis mossambicus*, metal accumulation and risks associated with the consumption of the two species in the Shongweni Dam.

**Objectives**

- To assess the environmental quality of the Shongweni Dam by measuring selected water and sediment quality parameters.
- To evaluate the impact of water pollution on the health of *Coptodon rendalli* and *Oreochromis mossambicus*, using various fish condition indices and histopathology-based health assessments.
- To assess metal concentrations in the muscle of the two tilapia species and determine whether they are safe for human consumption.

**Research questions**

- How do the anthropogenic activities in the uMlazi River catchment impact the water and sediment quality of the Shongweni Dam?
- How do *Oreochromis mossambicus* and *Coptodon rendalli* respond to water quality?
- What are the levels of metal concentrations in the muscle tissues of these fish species and are they safe for human consumption?

**Hypotheses**

The influx of effluents into the uMlazi River fluctuates throughout the year and the pollutant level in the water and sediment may differ depending on the dilution capacity of the river. It was, therefore, hypothesized that:

- The Shongweni Dam would exhibit high concentrations of pollutants in the water and sediment during the dry season due to low dilution capacity.

- *Coptodon rendalli* and *Oreochromis mossambicus* would exhibit significant histopathological changes in organs and tissues due to heavy metal exposure in the dam.
- *Coptodon rendalli* and *Oreochromis mossambicus* would exhibit elevated levels of metals, surpassing permissible limits for safe human consumption.

**Dissertation outline**

The dissertation comprised of six chapters:

**Chapter 1:** General introduction, including study aims, objectives, research questions, and hypotheses

**Chapter 2:** Literature review

**Chapter 3:** Water and sediment quality in the Shongweni Dam

**Chapter 4:** Histopathology-based health assessment of *Coptodon rendalli* and *Oreochromis mossambicus* from the Shongweni Dam

**Chapter 4:** Metal accumulation in the muscle of fish and health risk assessment

**Chapter 6:** Summary and conclusion

# Chapter 2
## Literature review

### 2.1 The uMlazi River catchment

Situated in a wet region of the country, the uMlazi River catchment spans 172 km in length and covers an area of 970 km$^2$. This river is one of the ten tertiary catchments within the proposed Mvoti and Umzimkulu water management area, spanning the east coast of the KwaZulu-Natal province and borders Lesotho to the west. The river originates at an elevation of 5000 m above sea level near the Entembeni area (Feysse and Gumbo, 2004) and is formed by the convergence of multiple tributaries. As it flows, the uMlazi River passes through regions with commercial farms and forested areas before reaching the Baynesfield Dam. It continues through the Mapstone Dam, the Hopewell area, and the Tala Valley, eventually reaching the Shongweni Dam and flowing into the Indian Ocean via a man-made canal near Durban (WRC, 2002). This river catchment has several weirs, small and large dams, that store water for both municipal and agricultural purposes (DSW, 2002). Population growth and various uses of the uMlazi River have pressured it resulting in increased strain on the ecosystem (Chikodzi et al., 2021).

#### 2.2.1 Topology and land use

The uMlazi River catchment is characterized by wetlands and supports about 690,800 residents (WRC, 2000; Faysse and Gumbo, 2004). The upper catchment is dominated by agricultural activities, forestry, and small and peri-urban settlements. The economy of the region depends on commercial forestry and irrigated agriculture, mainly growing vegetables, sunflowers, maize, and sugarcane, and raising livestock such as chickens, cows, pigs, and dairy farming (Umlaas Irrigation Board, 1997). The upper catchment suffers from high nutrient inputs from industrial and agricultural activities, while the middle catchment has small-scale farmers and the lower has small-peri urban communities facing significant air and water pollution issues. Eutrophication is a concern due to the presence of four wastewater works in the middle and lower catchments. Furthermore, rapid urbanization leads to higher population densities that result in more fecal and polluted runoff contamination (Umlaas Irrigation

Board, 1997). Despite the implementation of biomonitoring programs to reduce pollution, the lower uMlazi River catchment remains in a severely deteriorated state (WRC, 2002).

### 2.2.2 Hydrology

The river emanates at an elevation of 1500 m above sea level in the southwest of Pietermaritzburg (WRC, 2000). It has an average annual runoff of 72 – 173 mm, mean annual evaporation, and precipitation of 1070 – 1360 mm and 410 – 1100 mm, respectively (DWAF, 2002). Typically, summers receive a higher annual rainfall of 800 – 1000 mm while winters experience low rainfall, averaging 12.5 m per year (Everson et al., 2011). Good water quality is observed in the upper reaches but declines in the middle to lower reaches (WRC, 2000).

## 2.2 Shongweni Dam

The Shongweni Dam is located in the uMlazi River catchment area ($29^0$ 50’ 44” S, $30^0$ 44’ 38” E), approximately 30 km west of Durban. The dam supports a diverse range of bird, wild and aquatic life (WRC, 2000; Shah et al., 2019). It also serves as an important water reservoir for small-scale agriculture and provides recreational opportunities including fishing activities (Mavundla et al., 2020). The Shongweni Dam, however, faces significant challenges related to water quality, largely due to eutrophication and metal contamination from agricultural runoff, industrial discharges, and sewage effluents in the surrounding area (Tokatli, 2019). The treated effluents discharged from the wastewater treatment flow through the Sterkspruit River and then enter the Shongweni Dam (Pillay et al., 2004; Strassburg, 2004).

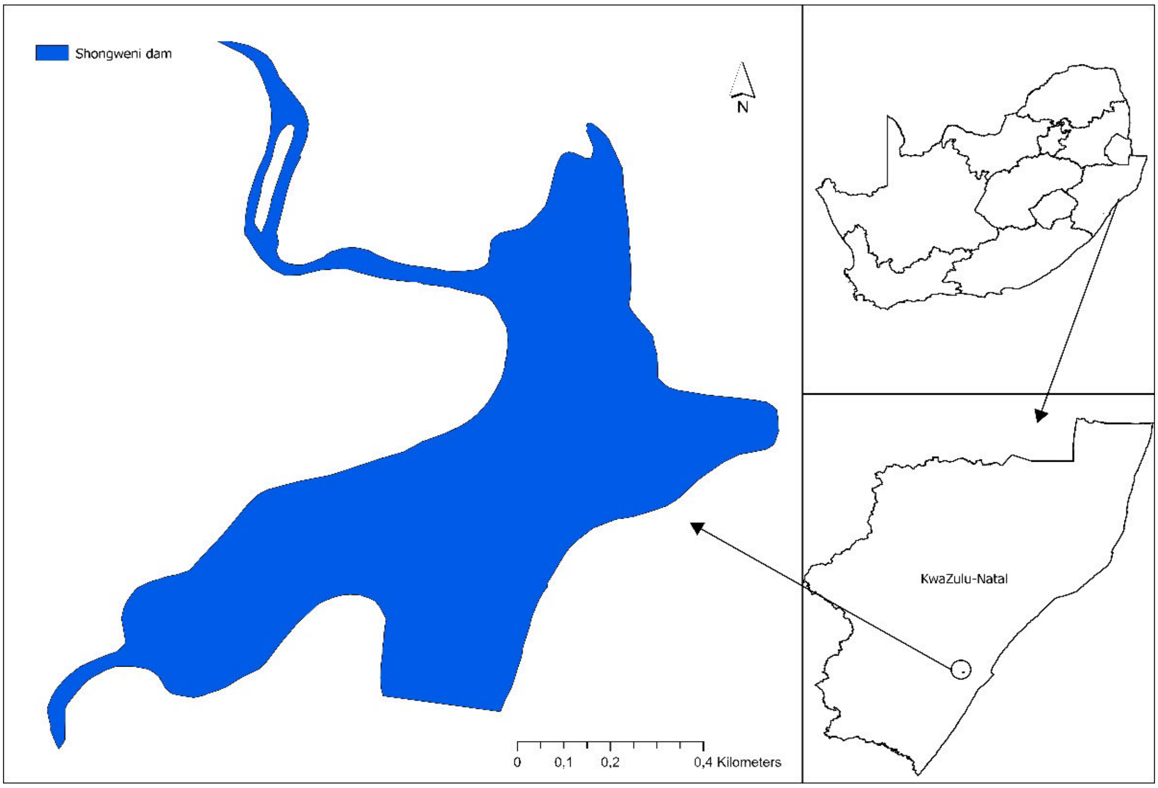


**Figure 2.1:** The map of South Africa showing the Shongweni Dam.

**Figure 2.2:** An image of a hypertrophic Shongweni Dam captured during the 2021 surveys.

### 2.3 Pollutants in surface water and sediment

Water acts as a major conduit for pollutants such as heavy metals, other chemicals, inorganic and organic matter, which can enter the aquatic environment through various pathways, including agricultural runoff, domestic waste, industrial effluents, waste disposal, and urban expansion (Akhtar et al., 2021; Edo et al., 2024). These pollutants not only pose serious threats to the freshwater ecosystem but also impact aquatic life and cause a decline in water quality (Kumar et al., 2018; Kamboj et al., 2020). Nutrients are among the most significant pollutants affecting the aquatic environment, presenting a significant threat to food security and future water supply (Birk et al., 2020; Yao et al., 2021). Excess nitrogen (N) and phosphate ($PO_4^{3-}$) from various anthropogenic activities including agricultural runoff, industrial processes, and municipal sewage discharges, often enter freshwater bodies and lead to eutrophication (Dodds and Smith, 2016). Research estimates that nitrogen and phosphates account for approximately 20 to 50% and 35 to 55%, respectively, of the contribution to eutrophication in aquatic environments (Akinnawo, 2023). Eutrophication results in increased phytoplankton biomass and the proliferation of harmful algal species, including cyanobacteria, green algae, and diatoms, which deteriorate water quality (Ballah et al., 2019; Sonarghare et al., 2020).

Other pollutants of great concern are metals, which enter the aquatic environment directly or indirectly from anthropogenic activities. Metals can be broadly categorized into essential, and non-essential. Essential metals, including iron (Fe), manganese (Mn), copper (Cu), selenium (Se), and zinc (Zn), are crucial for the growth and biological processes of aquatic organisms (Tchounwou et al., 2012; Rohani et al. 2022). However, excessive concentrations of these metals can be toxic, leading to adverse effects on aquatic organisms and ecosystem stability (Ali et al., 2019). A study conducted in Eleyele Dam reported Fe concentrations exceeded permissible limits and were associated with odour and taste problems in drinking water (Ojelabi et al., 2018). Excessive Fe has been linked to discoloration of water (Adetunde et al., 2011), thus, deteriorating water quality and negatively impacting recreational and fishing activities. Drinking water with elevated essential elements has been associated with gastrointestinal and immune system damage, leading to a condition called haemochromatosis (Ojelabi et al., 2018).

Non-essential metals; Hg, Pb, and Cd, have no known physiological and biological function even at low concentrations (Ali and Khan, 2018). These can bioaccumulate and/or biomagnify in aquatic food webs, endangering both aquatic life and human consumers (Bansal, 2023). Cadmium exposure in fish is associated with impaired reproduction, kidney damage, and skeletal deformities, ultimately reducing the survival and fitness of fish in polluted waters (Wood, 2002; Islam et al., 2020; Yan et al., 2020; Santos et al. 2021; Bansal, 2023). Previous studies documented high Pb levels in water to bioaccumulate in body tissues adversely causing teratogenic, neurotoxic, fetotoxic, and nephrotoxic effects on aquatic life and human consumers (Oyelek et al., 2016).

Heavy metals, a subset of both essential and non-essential metals, refer to metals or metalloids with a relative atomic density exceeding 5 $g/cm^3$, which can be toxic even at minimal concentrations (Ali and Khan, 2018b). These elements, including As, Cr, Cd, Pb, Cu, and Hg, are widely present in the environment (Ali and Khan, 2018; Kaur et al., 2018). The persistence, non-biodegradability, and bioaccumulation of heavy metals in food chains have raised significant environmental concerns (Moon et al., 2020; Naz et al., 2022; Zaynab et al., 2022). Heavy metal contamination can adversely affect ecosystem health, human safety, and overall water quality, as these typically accumulate in both water and sediments. Arsenic pollution in aquatic environments has been linked to diminishing fish populations as it disrupts cellular functions and DNA processes, resulting in mutations and increased mortality rates (Rahman et al., 2012).

Sediments often exhibit higher metal concentrations due to their ability to absorb and retain pollutants (Brady et al., 2015; Hama et al., 2023). The dynamics of sediment-bound metals are crucial; under certain conditions, they can either remain immobilized or be remobilized into the water column. This process further degrades water quality by altering key chemical and physical parameters such as pH, salinity, and temperature, thereby posing major risks to biodiversity, ecosystem integrity, and human health (Saleem et al., 2019; Ali et al., 2020). These physicochemical parameters are essential, as they substantially influence the water quality of any reservoir (Hakonde et al., 2020; Kormoker et al., 2022). The presence of metals in sediments is associated with various health complications (Huang et al., 2018; He et al., 2019; Nour et al., 2019; Tasher et al., 2020; Kormoker et al., 2022).

Thus sediments play a significant role in identifying metal pollution sources, potentially releasing accumulated metals into water and posing long-term exposure risks to aquatic ecosystems (Saha et al., 2017; Niu et al., 2021).

Given their role in assessing water quality, sediments are key indicators of heavy metal pollution (Abdel-Khalek et al., 2016). Research has documented substantial levels of metal pollution in water and sediments, contributing notably to water quality degradation. For example, in the Kafue River, pollution from metals Cd, cobalt (Co), Cr, Cu, and Pb, markedly intensifies downstream, demonstrating a complex sink-source-sink distribution driven by remobilization and pH shifts, particularly in the Kabwe transect (M'kandawire et al., 2017). Studies by Namugize et al. (2018) and others further corroborate the deteriorating water quality in rivers such as the Olifants River (Lynch et al., 2016; Lebepe et al., 2016; 2020). Effective management of aquatic ecosystems, especially in reservoirs, thus necessitates a combined approach of monitoring both water and sediment contamination to capture the full scope of metal inputs and ensure comprehensive water quality management (Aradpour et al., 2020).

### 2.3.1 Pollution effect on the aquatic life

Aquatic organisms are highly susceptible to metal pollution, as these readily accumulate in their tissues and often serve as indicators of water and sediment contamination (Annabi et al., 2013; Muhammad and Ahma, 2020). Prolonged exposure to metals has been associated with physiological and reproductive issues in fish and invertebrates, with the severity of toxicity varying based on species, concentration of metal, and exposure duration (Huang et al., 2023). Metals interact with components of biological molecules such as sulfur (S), oxygen (O), and N within aquatic organisms, altering the function and structure of hormones, proteins, and enzymes ultimately leading to organ damage (Banday et al., 2019). Specific studies highlight these effects, for instance, Nile tilapia (*Oreochromis niloticus*) exhibited decreased glucose levels when exposed to Cd and Zn (Shahjahan et al., 2022). Similarly, Banday et al. (2019) documented enzymatic fluctuations in *Clarias gariepinus* exposed to Cd, Cr, Cu and Ni from river Ganges. Such enzymatic changes reflect underlying metabolic disruptions caused by metal toxicity.

Metal exposure can disrupt the nervous system of aquatic organisms, and impair growth and reproduction. Studies showed reproductive failure in freshwater crustaceans due to sub-lethal Hg exposure, as well as liver damage and altered gill structures in fish exposed to high Cd levels (Smith et al., 2019; Garcia et al., 2021). Studies by Giri et al. (2021) and Fazio et al. (2022) reported accumulation of Cd in waterbodies negatively affected fish by reducing body weight and length, retarding growth, decreasing feed intake, and survival rate of fish populations. Additionally, fish exposed to metals have demonstrated lower GSI, reduced reproductive rates, fertilization and hatching rates success (Gárriz et al., 2019). These effects are further evidenced by changes in biochemical parameters and blood chemistry, often used to assess the physiological responses of fish to metal exposure (Banday et al., 2019; Javed and Usmani, 2019; Suchana et al., 2021). For example, Islam et al. (2020) documented decreases in red blood cell count and hemoglobin levels in *Pangasianodon hypothalamus* exposed to Cr concentrations.

Metals accumulate in specific organs such as the liver, gills, kidneys, and gonads often resulting in pathological changes. Studies have documented tissue damage ranging from liver necrosis to gill deformation in species exposed to heavy metals (Wagenaar and Barnhoorn, 2018; Kawade et al., 2020; Saleem et al., 2022; Izah et al., 2023). Hayati et al. (2017) and Garriz et al. (2019) found that Cd accumulation led to testicular fibrosis shrinkage and changes in spermatic lobules, cytoplasmic condensation, reduced GSI, and abnormal structures of oocytes.

**Figure 2.3:** Image illustrating abnormalities detected in the liver and gonads of tilapia collected from the Shongweni Dam during the 2021 surveys.

**2.4 Biomonitoring and bioindicator**

Biomonitoring is a vital approach for assessing the impact of metal pollution in aquatic environments, providing insights that complement traditional physical and chemical monitoring methods. While the latter reveals immediate effects, biomonitoring focuses on the combined and long-term impacts of pollutants on biotic communities and ecosystems (Ouma et al., 2022). This technique utilizes bioindicators to evaluate environmental contamination (Ouma et al., 2022). According to Zhou et al. (2008), biomonitoring is defined as a "scientific technique for assessing the environmental and biotic exposures to pollutants," relying on sampling and analysis of targeted organisms.

Bioindicators serve as markers that reflect the potential ecotoxicity of anthropogenic pollutants, helping to track both present conditions and historic patterns over time (Oertel and Salánki, 2003). This provides a more nuanced understanding of ecosystem health compared to conventional physicochemical indicators (Bartram et al., 1996; Lobo et al., 2004). Effective bioindicator organisms share a common trait: wide distribution, abundant, easily accessible, and have well-defined classifications (Ouma et al., 2022). Aquatic insects, plants, algae, fish, and other small aquatic organisms are frequently used to evaluate water quality and the overall state of aquatic systems (Zhou et al., 2008; Hamza-Chaffai, 2014; Ouma et al., 2022).

In South Africa, bioindicators such as bacteria, diatoms, macrophytes, macroinvertebrates, and fish have been employed in biomonitoring programs (Wepener et al., 2008; van Dyk et al., 2012; Fourie et al., 2014; Lebepe et al., 2016). Fish, in particular, are valuable bioindicators owing to their lengthy life spans, abundance across diverse habitat distribution, and ease of sampling. Moreover, fish are excellent bioindicators due to their sensitivity to low concentrations of metals, capacity to detoxify and metabolize quickly, and their ability to assimilate and reflect environmental changes over time (Weber et al., 2020; Dietrich et al., 2022; Sharma et al., 2024). Occupying higher trophic levels, they are effective indicator for evaluating the impacts of human activities on environmental conditions (Naz et al., 2022). Bioindicators can reflect a range of biological and environmental processes over extended periods (Armon and Hänninem, 2015; Gilbert et al., 2017). Therefore, biomonitoring using fish as bioindicators offers a comprehensive method for evaluating the impacts of heavy metal pollution on aquatic ecosystems, enhancing our understanding of environmental health and informing management strategies as well as public health guidelines.

## 2.5 Tilapia fish as bioindicator species

Tilapia species are extensively researched for their significance in both natural ecosystems and aquaculture. These Tilapiine cichlids are well-known for their successful invasiveness, attributed to their advantageous life-history traits, such as early maturation in response to environmental conditions, robust physiological tolerance, and omnivorous feeding habits (Lowe-McConnell, 2006; Martin et al., 2010). Their resistance allows tilapia species to withstand adverse environmental conditions, aided by their robust disease resistance and effective respiratory capabilities. These enables them to thrive in environments characterized by high ammonia levels and low dissolved oxygen (Zhou et al., 1998). Tilapia are among the most commonly used freshwater fish in toxicological studies as bioindicators due to their ease of breeding, top at the food chain, high demand for protein, rapid growth, and tolerance to environmental stressors (Farkas and Hyde, 2004; Mohktar et al., 2009).

### 2.5.1 *Oreochromis mossambicus* (Peters, 1852)

**Figure 2.4:** An image of the Mozambique tilapia (*Oreochromis mossambicus*) sampled from the Shongweni Dam.

*Oreochromis mossambicus* is widely known as a maternal mouthbrooder. The species is indigenous to the rivers of central and southern Africa (Bills, 2019). This cichlid is an omnivorous fish that has a slightly concave forehead, thick lips, and a protruding jaw, with a light cream or grey belly. It spawns in sand and mud (Skeleton, 2001; Froese and Pauly, 2020), can grow up to 1.2 kg, and can attain a maximum size of 39 - 42 cm. This species has a lifespan of 11 years (Luna, 2012) and can survive in temperatures ranging from 17 to 35$^0$C, with a tolerance of up to 42$^0$C under certain salinities (Ford et al., 2019; Froese and Pauly, 2020). Under favourable environmental conditions, *O. mossambicus* has become well-established in nearly every area where it has been introduced or cultured (Russell et al., 2012). As a result, it is considered one of the most widely distributed freshwater fish species worldwide, as they can thrive in freshwaters, brackish water, and hypersaline environments (Ford et al., 2019; Froese and Pauly, 2020). They can endure extremely low dissolved oxygen levels (approximately <1 mg/L), at least for brief periods, and appear to meet some of their oxygen needs by ''gulping'' air at the water's surface.

### 2.5.2 *Coptodon rendalli* (Boulenger, 1896)

*Coptodon rendalli* (Boulenger, 1897), also known as the redbreast is a tilapia fish widely distributed in south-eastern parts of Congo, South Africa (KZN). *Coptodon rendalli* has a slim head, small mouth, and red stomach. This cichlid species mainly feeds on plants but can ingest aquatic invertebrates and small fish (Skelton, 2001). This fish species is an indigenous cichlid that spawns in the substrate (Skelton, 2001; McMurtie et al., 2022) and is very resistant to diseases, low dissolved oxygen levels, and super-populations. Red breast has a maximum size of 42 cm, can grow to 2.5 kg, and has a life span of 7 years (Skelton, 2001; Frimpong and Angermeier, 2009). This fish can tolerate wide temperatures ranging from 11—37$^0$C and salinity ranging up to 19 parts per thousand (ppt) (Skelton, 2001; Awaïss et al., 2010). Furthermore, *C. rendalli* breeds mainly in summer, is popular for aquaculture, angling, and fisheries, and can be used for weed control in numerous dams in South Africa (Skelton, 2001). Both fish species were abundant in the research area and can withstand various levels of salt content, temperatures, and calm waters.

**Figure 2.5**: An image of the redbreast tilapia (*Coptodon rendalli*) from the Shongweni Dam.

## 2.6 Biometric indices

### 2.6.1 Condition factor (CF)

The condition factor (CF) is a morphological parameter utilized for evaluating the general condition of fish, which can differ based on age, environmental factors, sex, and breeding season. It is a biometric measure that assesses the overall success of fish populations by reflecting their production, growth, and survival rates (Hossain et al., 2012). Condition factor relies on the length-weight relationship of a fish species and is widely utilized in fisheries monitoring programs by fisheries scientists. Both biotic and abiotic factors have a pivotal role in CF (Prakash and Verma, 2019).

### 2.6.2 Hepatosomatic index (HSI)

The hepatosomatic index (HSI) represents changes in feeding habits and reproductive hormones, specifically in the spawning season, as a result of significant physiological alterations in fish at this time (Querol et al., 2002). This phenomenon was noticed in fish as they tend to have smaller livers when living in a resource-deprived environment with reduced energy reserves (Lee et al., 2012). The HSI serves as a useful indicator of fish health and overall aquatic ecosystem quality. A higher HSI indicates healthy and fast-growing fish in a favorable environment, while a lower value suggests poor fish growth and potential environmental issues (Kareem et al., 2015).

### 2.6.3 Gonadosomatic index (GSI)

The gonadosomatic index (GSI) serves as a useful marker of maturity and gonadal development (Hama et al., 2015). It is a practical biomarker that can indicate the impact of xenobiotics on growth, reproduction, and physiological capacity. The GSI can indicate exposure to toxic substances, while histopathology is a valuable method for evaluating contamination levels (Pieterse, 2004).

## 2.7 Fish histopathology as a biomarker of pollution

Histopathological studies, which involve macroscopic examination of tissues, provide valuable insights into the deleterious effects of heavy metal exposure on fish, illustrating the multifaceted impact on both organ health and reproductive success. Extensive literature documents the histological manifestations in fish exposed to heavy metals. Metal toxicity is a significant threat to fish, affecting vital organs like the gill, liver, and kidney. These organs are commonly studied for histopathological alterations resulting from exposure to various metal toxicants, serving as essential bio-monitoring tools to assess the toxic impacts of metal across different fish species (Bibi et al., 2021).

For instance, Javed et al. (2017) described significant gill changes including hyperplasia, leukocyte infiltration, and necrosis. Awasthi et al. (2019) reported that Cr exposure in *Channa punctatus* resulted in severe liver deformities, such as apoptosis, hypertrophy, and necrosis. Similarly, Ferreira et al. (2019) observed marked gill hyperplasia in *Oreochromis niloticus* exposed to Zn, while Chen et al. (2022) noted hemorrhages and lamellar disorganization in tilapia gills due to Cu exposure. Chronic exposure to heavy metals causes cytotoxic effects and degenerative alterations in fish organs, with the liver often accumulating higher concentrations, rendering it particularly vulnerable. Histopathological alterations in the liver include blood congestion, cirrhosis, necrosis, cellular edema, and degeneration (Benjamin and Kutty, 2019; Kawade, 2020).

Beyond organ damage, heavy metals adversely affect reproductive systems. Moustafa et al. (2023) noted significant histopathological alterations in the testes of tilapia subjected to Hg, including degeneration of germinal epithelium and reduced spermatozoa, which could lead to decreased fertility and threaten population sustainability in contaminated habitats. Additionally, heavy metal contamination disrupts the overall physiology of fish, affecting hemato-biochemical parameters and can lead to nuclear and cellular deformities in blood cells (Ahmed et al., 2013; Islam et al., 2020). Genetic damage and reproductive impairments, which include reduced gonadosomatic index (GSI), hatching rates, and fecundity have been documented (Gupta et al., 2021; Xiao et al., 2021; Edo et al., 2024).

### 2.8 Bioaccumulation of metals in fish

Fish are particularly susceptible to metal pollution, as they accumulate metals within their tissues through gill, diet and skin. This accumulation may lead to a range of adverse health effects, including behavioral changes, growth impairments, and physiological issues such as immunosuppression, reproductive problems, and organ damage (Muhammad and Ahma, 2020; Afzaal et al., 2022). Bioaccumulation, a concerning process, leads to the gradual build-up of heavy metals in living organisms, particularly fish, often resulting in elevated metals present, concentrations in top-tier predators and eventually humans (Eltholth et al., 2018; Atique et al., 2020). This accumulation is shaped by various factors, including the type and concentration of metals, various developmental stages, and food sources (Liu et al., 2019; Jiang et al., 2022; Pan et al., 2022).

Metal concentrations in fish tissues can become significantly higher than in their surrounding environment due to metal absorption along the gastrointestinal tract, gills, kidneys, and liver (Annabi et al., 2013; Habib et al., 2022). This bioaccumulation in organs like the gills, kidneys, and liver can lead to pathological changes and tissue damage (Ahmed et al., 2013; Al-Ghanim et al., 2019; Kawade et al., 2020; Naz et al., 2021). Bioaccumulation of metals can disrupt biological molecules, thereby altering the function and structure of critical enzymes, proteins, and hormones, which ultimately damages various fish organs (Banday et al., 2019). Heavy metal bioaccumulation negatively impacts embryonic and larval development, resulting in increased deformities, impaired cardiac function during critical developmental stages, and mortality (Luo et al., 2014; Castaldo et al., 2020).

Additionally, metal bioaccumulation in fish poses significant threats to biodiversity when these contaminated fish enter the food chain (Huang et al., 2021). Research has demonstrated that consuming fish with elevated levels of metals can cause significant health implications (Maurya and Malik, 2018; Lebepe et al., 2020; Mannzhi et al., 2021; Misra et al., 2024). When metal concentrations exceed maximum permissible limits, they may present potential health hazards to those who consume contaminated fish (Habib et al., 2022). Specifically, consuming catfish and tilapia contaminated with metals such as baron (Ba), cobalt (Co), Cr, antimony (Sb), Pb, and As from surface waters has been reported to pose health risks to humans (Sara et al., 2018; Lebepe et al., 2020; Marr et al., 2020; Addo-Bediako et al., 2021; Hlatshwayo et al., 2024; Misra et al., 2024). Consequently, the cumulative impact of heavy metal pollution on natural water systems is multifaceted, presenting both ecological challenges and human health implications (Xiao et al., 2021; Edo et al., 2024)

**2.9 Freshwater fish consumption in the Anthropocene era**

Freshwater fish consumption has become increasingly popular worldwide, largely due to their easy access, and substantial health and nutritional benefits, including its high-quality protein, minerals, omega-3 fatty acids, and vitamins (FAO, 2020). This rise in demand can be attributed not only to a global growing population but also to the recognition of fish as a preventive food against various health conditions (WHO, 2019). Fish consumption has been shown to lower heart disease, stroke, and other cardiovascular conditions due to its high omega-3 fatty acid content, recognized for their ability to decrease inflammation, improve blood lipid profiles, and enhance vascular function (Lee and Lim, 2020).

However, along these nutritional advantages, freshwater ecosystems have now become polluted due to increasing industrialization, urbanization and increase in agricultural activities etc. (Adams et al., 2020; Ahmed et al., 2022). Fish have the capacity to accumulate metals and pass them to the next predator such as human. Therefore, fish consumption has now become another route of metal exposure to humans (Kumar et al., 2022). Consuming contaminated fish allows metals to enter the human food chain, resulting to potential health risks (Zhang et al., 2022). Long-term exposure to metals through dietary intake has been linked to severe health outcomes, including neurological disorders, increased cancer risk, and kidney damage, according to WHO (2019) and Faisal et al. (2019). A recent study by Zhang et al. (2022) found that frequent consumers of freshwater fish contaminated with As and Cd had significantly higher lifetime cancer risks. Tilapia from polluted waters, for instance, had been found to contain Hg, As and Cd at levels above recommended limits, raising public health concerns (Lebepe et al., 2020; Ibrahim et al., 2021; Hlatshwayo et al., 2024; Misra et al., 2024).

To mitigate health risks associated with consumption of contaminated fish, it is essential to adhere to safe fish consumption guidelines. The World Health Organization (WHO, 2019) recommends limiting intake of predatory fish species, which tend to bioaccumulate higher contaminant levels, and choosing smaller fish lower on the food chain that generally contain fewer pollutants.

# Chapter 3

## Water and sediment quality in the Shongweni Dam

### 3.1 Introduction

Freshwater is a limited resource on Earth and crucial for both ecological integrity and human health (Avtar et al., 2011; Edokpayi et al., 2022). In South Africa, the right to safe drinking water is enshrined in the constitution, yet approximately 2.11 million residents, predominantly in rural areas, still lack this basic provision (Edokpayi et al., 2018). Freshwater ecosystems, thus, play a critical role as the primary source of domestic water supply for many rural communities. These ecosystems, though accounting for only 0.01% of Earth's water, sustain more than 6% of global biodiversity, providing essential ecosystem services, such as water supply, transportation, and access to food resources, while holding cultural significance for many African communities (de Graaf and Garibaldi, 2015; Hunt et al., 2020). Despite their ecological and social value, freshwater ecosystems face significant threats from anthropogenic disturbances such as mining, urbanization and industrial and agricultural activities that are overusing the water and on the other end polluting it (Rodell et al., 2018; Plessl et al., 2019).

Effluents from municipal, industrial, and domestic wastes, coupled with agricultural runoff has been reported to impact most freshwater ecosystems (Singh et al., 2020; Edokpayi et al., 2022). Pollutants such as pathogens, nutrients, pesticides, and metals introduced by these anthropogenic activities pose significant risks to freshwater ecosystems (USEPA, 2005; Varol, 2012). Among these pollutants, nutrient and metal contamination present major challenges to the sustainability of freshwater ecosystems globally (Izah et al., 2023; Lebepe et al., 2024). Nutrient pollution, primarily from N and phosphorus (P), leads to eutrophication characterized by harmful algal blooms (HABs), oxygen depletion, and reduced water clarity (Izah et al., 2023; Zahoor and Mushtaq, 2023). These conditions disrupt food webs, reduce aquatic biota, and impair water utility for drinking and irrigation (Izah et al., 2023; Zahoor and Mushtaq, 2023)

Metals are persistent in freshwater ecosystems, therefore, elevated concentrations cause an equally significant threat to freshwater systems. Upon reaching aquatic ecosystems, metals sink down to bottom sediment and remain fixed for a long period of time (Lebepe et al., 2020).

Sediment serves as reservoirs for metals, however, they can be resuspended into the water column should the environmental condition change. Environmental changes through mechanical disturbances or changes in physicochemical conditions like dissolved oxygen, pH, and temperature are known to influence metal resuspension from the bottom sediment, thereby posing threats to water quality and aquatic biota (Huang et al., 2017; Islam et al., 2018). This biogeochemical cycling of metals in sediments and water exacerbates their toxicity and persistence in aquatic ecosystems, highlighting the need for integrated assessment of water and sediment quality to evaluate ecosystem health (Wang et al., 2012; Tokatli, 2019).

The uMlazi River catchment is characterized by urbanization, wastewater treatment works, and industrial and agricultural activities (DWS, 2002). The Shongweni Dam, located in the lower uMlazi River, is therefore, serving as a repository for contaminants from the upper catchment. Despite the dam receiving effluents and runoffs from all the aforementioned activities, no study has been conducted to explore the effect on water quality and the aquatic biota thereof. Of particular concern is that local communities from the surrounding are depending on this dam for their livelihood. As a result, this study aims to assess water and sediment quality of the Shongweni Dam, and determining whether it is safe for sustaining aquatic ecosystem. It was hypothesized that there would be seasonal fluctuation of water and sediment parameters at the Shongweni Dam with the dry season showing high levels for numerous contaminants.

## 3.2 Methods and materials

### 3.3.1 Water and Sediment Sampling

Physicochemical parameters, including pH, dissolved oxygen (DO), water temperature (℃), total dissolved solids (TDS), and electrical conductivity (EC) were measured at each sampling point (inflow, middle, dam wall) using a HANNA multi-parameter. Water and sediment samples were collected using 1-liter polyethylene bottles at three sites; the inflow, the midsection, and near the dam wall. Hydrochloric acid ($HNO_3$) was added to each container to keep the metals dissolved. Surface water samples were collected and stored in a cooler box, then transferred to a field refrigerator. Sediment samples were taken using a Van Veen grab, transferred to a 5-liter bucket, and then stored in polyethylene bottle before being moved to a refrigerator for further metal analysis at the School of Life Sciences, UKZN-Westville.

### 3.3.2 Water and sediment processing

Upon arrival at the laboratory, sediment samples were oven-dried at $110^0$C for 48 hours. Approximately 0.2 g of the dried sediment was crushed into a fine powder using a mortar and pestle, then placed in a 200 ml beaker and digested with aqua regia. The mixture was digested at a moderate temperature for an hour, with additional aqua regia added as necessary. In the final 10 minutes, the solution was concentrated to approximately 10 ml by boiling. The mixture was then vacuum-filtered, transferred to a 150 ml volumetric flask, and diluted with distilled water. Finally, 50 ml of the solution was syringed into a 50 ml vial for metal analysis. Water and sediment samples were then analyzed for metal elements at an accredited (ISO 17025) chemical laboratory (WATERLAB (PTY) LTD in Pretoria, South Africa. The measured concentrations of metals, aluminium (Al), antimony (Sb), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), selenium (Se) and strontium (Sr), nutrients, and physicochemical parameters were then compared with the target water quality range (TWQR) as stipulated by DWAF (1996) and guidelines by CCME (2012) and WHO (2006, 2017).

### 3.3.3 Statistical analysis

Normality of data was assessed using the Shapiro-Wilk test, while variance homogeneity was verified through Levene's test. The Mann-U test was applied to compare means for physicochemical parameters, nutrient constituents and metal concentrations across seasons using R-3.1.1. The significance was considered at $p < 0.05$.

## 3.4 Results

The physical parameters, nutrients and metal levels in the water are presented in Table 3.1. The pH ranged from neutral to alkaline throughout the study and no seasonal variation was observed ($p > 0.05$). Moreover, seasonal variations were observed for TDS ($p < 0.05$) and EC ($p<0.05$) with higher levels being observed during dry season. Sulphate was significantly higher in dry than wet season ($p < 0.05$) (Table 3.1). Most metals were below detection limits in the water, except for Fe and Pb in both seasons, with aluminum (Al) exceeding set

**Table 3.1:** Water parameters observed in the Shongweni Dam during dry and wet seasons in 2021 and the standard international guidelines. Units are mg/l, unless stated otherwise. Target Water Quality Range (TWQR) as stipulated by DWAF (1996) and guidelines for aquatic ecosystems according to CCME (2012) and WHO (2006; 2017).

| Parameter | Dry | Wet | TWQR | Guidelines |
|---|---|---|---|---|
| Temp ($^0$C) | 19.42±0.88 | 20.28±0.18 | — | <25 |
| DO (%) | 63.07±9.03 | 56.63±6.25 | — | 80—120% |
| pH | 7.91±0.22 | 8.19±0.14 | 6.0 — 9.0 | 6.5—9 |
| TDS | 287.67±11.55 | 166.00±4.36 | 0 — 450 | <1200 |
| EC (µS/cm) | 572.33±19.63 | 337±1 | 0 —70 | <1700 |
| | | **Nutrients** | | |
| Sulphate ($SO_4^{2-}$) | 35 | 28 | 0 —200 | 100** |
| Nitrate ($NO_3^-$) | 4.7 | 3.4 | — | 0.5 |
| Nitrite ($NO_2^-$) | <0.05 | <0.05 | — | 0.06 |
| Orthophosphate ($PO_4^{3-}$) | 0.6 | 0.2 | 0 —70 | 0.1* |
| | | **Metals** | | |
| Aluminum (Al) | 0.877 | <0.100 | 0.15 | 0.01 |
| Antimony (Sb) | 0.001 | 0.001 | — | 0.02 |
| Arsenic (As) | <0.001 | 0.001 | 0.0001 | 0.01 |
| Cadmium (Cd) | <0.001 | <0.001 | 0.005 | 0.003 |
| Copper (Cu) | <0.010 | <0.010 | 1 | 0.5 |
| Iron (Fe) | 1.33 | 0.15 | 0.1 | 0.01* |
| Lead (Pb) | 0.108 | 0.07 | 0.01 | 0.012 |
| Manganese (Mn) | 0.097 | <0.025 | 0.05 | 0.18 |
| Selenium (Se) | 0.005 | 0.002 | 0.3 | 1 |
| Strontium (Sr) | 0.076 | 0.07 | — | — |

guidelines during dry season (Table 3.1). Metal concentrations followed a descending order of Fe (1.33 mg/l) > Al (0.877 mg/l) > Pb (0.108 mg/l) > Mn (0.097 mg/l) > strontium (Sr) (0.076 mg/l) > Sb (0.001 mg/l) in wet and Fe (0.15 mg/l) > Pb and Sr (0.07 mg/l) > Se (0.002 mg/l) > Al and As (0.001 mg/l) in dry seasons (Table 3.1). No seasonal variations were observed for most metals in water ($p > 0.05$), however, Fe, Pb and Al differed significantly between seasons ($p < 0.05$) with higher concentrations observed during dry season (Table 3.1).

Metal concentrations in sediments are presented in Table 3.2. The mean metal concentration followed a descending order: Fe > Al > Mn > Cu > Sr > Pb > As > Sb and Fe > Al > Mn > Cu > Sr > Pb during wet and dry seasons, respectively (Table 3.2). Contrasting the water trend, metals in sediment were significantly higher during wet season compared to dry season ($p < 0.05$) (Table 3.2).

**Table 3.2:** Mean± standard deviation of metal concentrations (mg/kg, dry weight) in the sediments of the Shongweni Dam.

| Sediment | Wet | Dry | Guidelines (CCME 2012) |
|---|---|---|---|
| Aluminum (Al) | 166333.3±19.38 | 87500±98.47 | — |
| Antimony (Sb) | 9.33±1.85 | 0.733±0.46 | — |
| Arsenic (As) | 19.67±3.21 | 10.4±8.21 | 5.9 |
| Cadmium (Cd) | 1 | <0.001 | 0.6 |
| Copper (Cu) | 470±177.39 | 185.6±138.24 | 37.5 |
| Iron (Fe) | 171000±28.59 | 99733.33±62.14 | — |
| Manganese (Mn) | 5333±71.59 | 2805.67±58.16 | 0.2 |
| Lead (Pb) | 151±40.58 | 72±56.15 | 35 |
| Selenium (Se) | <0.001 | <0.001 | — |
| Strontium (Sr) | 204±85.66 | 88.2±65.48 | — |

## 3.5 Discussion

### 3.5.1 Physico-chemical parameters in water

The water pH was alkaline and remained within the CCME (2012) guideline throughout the study. This pH is comparable to those observed in Flag Boshielo Dam impacted by acid mine drainage (Madanire-Moyo et al., 2012), Tercan Dam impacted by agricultural practices, domestic and industrial waste (Gunes et al., 2019), and Doorndraai Dam impacted by mining activities (Molekoa et al., 2022). Moreover, the Shongweni Dam pH was higher than those reported in the pristine Luphephe-Nwanedi Dam which exhibited a neutral pH (Madanire-Moyo et al., 2012). The pH at Shongweni Dam has not deviated from that expected from pristine, therefore, the anthropogenic activities in the uMlazi River catchment have not significantly impacted the water pH.

Similarly, DO level has not deviated from that expected from pristine water body. No seasonal variation was observed during 2021 surveys. The DO levels observed from the Shongweni Dam was comparable to those reported by Gaine et al. (2012), Singh et al. (2013), and Panda et al. (2017) which associated with increased biological oxidation, low flow of water, high evaporation rates, and reduced oxygen solubility during dry season. Dissolved oxygen is among the key drivers of biological and physical activities in aquatic ecosystems (Wetzel, 2001), however, the DO-related impacts were not observed in the Shongweni Dam. Associated with the DO, is the water temperature which plays a critical role in shaping water quality as it directly affects factors such as pH, DO, and EC (Saik and Yeragi, 2003). Higher temperatures accelerate an increasing oxygen demand often leading to a decline in DO levels (Panda et al., 2018).

Another important physical variable in the context of water quality is the TDS which is described as the overall concentration of both organic and inorganic material, and other soluble substances in a water body (Weber-Scanell et al., 2007; Benham et al., 2011). The TDS exceeded the FAO (2018) permissible limit of 100 mg/l during both seasons. The TDS often increases during the wet season primarily due to runoffs from land surfaces, particularly those characterized by agricultural and industrial activities (Ndubi et al., 2015; Panda et al., 2017). However, this depends on the time of sampling after rainfall. Dallas and Day (2004) reported that there is a positive association between TDS and EC, which was evident during the present study. The higher EC during the dry season could be explained by increased evaporation which concentrates the dissolved solids. Despite these variations, all physicochemical parameters were within or below set guidelines for irrigation, protection of aquatic life, and drinking water. Although physical parameters are barely toxic, their dynamics influence the toxicity of metals and other contaminants in aquatic ecosystems.

### 3.5.2 Nutrient constituents in the surface water

In the current study, $SO_4^{2-}$ levels peaked during the dry season, reaching 35 mg/l, but remained within permissible limits set by CCME (2012) and WHO (2016) of 13 mg/l and 50 mg/l, respectively. In contrast, higher $SO_4^{2-}$ levels have been documented in other South African water bodies, such as the Roodeplaat, Flag Boshielo, Return Water, Albasini and Loskop Dams, while the Luphephe-Nwanedi Dam and Mvudi River recorded significantly lower concentrations (Madanire-Moyo et al., 2012; Marchand et al., 2012; Edokpayi et al., 2015; Nibumereke et al., 2015; Lebepe et al., 2020). Sulphate is primarily used as an indicator of acid mine drainage, however, there were no signs of acid mine drainage pollution in the uMlazi River catchment which could be the explanation for the low $SO_4^{2-}$ concentrations at the Shongweni Dam.

However, $PO_4^{3-}$ and $NO_3^-$ levels in the dam exceeded the CCME (2012) set guideline of 0.1 mg/l and 0.5 mg/l, respectively. This condition provided suitable environment algal blooms (DWAF, 2006; Shabalala et al., 2013), hence, the eutrophic condition. The observed $NO_3^-$ levels surpassed those reported by Madnire-Moyo et al. (2012), Marchand et al. (2012), and Nibumereke et al. (2015). On the other hand, $NO_2^-$ levels were below detection limits and significantly lower than the 0.055 — 0.075 mg/l range reported by Nibumereke et al. (2015). Nutrient levels of $NO_3^-$, $NO_2^-$, $PO_4^{3-}$, and $SO_4^{2-}$ showed seasonal variation, with higher levels observed during the dry season. This seasonal trend aligns with findings by Nibumereke et al. (2015), Edokpayi et al. (2015) and Panda et al. (2017), who attributed such increases to the influx of effluents, agricultural runoffs, urban and industrial wastes containing biological residues. Therefore, effluents from wastewater works as a driver for increased nutrient concentrations during the dry season in the Shongweni Dam may not be dismissed.

### 3.5.3 Metal concentrations in water and sediments

The study revealed seasonal variations in metal concentrations in the water with concentrations being notably higher during the dry season. This pattern aligns with the reduced dilution of pollutants in dry seasons due to lower water inflows. Aluminium exceeded the DWAF (1996) TWQR during dry season. Moreover, Fe and Pb were also above DWAF (1996) TWQR and CCME (2012) guidelines for aquatic ecosystems during both seasons. Other metals were either below detection level or within the TWQR at both seasons. Upon reaching lentic water bodies, metals sink down to the bottom sediment and remain fixed until environmental conditions such as pH, temperature, and salinity changes to cause resuspension (Dallas and Day, 2004). In the present study, most metals were below detection levels in surface water, however, significant concentrations were recorded in sediment. There are no sediment guidelines for Al and Sb levels, however, the two metals exhibited elevated concentrations with dry season showing a significantly higher compared to wet season. Concentrations of As, Cd, Cu, Fe, Mn and Pb exceeded the CCME (2001) guideline for aquatic ecosystems (Table 3.2) which become a cause for concern since sediment becomes an internal source of metals.

Aluminium was recorded in minute concentration in surface water whereas a significantly elevated concentrations were observed in sediments during both seasons. Aluminium is barely toxic, nevertheless, elevated concentrations could result in fish health disruption (Dallas and Day, 2004). Aluminium concentrations in the bottom sediment of the Shongweni Dam were higher than those reported at Loskop and Flag Boshielo Dams (Lebepe et al., 2016), but relatively lower than that reported at Tercan Dam (Gunes et al., 2019). Antimony has also shown a notable concentration in sediment. Moreover, the observed Sb concentrations were lower than those reported at Loskop and Flag Boshielo dams (Lebepe et al., 2016; Lebepe et al., 2020) impacted by mining and industrial activities. Therefore, the wastewater works, and agricultural and industrial activities in the uMlazi River catchment could be the explanation for increased Al and Sb in the Shongweni Dam.

Arsenic was below detection level in the water during the dry season whereas a concentration of 0.001 mg/l was recorded during the wet season. Recorded As the level in the water was within the guideline for the aquatic ecosystem, however, substantially elevated concentrations were observed in sediment. Arsenic concentrations recorded in the water were comparable to those reported at Loskop Dam which were explained by extensive mining in the upper Olifants River catchment (Lebepe et al., 2020) and Vaal Barrage (Gilbert and Avenant-Oldewage, 2014) which were explained by industrial and mining activities. Cadmium was below detection level in the surface water during both seasons, however, elevated concentrations exceeding the guideline (0.6 mg/kg) were observed in sediment. A similar trend was observed by Gilbert et al. (2017), Teta et al. (2017), and Lebepe et al. (2020) in water bodies impacted by mining, industrial and agricultural activities. According to Proshad et al. (2021), the presence of Cd can be attributed to industrial effluents. Moreover, Cd is particularly worrisome, given its high toxicity and industrial origins, which could lead to adverse effects and bioaccumulation on aquatic life.

Another metal of concern is the Cu, which is one of the metals from wastewater works and agricultural activities (Chapman et al., 2019). The Cu concentration observed in Shongweni Dam sediment was substantially higher than those reported by Lebepe et al. (2016) and Gilbert et al. (2017) in the Olifants and Vaal rivers impacted by coal mining and industrial activities. Iron concentrations were concerning as they exceeded set guidelines in surface water for both seasons. The Fe observed during wet season was comparable to that reported by Madanire-Moyo et al. (2012), while that observed during dry season aligned with those reported by Wagenaar and Barnhoorn (2018). Conversely, concentrations of Fe in Roodeplaat, Tercan, Orlando, and Dams were considerably higher compared to that reported in the present study (Marchand et al., 2012; Bengu et al., 2017; Wagenaar and Barnhoorn, 2018; Gunes et al., 2019). There is no guideline for Fe in sediment, however, a substantially high concentrations were observed during both seasons. Iron is an essential metal but its elevated concentration could be detrimental to aquatic biota (Dallas and Day, 2004). The Fe concentrations observed in the Shongweni Dam were relatively higher than those reported in water bodies impacted by similar anthropogenic activities (Jooste et al., 2005; Abdel-Baki et al., 2013; Algül and Beyhan, 2020). Although high Fe concentrations in water typically pose minimal risk to human health due to dietary sources being primary contributor, their ecological implications are, however, significant (USEPA, 2007). Elevated Fe concentrations may affect aquatic biota, particularly benthic species.

Another metal which has been overlooked in many water quality studies is Mn which can be detrimental to aquatic life although is an essential metal that plays a role in enzymatic and biochemical reactions (Huang et al., 2021). Manganese exhibited significant concentration in surface water during dry seasons. The concentrations were within the DWAF (1996) TWQR for aquatic ecosystems. Moreover, Mn concentrations in the water were comparable to those reported in the Luphephe-Nwanedi Dam, however, were relatively higher than Klipvoor, Bospoort, and Marco-Bosveld Dams (Madanire-Moyo et al., 2012; Marchand et al., 2012; Wagenaar and Barnhoorn, 2018). However, Mn concentration in sediment exceeded the guideline for aquatic ecosystems at the Shongweni Dam. The concentration was relatively higher than that observed in the Phalaborwa Barrage (Jooste et al., 2015) and Vaal River (Gilbert and Avenant-Oldewage, 2014). Contrastingly, Pb concentration in surface water exceeded TWQR for aquatic ecosystems during both seasons. Similarly, Pb concentrations exceeded the guideline of 35 mg/kg in sediment. The Pb concentration observed in the present study is comparable to those observed in Flag Boshielo and Khami Dams (Madanire-Moyo et al., 2012; Teta et al., 2017). In contrast, the concentrations were relatively lower than those observed in Orlando, Luphephe-Nwanedi, Klipvoor, and Marco-Bosveld Dams (Madanire-Moyo et al., 2012; Bengu et al., 2017; Wagenaar and Barnhoorn, 2018). Significantly higher than the current study's Pb concentrations were those found in the Lower and Upper uMguza and Return Water Dams (Marchand et al., 2012; Teta et al., 2017). Sources of Pb include waste from mining, industrial, and municipal processes, therefore, the wastewater effluents could be the driver of the elevated Pb concentration in the Shongweni Dam.

Selenium is an essential metal with a role in enzymatic metabolism and biochemical reaction, however, increased concentration can be detrimental to aquatic life (Lino et al., 2020). Moreover, Se is known to have antagonistic relationship with some toxic metals (Misra et al., 2024). In the present study, Se was within the guideline in surface water and below detection limit in sediment. This could be explained by the fact that Se could be in demand in aquatic ecosystem and organisms are quickly taking it up before it can sink down to sediment. Contrasting Se trend, Sr showed significant concentrations in surface water and sediment during both seasons. Strontium concentrations observed in surface water were comparable to those reported in the Orlando Dam (Bengu, 2017). Moreover, there are no established guidelines for Sr concentrations in water for protection of aquatic life, irrigation, or drinking purposes. However, its bioavailability and toxicity could be influenced by Ca (Klaczek et al., 2022).

The cumulative presence of metals, even within permissible limit for whether in surface water or sediment, suggest a latent risk to both aquatic ecosystem and human health. Chronic exposure to sublethal metal concentrations may impact benthic organisms and bioaccumulate in the food web, increasing risks for communities relying on the dam for water and fish consumption. Most metals showed non-significant concentrations in the water column with sediment exhibiting substantial concentrations. The hypothesis that metal concentration would be higher during dry season was supported for surface water whereas sediment showed opposite trend. Nevertheless, metals reaching aquatic ecosystems are known to sink down to bottom sediments making sediments become potential source in the near future. This is what was observed in the present study, hence, metal concentrations settled in the bottom sediment becomes cause for concern.

# Chapter 4

## Histopathology-based health assessment of *Oreochromis mossambicus* and *Coptodon rendalli* from the Shongweni Dam

### 4.1 Introduction

Human activities exert widespread and adverse impacts on aquatic ecosystems, primarily through agricultural runoff and industrial wastewater, which introduce a variety of toxic pollutants into freshwater systems (Reid et al., 2019; Aradpour et al., 2020). These pollutants can affect aquatic life through additive, antagonistic, or synergistic interactions (Jackson et al., 2016). Environmental stressors, including metal pollution, may also exacerbate infectious diseases in aquatic species, decreasing biota survival rate and potentially leading to population decline in heavily polluted waters (Milla et al., 2011; Penttinen et al., 2016). Consequently, there is an increasing interest in biological effect monitoring to identify and assess at-risk ecosystems utilizing fish as bioindicators to evaluate the extent of pollution (Wagenaar and Barnhoorn, 2018; Lebepe et al., 2020; Misra et al., 2024).

Fish serve as critical bioindicators for monitoring metal pollution in freshwater systems due to their trophic position and their continuous exposure in the ecosystems (Rahman et al., 2012). Fish inhabit diverse aquatic environments used for human recreation and drinking water, and through continuous exposure, accumulate pollutants over time. The health status of fish has been utilized as an indicator of the overall well-being of aquatic ecosystems (Adebayo, 2017). Studies have shown how water pollution affects the overall health of fish (Lebepe et al., 2020; Hlatshwayo et al., 2024; Misra et al., 2024). According to Carmago and Martinez (2007), the pollution effect starts at the tissue level before gross change manifests at an organ level.

Histopathology, the microscopic examination of tissue changes, is instrumental in detecting the pathological effects of contaminants within aquatic organisms. This method provides early indicators of tissue damage, often before any external symptoms manifest (Marchand et al., 2008). Histopathological alterations in fish, particularly in gills, liver, gonads, and muscle tissue, have been linked to toxic pollutants in various ecosystems (McHugh et al., 2011; Wepener et al., 2011; Marchand et al., 2012; Wagenaar and Barnhoorn, 2018). Studies using this approach have successfully identified organ-specific tissue changes, which can be quantified and compared across species, locations, and pollutant levels using standardized protocols, such as those established by Bernet et al. (1999) and modified by van Dyk et al. (2009).

The Shongweni Dam, known for its eutrophic state, is subject to pollution from industrial effluents, agricultural runoff, and sewage discharge, particularly from Hammersdale textile industries (Graham et al., 1998; Dickens, 2010). This dam is home to a number of fish species which are providing ecological balance to the integrity of the dam. Despite the dam receiving effluents from wastewater work and its eutrophic status, no study was conducted to explore the effect of this pollution level on the health of inhabitant fish. Mozambique tilapia (*O. mossambicus*) and redbreast tilapia (*C. rendalli*) play key ecological roles in the Shongweni Dam. Their position within the food web and capacity to reflect cumulative impacts of local pollution make them good indicators of water pollution. Therefore, this study investigated the health of the two tilapia species using general health indices and a histopathology-based health assessment approach. Given that water bodies exhibit seasonal variation with regard to flow levels and the concentrations of contaminants may increase as the water level drops, it was hypothesized that fish will be highly affected during low flow season.

## 4.2 Methods and Materials

### 4.2.1 Sampling and Health Assessment Procedures

Sampling was conducted during the dry (July — August) and wet seasons (November — December) in 2021 at the Shongweni Dam, KwaZulu-Natal, using gill nets and an electroshocker. In total, sixteen *O. mossambicus* (12 males, 4 females) were sampled, with 9 collected in dry and 7 in the wet season, while fourteen *C. rendalli* (5 males, 9 females) were sampled, including 4 in dry and 10 in wet season. The study was approved by the University of KwaZulu-Natal Animal Research Ethics Committee (Ref: AREC/019/018). Captured tilapia fish were transferred to aerated containers with dam water to ensure their survival prior to processing.

### 4.2.2 Morphometric Measurements and Condition Indices

Tilapia fish were weighed, length measured and then euthanized by severing the spinal cord, followed by ventral dissection. Morphometric data, weight (g), and length (cm) were used to calculate the length-weight relationship (Equation 4.1) and condition factor (CF) (Equation 4.2). Liver and gonads were weighed to compute the hepatosomatic index (HSI) (Equation 4.3) and gonadosomatic index (GSI) (Equation 4.4).

$W = a.L^b$ ..........................................................................................................Equation 4.1

where W represents fish weight (g), L is the total length (cm), α is the intercept parameter and is the allometric growth parameter. The parameters α and *b* were obtained from a least of square linear regression of weight and length of Eq. (1), to obtain:

$Log_{10}W = Log_{10}\,\alpha + b\,Log_{10}\,L$

$CF = \frac{W \times 100}{L^3}$..........................................................................................................Equation 4.2

$HSI = \frac{liver\ mass\ (g)}{body\ mass\ (g)} \times 100$..........................................................................................Equation 4.3

$GSI = \frac{gonad\ mass\ (g)}{body\ mass\ (g)} \times 100$.......................................................................................Equation 4.4

### 4.2.3 Health Assessment Index

Fish health was profiled by assigning scores based on the severity of abnormalities in both external (eye, skin, fin, and gills) and internal organs (kidney, liver, and spleen) following the protocol established by Adams et al. (1993) where anomaly scores ranged from 0 to 30, with higher values reflecting greater severity. These scores were summed to determine the Health Assessment Index (HAI) for each fish. The mean HAI for the entire sample group was calculated by averaging individual fish HAI values, as described by Adams et al. (1993). This method has been extensively used in studies assessing fish health in South African Dams and has proven effective in evaluating fish condition (Marchand et al., 2012; Sara et al., 2014; Lebepe et al., 2020).

### 4.2.4 Histopathology-based Assessment

Liver, gill, and gonadal tissues were dissected out from each fish and weighed. Tissue samples were fixed in 10% formalin for preservation. After fixation, samples were washed in tap water, dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax (Wagenaar and Barnhoorn, 2018). Sections were cut to 5 µm thickness, stained, and prepared for microscopic examination at the Sefako Makgatho Health Sciences University. Digital images of damage were captured using an Optika Proview Digital Camera and microscope. Tissue sections were scanned at 10X and 40X magnifications, and histopathological alterations were assessed following a modified version of the Bernet et al. (1999) protocol. Histopathological lesions were categorized into four reaction patterns, circulatory disturbances, inflammatory response, and progressive and regressive changes.

### 4.2.5 Statistical Analysis

Quantitative data was checked for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Variations CF, HSI, GSI, and HAI between species were evaluated for statistical significance using the Mann-U test or an independent t-test. Statistical significance was determined at $p < 0.05$

## 4.3 Results

### 4.3.1 Weight and Length

The weight of *C. rendalli* ranged from 390 g to 1710 g, while *O. mossambicus* weights varied between 760 g to 1530 g. Mean weight showed no significant difference between species (Mann-U = 47; $p > 0.05$) (Figure 4.1). Moreover, no seasonal variation was observed for each fish species (Mann-U = 83; $p > 0.05$). The length of *C. rendalli* ranged from 27.6 cm to 43.5 cm, and *O. mossambicus* from 32 cm to 41.5 cm, with no statistical difference between the species (Mann-U = 49; $p > 0.05$) (Figure 4.1). Moreover, no seasonal variation was observed for fish length in either species (Mann-U = 85; $p > 0.05$).

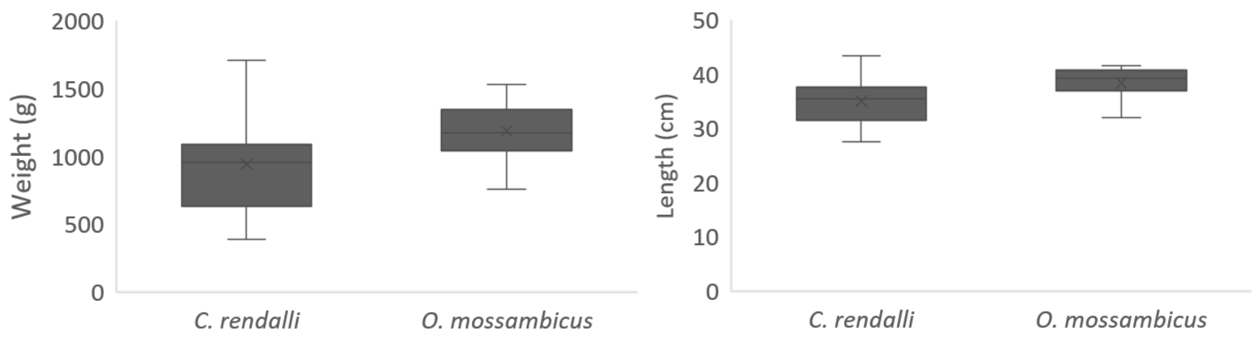


**Figure 4.1**: Mean weight (g) and length (cm) of two tilapia species (*Oreochromis mossambicus* and *Coptodon rendalli*), sampled from the Shongweni Dam.

### 4.3.2 Weight-length relationship

Both *C. rendalli* and *O. mossambicus* populations exhibited negative allometric growth (Table 4.1). The length-weight equations for *O. mossambicus* were W = $0.097L^{2.586}$ for males and W = $0.017L^{2.578}$ for females. For *C. rendalli*, the equations were W = $0.058L^{2.548}$ for males and W =$0.061L^{2.541}$ (Table 4.1). The coefficient of determination ($R^2$) was below 0.90 for both male and female *O. mossambicus*, whereas it exceeded 0.90 in *C. rendalli*, indicating a stronger model fit in the latter species (Table 4.1).

**Table 4.1**: The length-weight relationship of *Oreochromis mossambicus* and *Coptodon rendalli* from the Shongweni Dam during 2021 survey.

| **Species** | | **N** | **α** | **b** | **$R^2$** | **Growth** |
|---|---|---|---|---|---|---|
| | Males | 12 | 0.097 | 2.586 | 0.52 | -Allometric |
| | Females | 4 | 0.017 | 2.578 | 0.527 | -Allometric |
| *O. mossambicus* | Combined | 16 | 0.068 | 2.584 | 0.2 | -Allometric |
| | Males | 5 | 0.058 | 2.548 | 0.995 | -Allometric |
| | Females | 9 | 0.061 | 2.537 | 0.908 | -Allometric |
| *C. rendalli* | Combined | 14 | 0.060 | 2.541 | 0.939 | -Allometric |

Note: α is the intercept parameter, b is the allometric growth parameter, $R^2$ is the coefficient of determination

### 4.3.3 Biometric Indices and Necropsy

Table 4.2 provides a summary of sample size and biometric indices; CF, HSI, GSI, and HAI, for *O. mossambicus* and *C. rendalli* sampled from the Shongweni Dam. The highest mean CF was recorded for *O. mossambicus* at 2.124, followed closely by *C. rendalli* at 2.079. The HSI values for *O. mossambicus* ranged from 1.28 to 3.57%, and for *C. rendalli* from 1.76 to 2.67%. Gonadosomatic indices ranged from 0.003 to 2.81% and 1.67 to 2.92% for *C. rendalli* males and females, respectively. For *O. mossambicus*, GSI ranged from 0.01 to 0.76% in males and

1.43 to 3.62 in females. There was no seasonal variation in CF (Mann U = 78; $p > 0.05$), HSI (Mann-U = 65; $p > 0.05$), and GSI (Mann-U = 58; $p > 0.05$).

**Table 4.2**: Biometric indices of tilapia species caught at the Shongweni Dam during the 2021 survey.

| **Biometric indices** | ***Coptodon rendalli* (n=14)** | ***Oreochromis mossambicus* (n=16)** |
|---|---|---|
| Condition factor (CF) | 2.079±0.238 | 2.124±0.505 |
| Hepatosomatic index (HSI) | 1.363±0.574 | 1.387±0.556 |
| Gonadosomatic index (GSI) Female | 2.312±0.474 | 2.500±0.946 |
| Gonadosomatic index (GSI) Male | 0.754±1.164 | 0.414±0.202 |

No external macroscopic anomalies were detected in the skin, eyes, fins, or opercula of sampled tilapia fish, resulting in a score of 0 for these organs. Internal macroscopic anomalies were observed in the liver, gills, and gonads. *Oreochromis mossambicus* showed HAI scores ranging from 30 to 60, with more significant anomalies in the liver and *C. rendalli* exhibits HAI scores between 0 to 120, with moderate anomalies in gills and kidney, and more significant anomalies in the liver. The HAI was highest during the dry season for *C. rendalli* and vice versa for *O. mossambicus*, with the highest mean (42.0± 35.21) recorded for *C. rendalli* (Figure 4.2). Seasonal comparisons revealed no significant variation in the HAI scores for *O. mossambicus* and *C. rendalli* across dry and wet seasons (Mann-U = 81; $p > 0.05$). Additionally, there was no significant difference in HAI between *C. rendalli* and *O. mossambicus* (Mann-U = 80; $p > 0.05$).

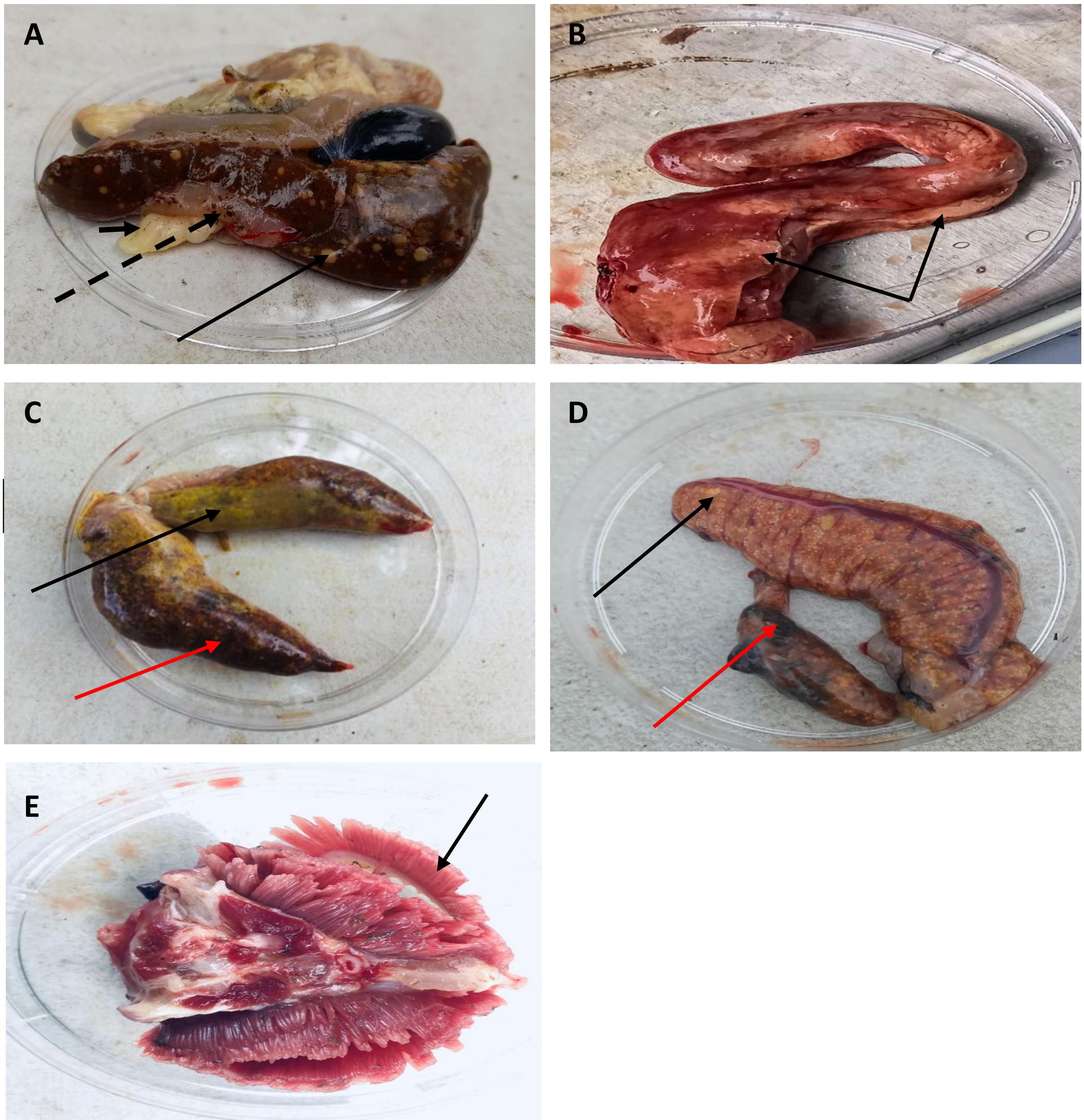


**Figure 4.2:** Macrographs observed in two tilapia species from the Shongweni Dam. **A.** Dark spots (dotted arrow), Nodules in the liver (solid arrow); **B.** Liver discoloration (solid arrows); **C & D.** Yellowish discoloration (solid arrow), abnormal gonads (red arrows); **E.** gill discoloration (solid arrow).

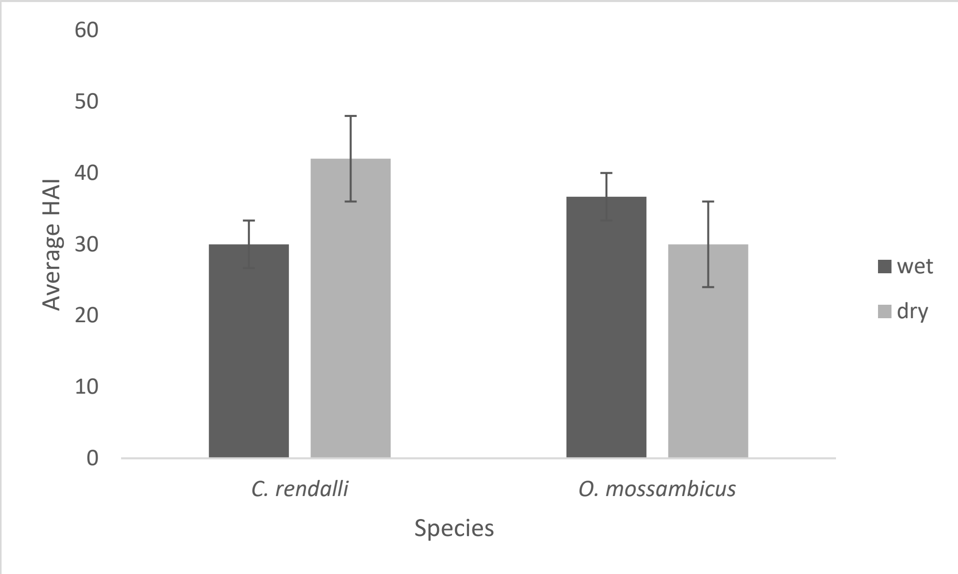


**Figure 4.3:** Bar-plot illustrating the mean± standard deviation of health assessment index for *Coptodon rendalli* and *Oreochromis mossambicus* during the dry and wet season at Shongweni Dam, KwaZulu-Natal.

### 4.3.4 Qualitative histopathology assessment

Table 4.3 presents the percentage prevalence of histopathological lesions observed in the gills of the two tilapia species sampled from the Shongweni Dam. Four reaction patterns were identified in the gill tissues of both species; circulatory disturbances, regressive changes, progressive changes, and inflammation. Circulatory disturbances, including hemorrhage/aneurysm and intercellular oedema, were the most prominent lesions, with a prevalence of 92.50% in *C. rendalli* and 77.38% in *O. mossambicus* (Table 4.3; Figure 4.4). Regressive changes, characterized by architectural and structural alterations, pillar cell rupture, and plasma alterations, were also highly prevalent, showing 90 to 100% lesions in both species (Table 4.3). Although not prominent, progressive changes such as epithelial hyperplasia were observed (Figure 4.4). Statistical analysis revealed no significant differences in gill index between the two species (Kruskal-Wallis $H = 0.221$; $p > 0.05$) and across seasons (Kruskal-Wallis $H = 0.205$; $p > 0.05$).

**Table 4.3:** Reaction patterns and percentage prevalence of histopathological alterations in the gill of *Oreochromis mossambicus* and *Coptodon rendalli* sampled from the Shongweni Dam during the dry and wet seasons in 2021.

| **Gill** | | | | |
|---|---|---|---|---|
| Reaction pattern | Functional unit of the tissue | Alteration | *O. mossambicus* | *C. rendalli* |
| Circulatory disturbance | | Hemo/hyper/aneur | 67.46 | 95 |
| | | Intercellular oedema | 87.3 | 90 |
| | Epithelium | Architec and struct alterations | 100 | 100 |
| Regressive changes | | Plasma alterations | 100 | 95 |
| | | Deposits | 67.46 | 52.5 |
| | | Nuclear alterations | 47.62 | 65 |
| | | Atrophy | 67.46 | 12.5 |
| | | Necrosis | 81.75 | 60 |
| | | pillar cell rupture | 100 | 100 |
| | Supporting tissue | Architec and struct alterations | 78.57 | 87.5 |
| | | Plasma alterations | 92.86 | 47.5 |
| | | Deposits | 76.19 | 42.5 |
| | | Nuclear alterations | 63.49 | 95 |
| | | Atrophy | 63.49 | 0 |
| | | Necrosis | 60.32 | 70 |
| Progressive changes | Epithelium | Hypertrophy | 50.79 | 85 |
| | | Hyperplasia | 47.62 | 42.5 |
| | Supporting tissue | Hypertrophy | 60.32 | 55 |
| | | Hyperplasia | 74.6 | 47.5 |
| Inflammation | | | | |
| | | Exudate | 0 | 42.5 |
| | | Activation of RES | 0 | 35 |
| | | Infiltration | 81.75 | 55 |

Hemo=hemorrhage, hyper=hyperaemia, aneury=aneurysm, architec=architectural, struct=structural

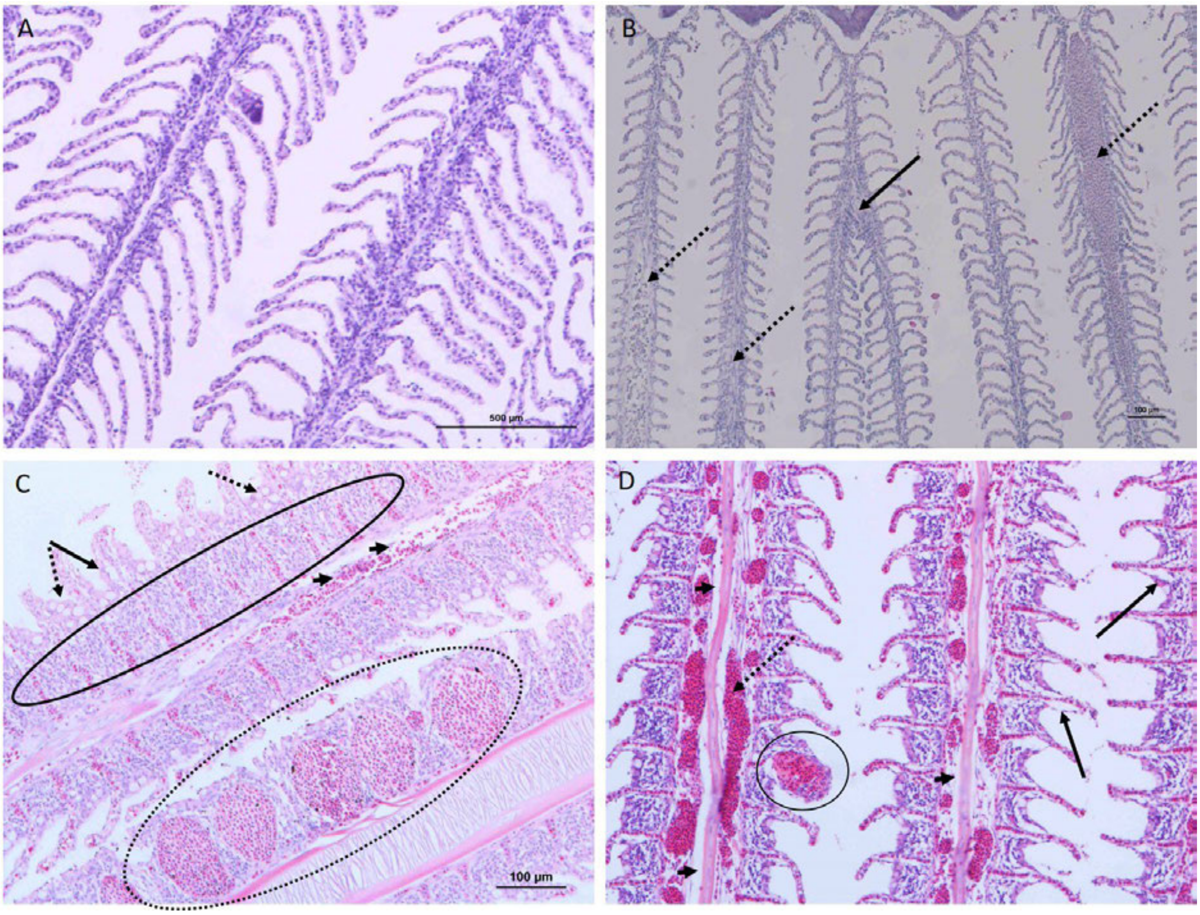


**Figure 4.4:** Histopathological alterations observed in the gills of *Oreochromis mossambicus* and *Coptodon rendalli* from the Shongweni Dam during 2021 surveys. **A.** Normal gill histology; **B.** Branching of primary lamella (solid arrow), hemorrhage resulting in structural alteration (dotted arrows); **C.** Aneurism and epithelial hyperplasia resulting in fusion of secondary lamella (dotted circle), epithelial hyperplasia (solid-lined circle), epithelial lifting (solid arrow), hyperplasia of mucus cells (dotted arrows), hemorrhage and oedema (arrowhead); **D.** Red blood cells congestion (dotted arrow), aneurism (encircled), epithelial lifting (solid arrows), oedema (arrowhead)

Table 4.4 provides a summary of the percentage prevalence of histopathological alterations in the liver of *O. mossambicus* and *C. rendalli*. Progressive changes, including necrosis, steatosis, and vacuolation, were the most prevalent lesions, occurring in 60.77% of *O. mossambicus* and 53.93% of *C. rendalli*. These were followed by circulatory disturbances, with a prevalence of 47.22% and 46.25% prevalence for *O. mossambicus* and *C. rendalli*, respectively. Inflammatory responses were the least prevalent, occurring in 37.04% of *O.*

*mossambicus* and 31. 67% of *C. rendalli*. (Table 4.4; Figure 4.4). The liver index showed no significant difference between *O. mossambicus* and *C. rendalli* (Kruskal-Wallis H = 2.456; $p < 0.05$). Similarly, no seasonal variation was observed for liver index for each species (Kruskal-Wallis H = 0.213; $p > 0.05$).

**Table 4.4:** Reaction patterns and percentage prevalence of histopathological alterations in the liver of *Oreochromis mossambicus* and *Coptodon rendalli* sampled from Shongweni Dam during dry and wet seasons in 2021.

| **Liver** | | | | |
|---|---|---|---|---|
| Reaction pattern | Functional unit of the tissue | Alteration | *O. mossambicus* | *C. rendalli* |
| Circulatory disturbance | | Hemo/hyper/aneury | 43.75 | 82.86 |
| | | Intercellular oedema | 68.75 | 51.43 |
| Regressive changes | Liver tissue | Architec and struct alterations | 75 | 68.57 |
| | | Plasma alterations (steatosis, hydropic deg) | 81.25 | 92.86 |
| | | Deposits | 81.25 | 92.86 |
| | | Nuclear alterations | 75 | 80 |
| | | Atrophy | 68.75 | 41.43 |
| | | Necrosis | 56.25 | 55.71 |
| | | Vacuolar degeneration | 81.25 | 82.86 |
| Progressive changes | Liver tissue | Hypertrophy | 68.75 | 55.71 |
| | | Hyperplasia | 31.25 | 38.57 |
| Inflammation | | Exudate | 37.5 | 27.14 |
| | | Activation of RES | 0 | 0 |
| | | Infiltration | 68.75 | 80 |

Hemo=hemorrhage, hyper=hyperaemia, aneury=aneurysm, architec=architectural, struct=structural

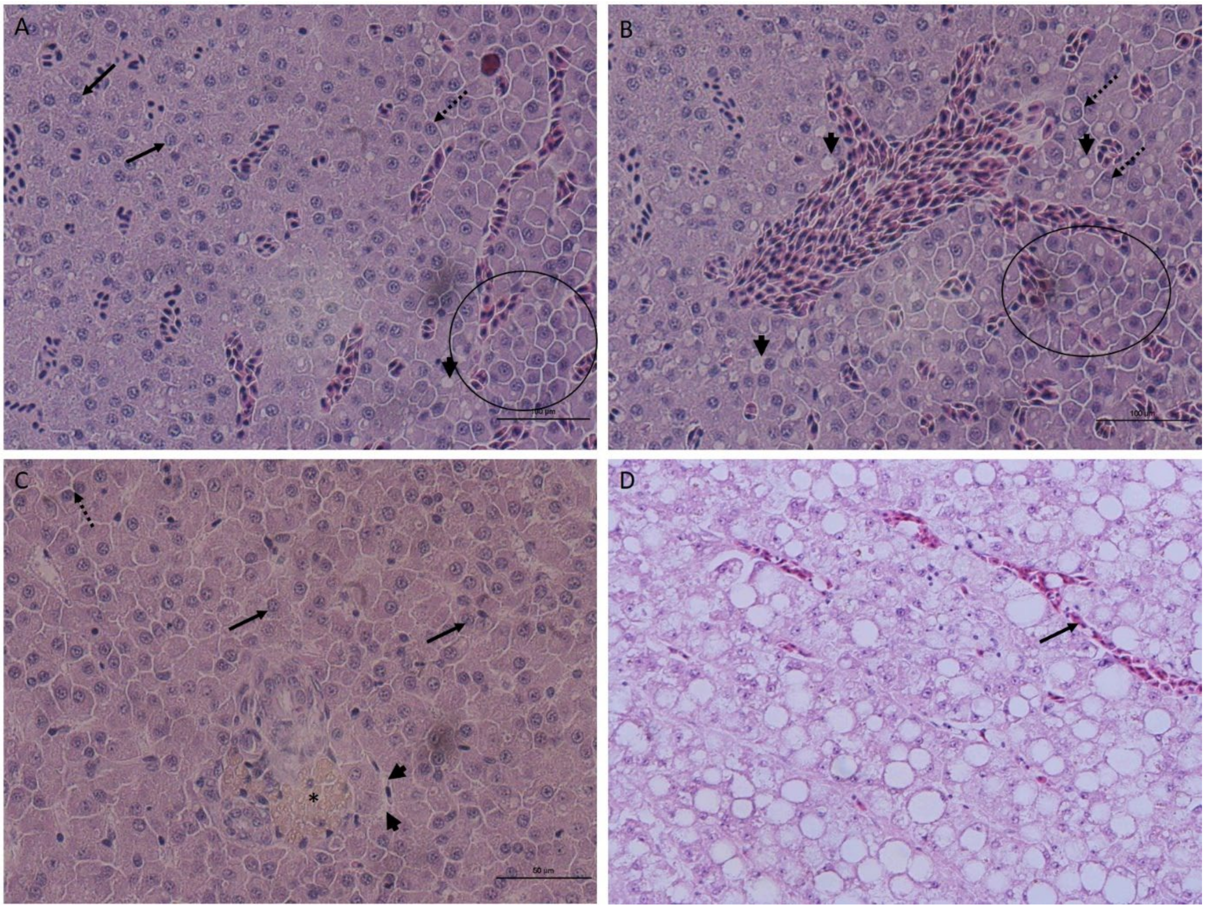


**Figure 4.5:** Histological lesions observed in the livers of *Oreochromis mossambicus* and *Coptodon rendalli* from the Shongweni Dam during the 2021 surveys. **A.** Hepatocellular pleomorphism (encircled), vacuolation (arrowhead), peripheral nucleus (solid arrows), disintegrated nucleolus resulting in necrosis (dotted arrow); **B.** Hepatocellular pleomorphism (encircled), peripheral nucleus as a result of vacuolation, vacuolation (arrowheads), **C.** Fibrosis of blood sinusoid (arrowhead), peripheral nucleus (dotted arrow), necrosis characterized by disintegrated nucleolus (solid arrows), melanomacrophages (asterisk); **D.** steatosis, red blood cells congestion in sinusoids (arrow)

Histopathological examinations of gonads of *O. mossambicus* and *C. rendalli* revealed distinct pathological changes in both male and female specimens (Table 4.3; Figures 4.6 and 4.7). Pigmented macrophages were observed in both sexes, with a higher prevalence in ovaries (81.75%) compared to testes (56.35%) of *O. mossambicus* and equal prevalence (50%) in both gonadal types of *C. rendalli*. Testes exhibited interstitial cell hyperplasia, condensed nuclei, red blood cell infiltration, basal membrane epithelial lifting, vacuolation, and interstitial fibrosis, whereas ovaries showed perifollicular cell hyperplasia, ovarian oedema, reduced yolk formation, cortical alveoli, cystic perifollicular cells, oocyte atresia, and degeneration of perifollicular wall.

Male gonad indices showed significant differences between *O. mossambicus* and *C. rendalli* (Kruskal-Wallis H = 0.995; $p < 0.05$), with no seasonal variation being observed (Kruskal-Wallis H = 1.981; $p > 0.05$). Similarly, ovary indices showed a significant difference between the two species (Kruskal-Wallis H = 0.816; $p < 0.05$), nevertheless, no seasonal variation was observed in each species (Kruskal-Wallis H = 0.054; $p > 0.05$).

**Table 4.5:** The percentage prevalence of histopathological alterations in the ovaries and testes of *Oreochromis mossambicus* and *Coptodon rendalli* sampled from Shongweni Dam during dry and wet seasons in 2021.

| **Organ** | | | |
|---|---|---|---|
| Testes | Alterations | *O. mossambicus* | *C. rendalli* |
| | Pigmented macrophages | 56.35 | 50 |
| | Interstitial cell hyperplasia | 49.21 | 32.5 |
| | Condensed nuclei | 56.35 | 50 |
| | Infiltration of red blood cells | 38.10 | 32.5 |
| | Epithelial lifting of the basal membrane | 50.79 | 50 |
| | Interstitial fibrosis | 30.95 | 37.5 |
| Ovaries | | | |
| | Pigmented macrophages | 81.75 | 50 |
| | Perifollicular cell hyperplasia | 38.10 | 27.5 |
| | Ovary oedema/Vacuolation | 36.51 | 50 |
| | Postovulatory follicle complexes | 38.10 | 42.5 |
| | Atresia of oocytes | 50.79 | 40 |
| | Peripheral nucleus | 61.90 | 45 |

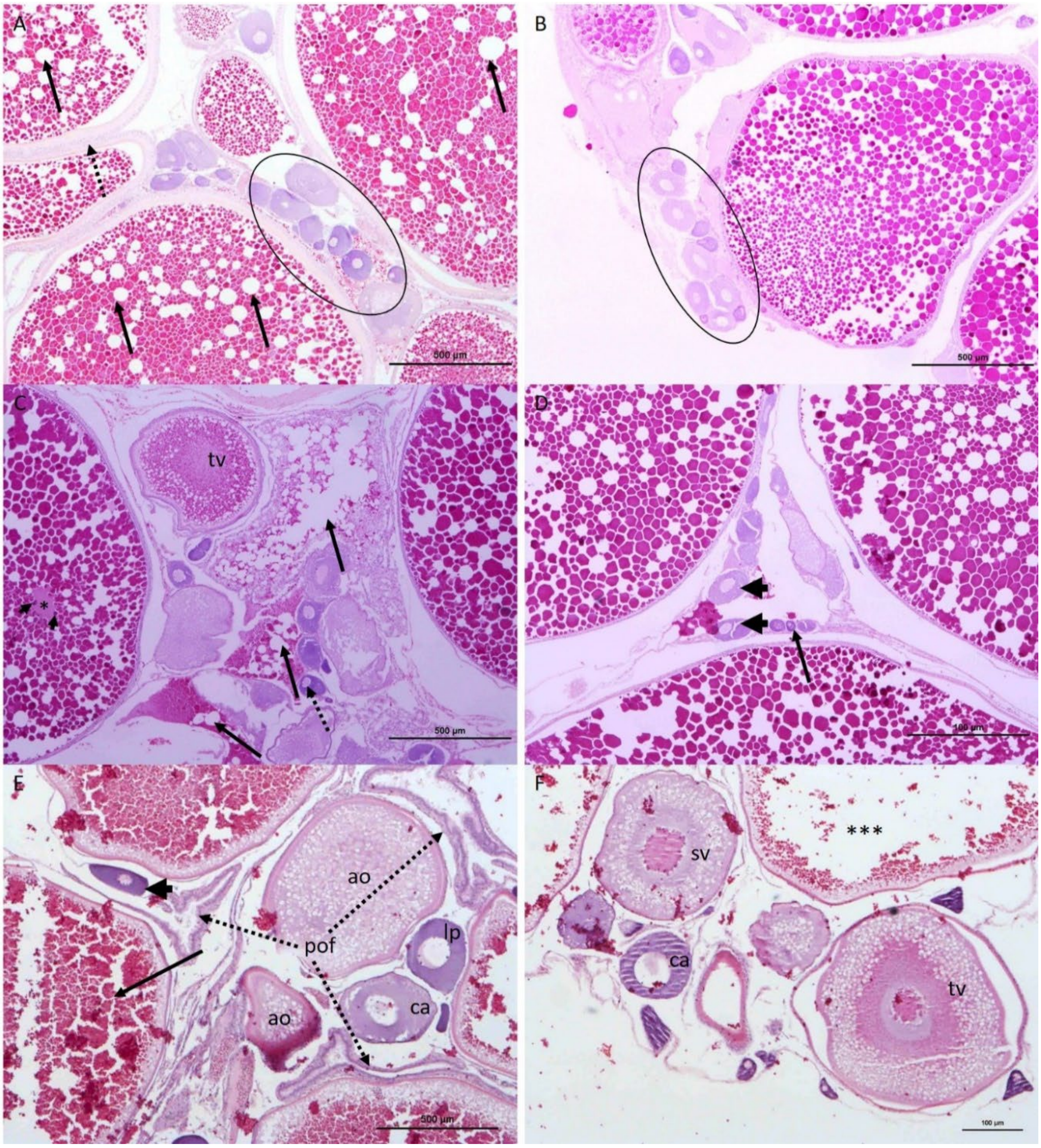


**Figure 4.6:** Different oocyte growth stages and pathologies observed for *Oreochromis mossambicus* and *Coptodon rendalli* from the Shongweni Dam. **A.** Perifollicular cell hyperplasia (dotted arrow), pre-vitellogenesis stages (encircled), atresia characterised by lipids vacuolation (solid arrows); **B.** Pre-vitellogenesis stages on connective tissue (encircled); **C.** Tertiary vitellogenesis (tv), vacuolation (solid arrows), nucleus (asterisk), nucleoli (arrowheads), peripheral nucleus (dotted arrows); **D.** late primary growth pre-vitellogenesis (arrowheads), early primary growth pre-vitellogenesis (arrow); **E.** Germinal vesicle migration (solid arrows), postovulatory follicle complexes (pof), atresia characterised by lipids vacuolation (asterisks); atresia or oocytes (ao), late primary growth pre-vitellogenesis (lp), late primary growth pre-vitellogenesis (arrowheads), quaternary vitellogenesis (qv), corticular

alveoli (ca); **F.** Germinal vesicle migration with liquefaction of yolk globules (asterisks), secondary vitellogenesis (sv), tertiary vitellogenesis (tv)

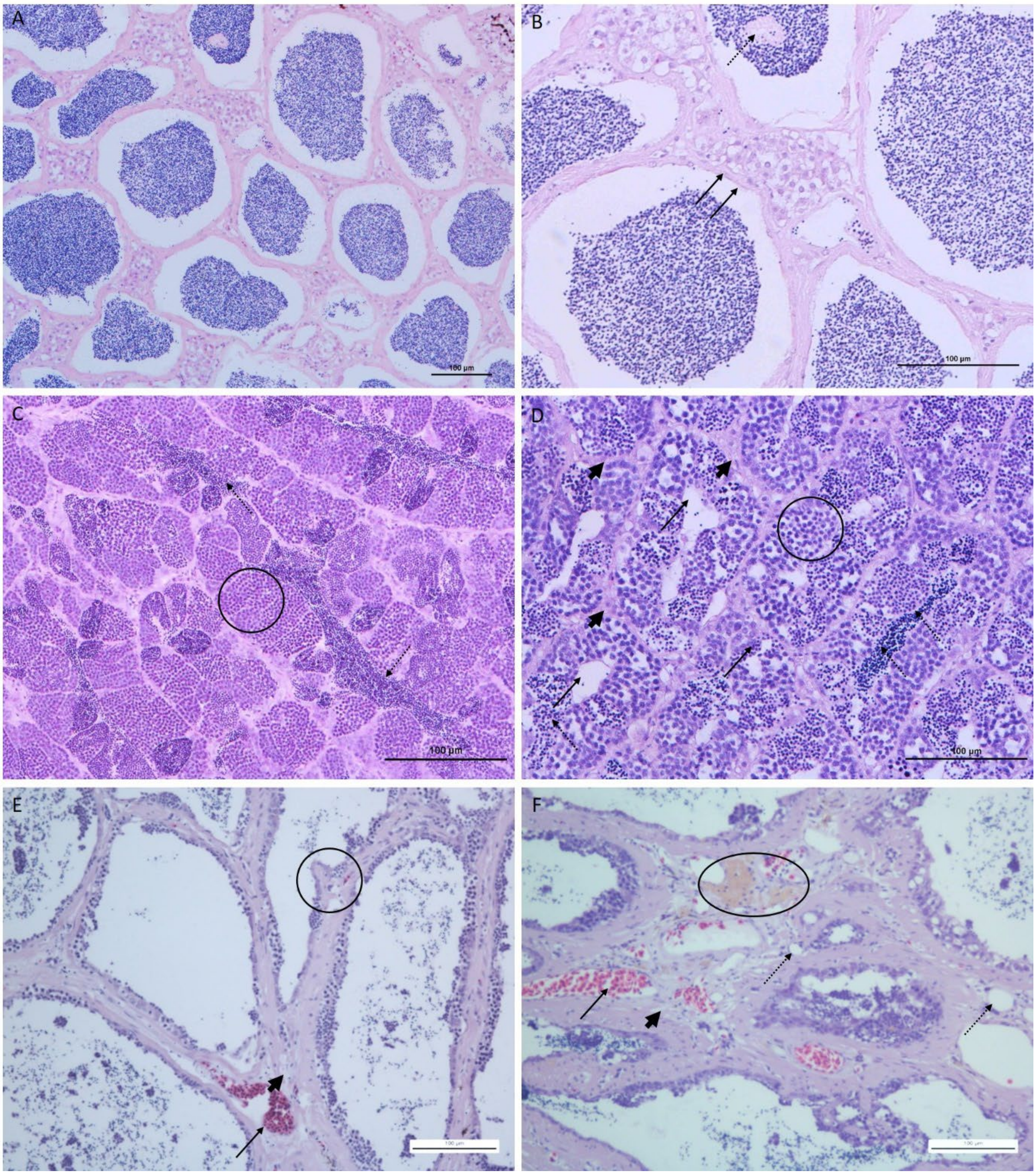


**Figure 4.7:** Histology of the testes observed in the two tilapia species from the Shongweni Dam. **A.** Active spermatogenesis in the lobular wall (spermatocytes, spermatids and sperm clusters) and wide lumen with free spermatozoa. **B.** interstitial tissue with Leydig cells (solid arrows); **C.** Primary spermatocytes (encircled); **D.** Spermatozoa (dotted arrows), lumen of the lobules (solid arrows), primary spermatocytes (encircled), interstitial tissue (arrowhead); **E.** Interstitial cell hyperplasia (encircled), infiltration of red blood cells which has also resulted in epithelial lifting of the basal membrane(arrow), interstitial fibrosis (arrowhead); **F.** Infiltration

of red blood cells (solid arrow), interstitial fibrosis (arrowhead), pigmented macrophages (encircled), vacuolation (dotted arrow)

## 4.4 Discussion

### 4.4.1 Length-weight relationships

The length-weight relationship (LWR) is a valuable indicator for assessing fish growth, maturity, survival, and overall health (Nasri et al., 2021; Tadesse et al., 2024). The LWR allows morphological comparisons of fish populations across various regions and among species within the same taxa (Famoofo and Abdul, 2020). The parameters of LWR can vary significantly due to factors such as diet, sexual maturity, sample size, sampling period, preservation techniques employed on the specimens, seasonal variations, and the degree of stomach fullness (Hossain et al., 2014; Jan and Ahmed, 2020). The values in LWR equations reflect the species' general well-being, linked to factors like fatness and body shape (Jisr et al., 2018). In this study, both tilapia species exhibited negative allometric growth ($b < 3$), suggesting fish were growing more in length than in weight. Similar findings of negative allometric growth in other fish species were observed in the upper River Awash and Thomas Dam (Tadesse et al., 2024; Usman et al., 2024) and in the polluted Inanda and Nagle dams (Misra et al., 2024).

Such growth patterns can suggest limitations in feeding or environmental factors including temperature and water quality, impacting metabolism and energy allocation (Kalita et al., 2016; Kuriakose, 2017; Rana and Kaur, 2023). Negative allometric growth may also result from physiological factors like spawning or maturity status (Jisr et al., 2018). Conversely, *O. mossambicus* in other habitats, such as polluted Phalaborwa Barrage and Tugwi-Mukosi Dam, have demonstrated positive allometry (Addo-Bediako et al., 2014; Mabika et al., 2024), underscoring the variability of growth responses to environmental conditions. The coefficient of determination ($R^2$) values for *C. rendalli* populations were above 0.90, indicating strong linearity between length and weight and a reliable model for predicting growth. This aligns with previous research on various fish species in different water bodies, reflecting robust predictive capacity (Egbal et al., 2011).

### 4.4.2 Overall fish health: Biometric indices

Condition factor (CF) reflects the general health of fish in relation to environmental variables, age, sex, and reproductive stage (Pandit et al., 2019; Prakash and Verma, 2019). The CF metric also incorporates ecological and biological aspects, such as gonad development, fitness, and the fish's adaptability to environmental conditions (Keyombe et al., 2017; Tadesse et al., 2024). Additionally, physiological factors such as maturity, reproductive cycles, water quality, and food availability may influence CF (Ujjania et al., 2012). Typically, CF values > 1 indicate favorable environmental conditions and adequate feeding (Ajibare et al., 2020). The present study also showed CF values for both tilapia species exceeded one, suggesting overall good health. These findings align with previous studies that reported similar trends for *O. niloticus* in Lake Naivasha, Kenya, and *O. mossambicus* and *C. rendalli* in Hout River, Inanda and Nagle dams (Sara et al., 2014; Keyombe et al., 2017; Hlatshwayo et al., 2024; Misra et al., 2024).

Although CF is a valuable component, this study revealed certain limitations in its application. While it remains a dependable measure for assessing the general condition of fish populations in environmental and fisheries studies (Uddin and Ghosh, 2021; Usman et al., 2024), and serves as an indicator of metabolic status (Khan et al., 2020), it proved to be inadequate for effectively responding to pollution level for the two tilapia populations in the Shongweni Dam. This limitation may stem from the fact that it responds late to pollution, hence, a need for it to be applied with other indices and microscopic analyses of tissues.

Another important index used in fish health studies is the HSI. The HSI for fish inhabiting a good-quality water may range from 1 to 2% (Wagenaar and Barnhoorn, 2018). In the present study, HSI values for *C. rendalli* were notably lower than the typical range of 1 to 2 reported for freshwater carp and tilapia species in good-quality water (Wagenaar and Barnhoorn, 2018). In contrast, most *O. mossambicus* remained within this range. Low HSI values may signify depleted energy reserves in the liver due to prolonged stress, as suggested by Aly and Abouelfald (2020). This depletion could arise from the physiological demands of coping with environmental challenges, such as metal contamination. Similar findings were documented in *Oreochromis niloticus* exposed to metals, where stress-induced energy utilization resulted in HSI values below 1 (Çiftiç et al., 2015). However, the reliability of HSI as a biomarker for water pollution remains subject to debate. Barišić et al. (2018) and Yancheva et al. (2022) emphasize that HSI values are modulated by a complex interplay of factors beyond contaminant exposure, such as seasonal variations, food availability, and reproductive cycles. Nevertheless, the HSI observed for both species was within the normal range and no seasonal variation was observed.

The gonadosomatic index (GSI) showed relatively lower values for *O. mossambicus* than *C. rendalli*, although no significant differences were observed between species or across seasons. This marked decrease in GSI values aligns with findings from other studies on freshwater fish species exposed to metal contaminants (Singh and Srivastava, 2015; Dewi and Probowo, 2017; Pandit et al., 2019). Similarly, decreased GSI values have been documented in freshwater species inhabiting in aquatic systems contaminated with industrial effluents and organic pollutants (Gilroy et al., 2012; Kaptaner et al., 2016). Notably, the mean GSI was significantly lower in males than females. Furthermore, prolonged exposure to pollutants has been reported to impair the development of reproductive organs, contributing to reduced GSI values (Pieterse, 2004). Therefore, the reduced GSI values in both tilapia species may be linked to pollution in the dam. Moreover, the lack of significant differences between *C. rendalli* and *O. mossambicus* indicates similar metabolic capacity and reproductive success, and that both species are equally impacted by pollutants.

Health assessment index (HAI) proved to be an important tool that can be used as the first screening for water quality condition (Adams et al., 1993; Avenant-Oldewage, 2009). Organs such as liver and gills are known to respond rapidly to water pollution due to their location and role in metabolism (Roberts, 2012). In the present study, high anomaly scores were observed in the gills and liver of *O. mossambicus* and *C. rendalli*. Liver anomalies included nodules and general discoloration, while common gill anomalies included excess mucus, clubbed and paleness. Moderate lesions were identified in the ovaries and testes, with no abnormalities detected in the kidneys or spleen. The HAI showed no significant differences between the two species and across seasons. The mean HAI for *C. rendalli* was comparable to values reported for *Clarais gariepinus* from Luphephe-Nwanedi Dams (Madanire-Moyo et al., 2012). In contrast, both *O. mossambicus* and *C. rendalli* exhibited lower mean HAI compared to those reported in freshwater fish species from Loskop Dam, Mamba River, Balule River, and Hout River (Watson et al., 2012; Sara et al., 2014). Furthermore, the HAI values observed in this study were lower than those reported for fish from Flag Boshielo and Return Water Dams (Madanire-Moyo et al., 2012). These findings suggest that both tilapia species exhibited a comparatively better health, potentially indicative of less severe environmental stress or lower pollution levels in the dam compared to these other water bodies. While the findings suggest relatively good health for fish in Shongweni Dam, the influence of pollutants and ecological conditions warrants further investigation.

### 4.4.3 Gill histopathology

The gill's proximity to the aquatic environment makes its role in assessing pollution indisputable. Most studies showed that gills respond rapidly to waterborne pollutants (Jabeen et al., 2018; Shahid et al., 2022). Among the lesions observed in the present study, the gill exhibited more pronounced damage compared to other tissues, with regressive changes being the most prominent. However, there was no significant variation in the severity of lesions between the two species and between seasons. The gills of fish, due to their direct contact with the external environment, are highly sensitive indicators of water quality (Getnet et al., 2024). Pollutant exposure often leads to significant histopathological changes that impair gill functioning (Shahid et al., 2022). In the present study, gill lesions such as hemorrhages, epithelial lifting, hyperplasia, aneurysms, and edema were prominent in *O. mossambicus* and *C. rendalli*. These lesions compromise the respiratory and osmoregulatory functions of the gills, as highlighted by Roberts (2012). Similar histopathological lesions, such as necrosis, sinusoidal dilation, and epithelial damage, have been widely reported in freshwater fish exposed to metal contamination and pollutants from industrial and sewage effluents (Jabeen et al., 2018; Shahid et al., 2022; Getnet et al., 2024).

Hyperplasia, a reversible adaptation to increase the protective barrier between the environment and blood, reflects a defense mechanism against moderate pollutants (Guevarra et al., 2020). However, irreversible lesions like aneurysms are indicative of severe stress and severely impair respiratory efficiency (Hassaninezhad et al., 2014; Abalaka, 2017). Edema and other degenerative changes in fish gills have been consistently associated with exposure to waterborne toxins and heavy metals, as seen in studies across varied geographies, including Portugal, where Zn and Pb were implicated (Fonseca et al., 2016), and in Lake Nasser, where fertilizers and salts contributed to observed damage (Ahmed et al., 2020). Furthermore, lesions identified in this study were similar to those reported in fish exposed to microcystin, a highly toxic hepatoxin frequently linked to algal blooms in hyper-eutrophic systems (Gupta and Guha, 2006; Nascimento et al., 2012; Wagenaar and Barnhoorn, 2018). It is evident that gill is susceptible to water pollution due to its location relative to the external water environment. Moreover, there was no substantial difference with regard to gill pathology between species, however, it is essential to closely monitor the condition of fish as the dam remains a repository for contaminants from the upper uMlazi River catchment.

#### 4.4.4 Liver histopathology

The liver, as a central metabolic organ responsible for processing chemical pollutants, is particularly vulnerable to contamination (Waweru et al., 2024). In this study, liver histopathology revealed changes such as blood sinusoid congestion, vacuolization, fibrosis of blood sinusoids, melanomacrophages, hepatocellular pleomorphism, and nucleolus necrosis. These findings are consistent with those observed after heavy metal exposure (Cuevas et al., 2016; Mahboob et al., 2020). The observed liver vacuolization is likely to be linked to pollutant exposure, as reported in similar studies (Luki et al., 2011; Hussain et al., 2021). Additionally, fibrosis, necrosis, and hemorrhage observed in this study, align with findings observed in metal and nutrient-polluted aquatic ecosystems (Tayel et al., 2018; Ahmed et al., 2020; Waweru et al., 2024).

Melanomacrophages, which were prominent in the liver, are commonly associated with environmental stressors, including pollutant exposure, disease, age, and starvation (Waweru et al., 2024). These cellular responses have also been linked to pathogenic bacteria, such as *Aeromonas hydrophila* in *O. niloticus* (Azadbakht et al., 2018; Waweru et al., 2024), and to elevated heavy metals, including manganese (Mn) (Alm-Eldeen et al., 2018). Feist et al. (2015) and Wagenaar and Barnhoorn (2018) observed hepatocellular pleomorphism in *Clarias gariepinus*, exposed to metal pollutants and nutrient enrichment. Similar lesions, including vacuolization, hyperplasia, aneurism, and epithelial lifting, were reported in *Labeo rasae* from Loskop Dam, which receives effluents from agricultural activities, metallurgic industries, and mining in the upper Olifants River catchment (Oberholster et al., 2017; Lebepe et al., 2020). These findings provide a comparable scenario to Shongweni Dam, which likely receives contaminants from its deteriorating catchment area and surrounding anthropogenic activities. Despite pathologies observed in the liver of both species, the severity is still moderate, hence, not influencing the functioning of the organ. Nevertheless, fish health should be monitored to ensure that any drastic deviation from the current condition is detected before gross changes manifest.

### 4.4.5 Gonads (ovaries and testes) histopathology

Gonads are sensitive organs that showed significant histopathological lesions from pollutant exposure (Zulfahmi et al., 2018; Garriz et al., 2019). Pollutants significantly impact the reproductive health and gonadal structures of fish, leading to impaired spermatogenesis and disruptions in lobular architecture (Sionkowski et al., 2017; Bhat et al., 2022). Moreover, pollutants compromise reproductive health in various ways including, decreased oocyte diameter (Alquezar et al., 2006), hormonal dysfunction (Ebrahimi and Taheriandfard, 2011), reduced gonadosomatic indices (Gerbron et al., 2014), altered reproductive behavior (Bertram et al., 2015), and increased larval abnormalities (Sfakianakis et al., 2015; Zhang et al., 2016). Severe pathological alterations in fish gonads, such as masculinization, sperm duct enlargement, interstitial tissue changes, and basal membrane detachment in testes, along with atretic oocytes in ovaries, have been documented in response to pollutant exposure (Luzio et al., 2016).

The present study recorded ovarian lesions in *O. mossambicus* and *C. rendalli*, including oocyte atresia, perifollicular cell hyperplasia, ovarian edema, and vacuolization. These findings align with earlier studies on species such as *Clarias gariepinus* and *Labeo rohita*, which showed disrupted oocyte development stages and atresia, indicative of endocrine disruption (Mehta et al., 2015; Abdel-Kader and Mourad, 2019; Okito et al., 2023). Atresia, an irreversible degenerative process, compromises reproductive success and is often linked to chemical and heavy metal toxicity (Soler et al., 2020; Ghosh et al., 2022). Additionally, hyperplasia of follicular cells and vacuolization observed in polluted waters reflect the direct impact of industrial and domestic effluents on fish’s reproductive health (Wahbi and El-Greisy, 2007). Such disruptions, as reported in diverse environments like the Harike Wetland (Jangu et al., 2020) and Verinag (Bhat et al., 2023), underscore the widespread threat posed by metal contamination to aquatic ecosystems.

The testes of *O. mossambicus* and *C. rendalli* from polluted waters of Shongweni exhibited impaired spermatogenesis, along with disruptions in lobular structure and basal membrane, consistent with previous findings by Elgaml et al. (2019). Comparable pathologies including interstitial fibrosis, vacuolation, and basal membrane detachment, were noted in *Tilapia guineensis* from Lake Nokoué and were linked to organic and inorganic pollutants, including toxic metals (Prudencio et al., 2023). Additionally, melanomacrophage centers, indicative of immune responses to pollution, were reported in *Clarias gariepinus* from Orlando Dam, a system heavily impacted by sewage discharges and mining activities (Bengu et al., 2017).

The findings reveal that Shongweni Dam is significantly affected by pollutants, including heavy metals and nutrients, likely originating from domestic, agricultural, mining, and industrial activities within the uMlazi River catchment or its surrounding areas. Moreover, they shed light on the widespread effects of pollution on fish health and emphasize the pressing need for strategies to mitigate contamination in aquatic ecosystems to protect fish populations and maintain the ecological functioning of the system.

# Chapter 5

## Metal accumulation in the muscle of *Oreochromis mossambicus* and *Coptodon rendalli* and health risk assessment

### 5.1 Introduction

Metal pollution in aquatic ecosystems has emerged as a global environmental issue due to its ecological and human health impacts (Kumar et al., 2017; Ali et al., 2020). Metals enter water bodies through various natural and anthropogenic processes (Ali et al., 2019), with rapid industrialization and urbanization exacerbating the release of industrial effluents and municipal discharges (Panda et al., 2023; Sharma et al., 2023). Such pollutants are concerning due to their persistence, toxicity, and capacity to accumulate in aquatic organisms (Saeed et al., 2020). Some metals biomagnify up the food chain, resulting in lethal doses in top predators (Rahman et al., 2013). In aquatic systems, fish are among the top predators and they get highly affected by metal contamination (Raknuzzaman et al., 2016). As a result, fish are regarded as valuable bioindicators of metal contamination (Ahmed et al., 2015; Alipour et al., 2015). Route of metal exposure to fish include through gills as they are in close contact with the water, through diet when feeding on contaminated food and through the skin (Eltholth et al., 2018; Rakocevic et al., 2018).

Despite fish accumulating metals, they are also crucial to human diets, providing essential lipids, proteins, minerals, and nutrients that help combat micronutrient deficiencies (FAO, 2020). Additionally, consuming high-quality fish has health benefits including reduced hypertension, diabetes, and coronary heart diseases as well as promoting healthy brain development in humans (Christensen et al., 2016; Eltholth et al., 2018). Therefore, consuming fish could be beneficial to humans but it can also serve as a route for metal exposure. Consuming fish from contaminated water bodies can pose significant health risks to humans, including neurological disorders, and renal and reproductive issues, particularly from metals such as As, Cd, and Pb, which are harmful even at low concentrations (WHO, 1993; Zhou et al., 2016; Ray and Vashishth, 2023). Other metals such as Fe, Mn, Cu, and Zn, although beneficial in moderate levels, can adversely affect human health when consumed in excess (Abbaspour et al., 2014; Prashanth et al., 2015).

According to Li et al. (2013), consuming metal-contaminated fish can present carcinogenic or non-carcinogenic risks. Consumption of fish with elevated levels of Cd, Cr and Pb has been linked to liver, kidney, and neurological disorders (Taweel et al., 2013; Ezemonye et al., 2019; Budi et al., 2024). Due to increasing metal pollution levels in freshwater bodies, there is a dire need to determine whether aquatic resources used as food are safe for human consumption. The Target Hazard Quotient (THQ) is widely used to assess the risk of metal exposure through contaminated fish, offering a measure of non-carcinogenic risks from fish consumption (USEPA, 2000; 2012; Ramish et al., 2024). Numerous studies showed that consuming fish from polluted water bodies is not safe for human health (Rahman et al., 2012; Alipour et al., 2014; Zhu et al., 2015; Lynch et al., 2016; Sara et al., 2017; Lebepe et al., 2020; Mannzhi et al., 2021; Misra et al., 2024). It is, therefore, necessary to determine the edibility of fish from water bodies used by humans for their livelihood.

In South Africa, rural communities residing in close proximity to rivers often depend on fish for dietary protein (Ellender et al., 2009; McCafferty et al., 2012; Morand et al., 2012). Various rivers have been studied across the country i.e. the Vaal (Crafford and Avenant-Oldewage, 2010), Olifants (Addo-Bediako et al., 2014; Jooste et al., 2014), Luvuvhu (Mannzhi et al., 2021), and uMgeni (Misra et al., 2024) rivers which are impacted by numerous anthropogenic activities such as wastewater works, acid mine drainage, industrial and agricultural activities. It is evident that the problem could be extending to many other rivers that are impacted by similar anthropogenic activities; therefore, knowing metal concentrations in fish that are used for human diet is crucial in safeguarding the health of communities, hence, enhancing food security (Li et al., 2015)

KwaZulu-Natal is a developing province with a high aggregation of industries and agricultural activities, however, its waterbodies have received little attention with regard to pollution studies. Shongweni Dam, an impoundment in the uMlazi River has received little attention despite its role as a repository for pollutants from the upper reach and its capacity to provide fish to local communities. *Oreochromis mossambicus* and *C. rendalli* are the commonly consumed fish in this dam, it is therefore, imperative to determine whether they are safe for human consumption. This study aims to assess metal concentrations in the muscle and evaluate the potential non-carcinogenic and carcinogenic health risks associated with their consumption. Given that the dam receives effluents from the wastewater work, it was hypothesized that metal concentrations in muscle tissue would exceed the limit for safe consumption.

## 5.2 Methods and Materials

### 5.2.1 Fish sampling

Fish were collected from the Shongweni Dam during dry and wet seasons using an electro-shocker and gill net. Two fish species, *O. mossambicus* (n = 16) and *C. rendalli* (n = 14), were collected and euthanized. Each fish was ventrally dissected, and a muscle sample was cut out, wrapped with foil, and stored in dry ice. Upon return to campus, muscle tissue samples were transferred to the -80$^0$C fridge until processing.

### 5.2.2. Laboratory analysis

Approximately 0.2 g of muscle tissue was oven-dried for 48 hours at a temperature of 100$^0$C. Following a 48-hour drying period, the dry sample was crushed and homogenized with a porcelain mortar and pestle. Powdered samples were transferred into 250 ml beakers and digested using Aqua regia solution (3HCl: $1HNO_3$). The combination was subsequently heated at a moderate temperature for one hour and/or until the solution got to 20 ml. Following full digestion, samples were then cooled and vacuum filtrated using a 0.45 µm membrane. The filtered solution was transferred to a 250 ml volumetric flask and topped to mark with distilled water. Then 50 ml of diluted solution was syringe filtered into a 50 ml vial for analysis and stored in the fridge until analyses. The metals, As, Sb, Cd, Cr, Fe, Mn, Pb, Se, and Sr were analyzed using ICP-OES at the Water Lab. The analysis was carried out with blanks and certified reference materials for quality assurance.

### 5.2.3 Bioaccumulation Factor (BAF) of metals in muscle tissue of selected fish

Bioaccumulation factor (BAF) was computed to evaluate the changes in metal levels as time progressed. BAF is determined by comparing metal levels in organisms to metal levels in water and sediment, through the following formula:

$$BAF = \frac{metal\ conc.in\ muscle\ (mg.kg)}{metal\ conc.in\ sediment\ (mg.kg)} \text{.....................................................Equation 5.1}$$

A BAF value below 1000 indicates no possibility of bioaccumulation in the organism, from 1000 and 1500 it is considered bio-accumulative, and values higher than 1500 are classified as very bio-accumulative (Arnot and Gobas, 2006).

### 5.2.4 Health Risk Assessment

A risk assessment on human health was carried out using the desktop approach developed by the United States Environmental Protection Agency (USEPA, 2000). This is calculated based on the estimation that an adult weighing 70 kg consumes 0.15 kg of fish muscle portion once per week. Chronic non-carcinogenic calculates the target quotient hazard (THQ) and carcinogenic cancer (CR) health risks were assessed:

$$THQ = \frac{C\times IRF\times EF\times ED}{RfD\times BW\times AT}$$ ......................................................................Equation 5.2

$$CR = \frac{C\times IRF\times EF\times ED\times OSF}{RfD\times BW\times AT}$$ ......................................................................Equation 5.3

In the targeted tissue, C represents the metal level (mg/kg), IRF is the fish consumption rate (150 g), EF is the exposure frequency (365 days per year), and ED is the duration of exposure (30 years for non-cancer risk according to USEPA, 2011). RfD represents the oral reference dose (mg kg/day). RfD levels published by the USEPA (2013, 2015) were used. BW is the body weight (70 kg), and the average time of assessment abbreviated as AT (70 years *365 days per year). The oral slope factor (OSF), representing the risk per unit exposure (mg/kg/day), is sourced from the Integrated Risk Information System for carcinogenic metals Pb ($8.5 \times 10^{-3}$), Cd (15), Cr (0.5), and As (1.5). Data for other metals is unavailable.

Non-carcinogenic risk was assessed by determining if the THQ value exceeds 1. The THQ > 1 indicates an acceptable risk that has a negative effect on human health but is not cancer-causing. Nevertheless, THQ < 1 is within the acceptable limit indicating a minimal or almost negligible impact on human health risk (Alipour et al., 2015).

Carcinogenic risk refers to the likelihood of an individual acquiring cancer over a lifetime due to exposure to a potential carcinogen (Ullah et al. 2017; Zhong et al., 2018). Acceptable lifetime CR levels range $10^{-4}$ (1 in 10,000 chance) and $10^{-6}$ (1 in 1,000,000 chance).

### 5.2.5 Statistical Analysis

Statistical analyses were carried out using R-3.1.1 statistical software (R Development Core Team). Normality of the data was conducted with a Shapiro-Wilk test, and homogeneity of variance was tested with a Levene's test. To assess whether metal content in the fish muscle varied significantly between fish species and seasons independent sample t-test. Data were statistically significant at $p < 0.05$. Box-and-whisker plots were drawn for metal concentrations in fish muscle at the Shongweni impoundment using R-3.1.1. Non-metric multidimensional scaling (NMDS) (Clarke and Warwick, 2001) was performed to visualize metal concentrations in fish muscle from the impoundments and species. Pearson and/or Spearman's correlation test were employed to assess the relationship between fish length and metal accumulation, and inter-metal relationships.

A distance-based test of homogeneity of multivariate dispersion and multiple analysis of variance (Anderson 2001a, 2001b) were performed to evaluate whether there is a statistical difference in the muscle metal concentration of *C. rendalli* and *O. mossambicus* in the Shongweni Dam using the betadisper and Adonis functions in VEGAN.

## 5.3 Results

### 5.3.1 Metal concentration in muscle tissue between species

Average metal accumulation in the muscle tissue of *C. rendalli* followed descending order: Fe (99.08mg/kg) > Cr (19.43mg/kg) > Mn (2.9mg/kg) > Sr (2.84mg/kg) > Pb (0.57 mg/kg) > Se (0.21 mg/kg) > Sb (0.13 mg/kg) > As (0.10 mg/kg) > Cd (0.00 mg/kg) and Fe (73.92 mg/kg) > Sr (3.48 mg/kg) > Cr (3.25 mg/kg) > Mn (1.44 mg/kg) > Pb (0.54 mg/kg) > Se (0.39 mg/kg) > Sb (0.11 mg/kg) > As (0.10 mg/kg) for *O. mossambicus* (Table 5.1). Among all the metals, Fe was the highest and Cd the lowest in both fish species.

**Table 5.1**: Metal concentrations (mg/kg dry wt; mean ± standard deviation) in tilapia fish collected from the Shongweni Dam in 2021 and permissible limits.

| **Metals** | ***C. rendalli*** | ***O. mossambicus*** | **Permissible limits** |
|---|---|---|---|
| **Sb** | 0.139±0.046 | 0.114±0.038 | 1.5 (FAO, 1983) |
| **As** | 0.104±0.009 | 0.100±0.007 | 2 (JECFA, 2002) |
| **Cd** | 0.002±0.001 | ND | 0.17 (FAO, 1983) |
| **Cr** | 19.756±4.059 | 3.250±0.941 | 2 (MOHSAC, 2006) |
| **Fe** | 98.145±23.924 | 73.921±12.941 | 333.30 (Mokhtar et al., 2009) |
| **Mn** | 2.899±0.899 | 1.435±0.433 | 3.52 (IAEA, 2003) |
| **Pb** | 0.538±0.163 | 0.536±0.128 | 0.02 (FAO, 2003) |
| **Se** | 0.208±0.120 | 0.386±0.311 | 1 (MOHSAC, 2006) |
| **Sr** | 2.654±1.687 | 3.479±0.703 | — |

ND= not detected, below detected levels in the muscle tissue

Cadmium was below the detection limit for *O. mossambicus* whereas a notable concentration was observed for *C. rendalli* (Figure 5.1). There was no significant difference observed in Sb ($W = 61.5$, $p > 0.05$), Pb ($W = 118$, $p > 0.05$), Sr ($W = 77.5$, $p > 0.05$), and Se ($W = 72$, $p > 0.05$) between species. However, Fe ($W = 177$, $p < 0.05$) and Cr ($W = 210$, $p < 0.05$) showed a significant difference between species with *C. rendalli* exhibiting relatively higher concentrations (Figure 5.1). Arsenic showed no significant difference between species ($W = 100$, $p > 0.05$). In contrast, a significant difference was observed for Mn between species ($W = 30$, $p < 0.05$) with *C. rendalli* exhibiting higher concentrations (Figure 5.1).

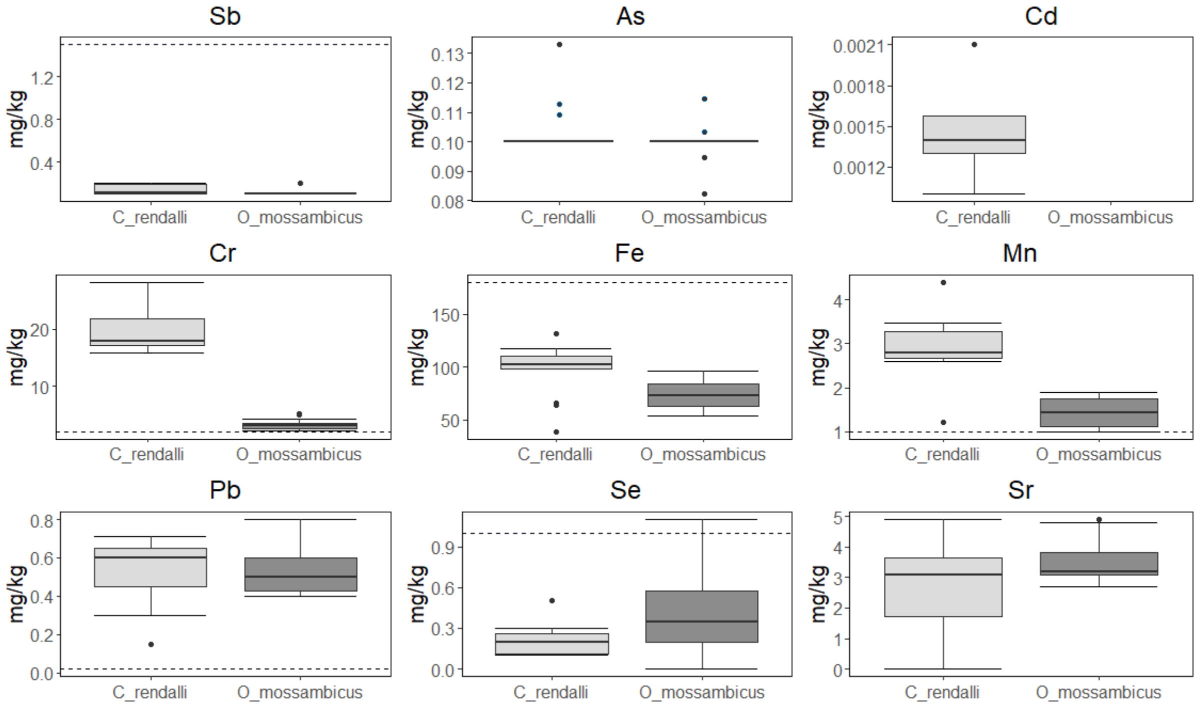


**Figure 5.1:** Box and whisker plots indicating metal concentrations in the muscle tissue of *Coptodon rendalli* and *Oreochromis mossambicus* from the Shongweni Dam.

The non-metric multidimensional scaling also showed a clear separation between species (MANOVA, $p < 0.001$) (Figure 5.2). Moreover, dispersion results also showed a significant difference (Permidisp, $p < 0.05$) with average distances to the median being 0.91 for *C. rendalli* and 0.65 for *O. mossambicus*.

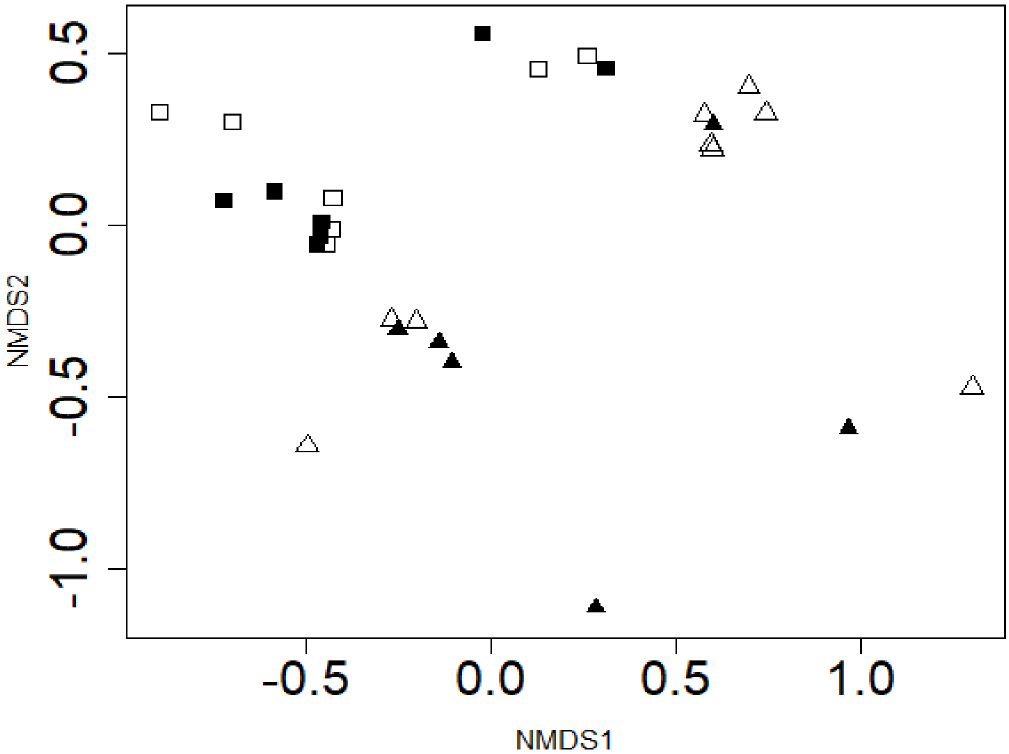


**Figure 5.2:** Non-metric multidimensional scaling presenting metal concentrations with seasons and between species (*Oreochromis mossambicus*, low flow (□) and high flow (■); *Coptodon rendalli* (Δ) and high flow (▲)).

5.3.2 Metal concentration in fish muscle between seasons

During the wet season, metal concentrations in *C. rendalli* followed descending order: Fe > Cr > Mn > Sr > Pb > Se > Sb > As > Cd and Fe > Cr > Sr > Mn > Pb > Se > Sb > As > Cd during dry (Figure 5.2). Descending rank for metal concentration in the muscle tissue of *O. mossambicus* was Fe > Cr > Sr > Mn >Pb > Se > As >Sb during the wet season and Fe > Sr > Cr > Mn > Se > Pb > Sb > As during the dry season (Figure 5.6). *Oreochromis mossambicus* showed no seasonal variation for Sb ($W = 12$, $p > 0.05$), As ($W = 13.5$, $p > 0.05$), Cr ($W = 9.5$, $p > 0.05$), Fe ($W = 20$, $p > 0.05$), Mn ($W = 20$, $p > 0.05$) and Sr ($W = 18$, $p > 0.05$) whereas seasonal variation was observed for Pb ($W = 7.5$, $p < 0.05$) and Se ($W = 47.5$, $p < 0.05$). Cadmium was below the detection level for *O. mossambicus* in both seasons while no seasonal variation was observed for *C. rendalli* for all metals.

### 5.3.3 Inter-metal and metal-fish length correlation

Figures 5.3 and 5.4 are showing correlation coefficients with the significance denoted by a red asterisk on top of the value. Weak relationships were observed for Sb-As ($r = 0.17$, $p > 0.05$), As-Cr ($r = 0.27$, $p > 0.05$), As- Fe ($r = 0.31$, $p > 0.05$), As-Pb ($r = 0.22$, $p > 0.05$), As- Sr ($r = 0.32$, $p > 0.05$), Cd-Pb ($r =0.32$, $p > 0.05$), Cr-Fe ($r = 0.33$, $p > 0.05$), Fe-Pb ($r = 0.22$, $p > 0.05$), Mn-Pb ($r = 0.24$, $p > 0.05$), Mn-Se ($r = 0.39$, $p > 0.05$), and Pb-Sr ($r = 0.12$, $p > 0.05$) in the muscle of *C. rendalli* (Figure 5.3). *Oreochromis mossambicus* exhibited weak positive relationships for all metals with the exception of Fe-Mn ($r = 0.57$, $p < 0.05$), Cr-Sr ($r = 0.41$, $p < 0.05$), Fe-Sr ($r = 0.43$, $p < 0.05$), Fe-Pb ($r = 0.40$, $p < 0.05$), Mn-Sr ($r = 0.41$, $p < 0.05$) (Figure 5.4). There were no relationships observed for Cd with other metals in the muscle tissue of *O. mossambicus* (Figure 5.4). Moreover, the length of *O. mossambicus* showed relatively weak relationships for all metals.

Muscle tissue of *C. rendalli* showed negative weak relationships for Sb-Se ($r = -0.27$, $p > 0.05$), Sb-Sr ($r = -0.21$, $p < 0.05$), Cd-Sr ($r = -0.32$, $p > 0.05$), Cr-Pb ($r = -0.18$, $p > 0.05$), Cr-Se ($r = -0.19$, $p > 0.05$), Fe-Mn ($r = -0.17$, $p > 0.05$), Fe-Se ($r = -0.15$, p >), Fe-Sr ($r = -0.20$, $p > 0.05$), Pb-Se ($r = -0.14$, $p > 0.05$) (Figure 5.3). A significant strong negative relationship was observed in *C. rendalli* for Cd-Se ($r = -0.95$, $p < 0.05$), while other metals showed moderate negative relationships; Sb-Mn ($r = -0.47$, $p < 0.05$), Sb-Pb ($r = -0.48$, $p < 0.05$), As-Mn ($r = -0.41$, $p < 0.05$), Cd-Mn ($r = -0.50$, $p < 0.05$) and Cr-Mn ($r = -0.55$, $p < 0.05$) (Figure 5.3). Moreover, a significant positive relationship was observed for Sb-Cd ($r = 0.95$, $p < 0.05$). Additionally, the length of *C. rendalli* exhibited moderate significant relationships for Sb ($r = 0.62$, $p < 0.05$) and Pb ($r = 0.48$, $p < 0.05$), and weak relationships with other metals (Figure 5.3).

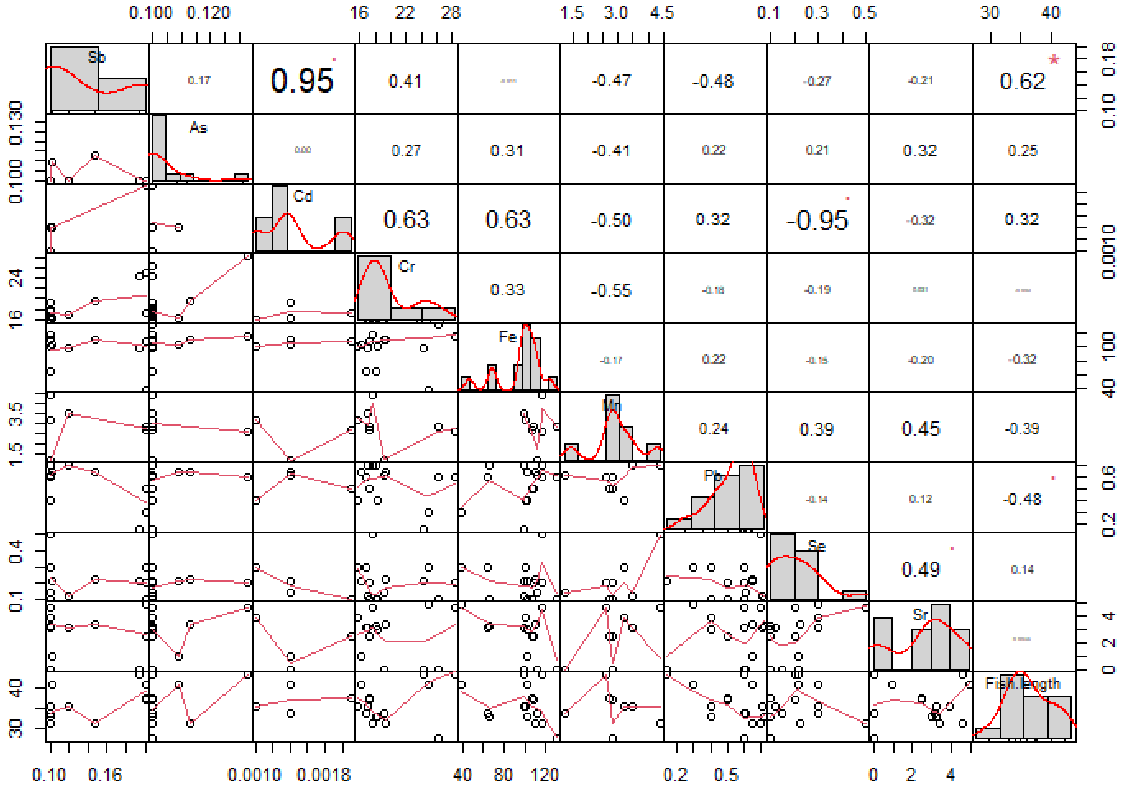


**Figure 5.3:** Inter-metal and metal-length correlation of the muscle tissue for *Coptodon rendalli* from the Shongweni Dam.

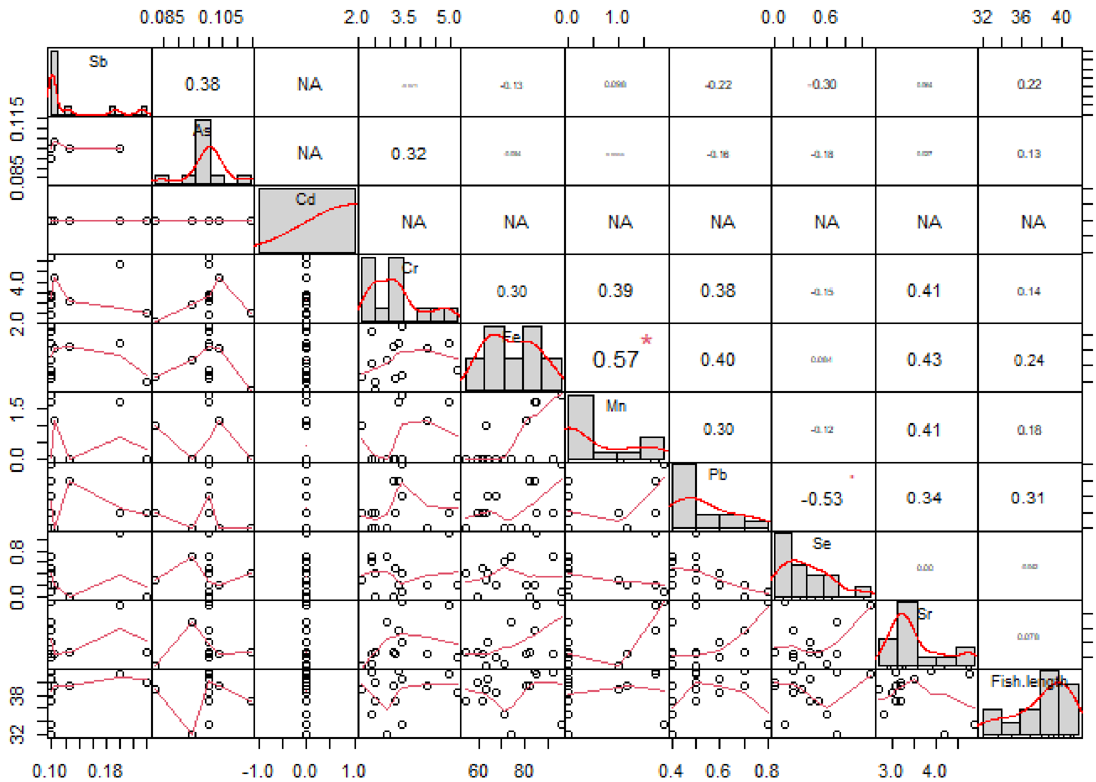


**Figure 5.4:** Inter-metal and metal-length correlation of the muscle tissue for *Oreochromis mossambicus* from the Shongweni Dam.

### 5.3.4 Bioaccumulation factor

The bioaccumulation factor for organ-water ($BAF_W$) was not determined since most metals were below the detection limit. However, BAF for organ-sediment ($BAF_S$) was calculated but not for Cd and Cr as these elements were undetected in the sediment (Figure 5.5). $BAF_S$ followed a descending order: Sb (26.023) > Sr (15.783) > As (6.172) > Pb (4.355) > Mn (0.664) > Fe (0.658) for *C. rendalli* and Sb (21.429) > Sr (20.685) > As (5.913) > Pb (4.338) > Fe (0.496) > Mn (0.329) for *O. mossambicus*. The $BAF_S$ values were below 1 for Fe and Mn but relatively greater than 1 for other metals in both species (Table 5.2). Moreover, there was no significant difference in $BAF_S$ between species and season (Mann U, $p > 0.05$).

**Table 5.2**: Bioaccumulation factor of tissue-sediment ($BAF_s$) recorded for *Coptodon rendalli* and *Oreochromis mossambicus* from the Shongweni Dam during 2021 survey.

| **Metals** | ***C. rendalli*** | ***O. mossambicus*** |
|---|---|---|
| **Sb** | 26.023 | 21.429 |
| **As** | 6.172 | 5.913 |
| **Cd** | NC | NC |
| **Cr** | NC | NC |
| **Fe** | 0.658 | 0.496 |
| **Mn** | 0.664 | 0.329 |
| **Pb** | 4.355 | 4.338 |
| **Se** | NC | NC |
| **Sr** | 15.783 | 20.685 |

NC= not calculated due to low or undetected levels in the medium/muscle tissue

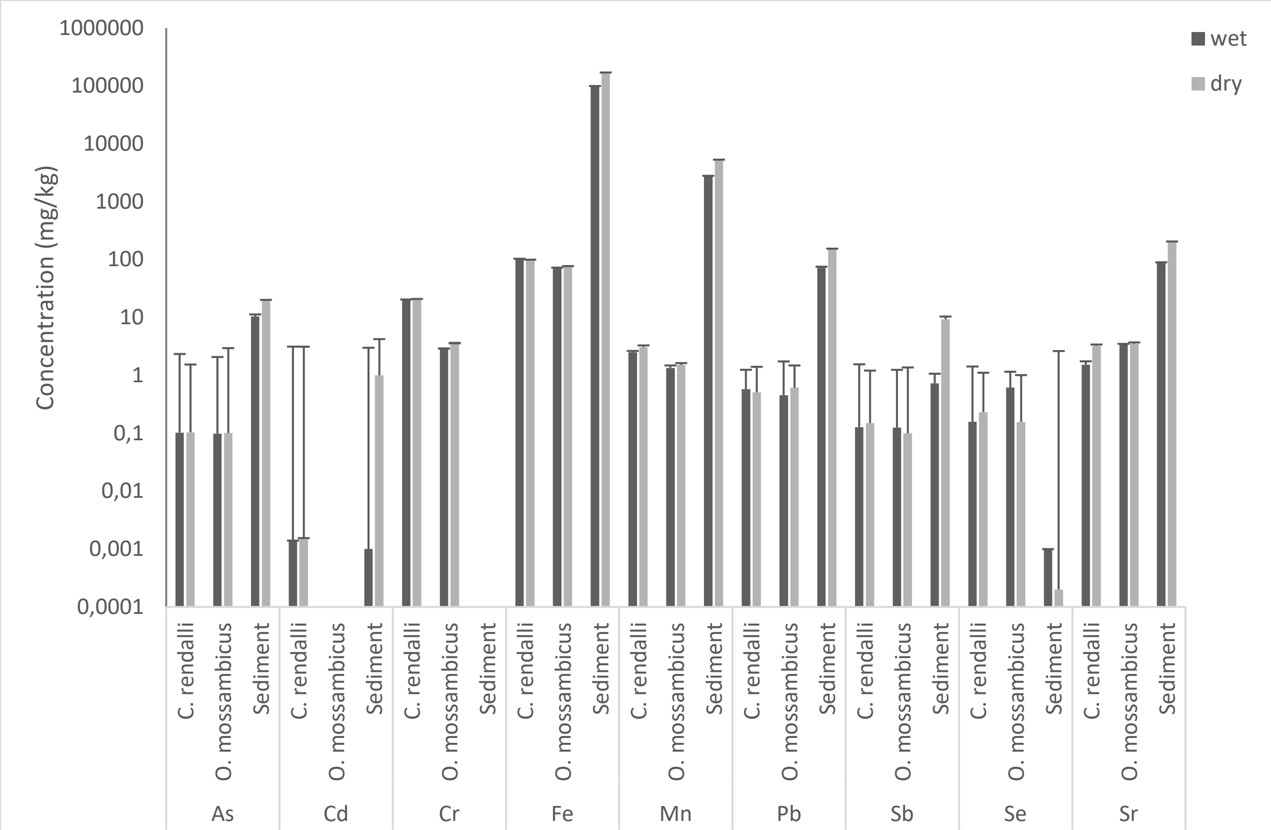


**Figure 5.5:** Mean metal concentrations (mg/kg) recorded in sediment and muscle tissue of *Coptodon rendalli* and *Oreochromis mossambicus* sampled at the Shongweni Dam during dry and wet seasons in 2021.

### 5.3.5 Non-carcinogenic and carcinogenic risk assessment

The THQs of *C. rendalli* and *O. mossambicus* showed potential health risks (>1) for Cr (Table 5.3). Hazards for adults were determined based on the assumptions from the USEPA where an adult weighing approximately 70 kg is assumed to consume 150 g of fish daily for 7 days per week. Lead and Cr showed THQ > 1 for 100% of the sampled individuals of *C. rendalli* and *O. mossambicus*, and other metals, including As, Sb, Fe, Mn, Sr, and Se had THQ < 1. However, As and Sb showed the THQs of 0.48 and 0.59, respectively.

The mean THQ values followed the descending order: Pb (18.332)> Cr (11.900)> As (0.592)> Sb (0.483)> Fe (0.257)> Se (0.076)> Mn (0.022)> Sr (0.009) > Cd (0) for *C. rendalli* and Pb (17.263)> Cr (1.990)> As (0.522)> Sb (0.262)> Fe (0.194)> Se (0.14)> Sr (0.011)> Mn (0.005)>

Cd (0) for *O. mossambicus* (Table 5.3). The THQs of most metals showed no statistical significance between the tilapia species ($F = 12.163$; $p > 0.05$), however, *C. rendalli* showed a significantly higher Cr THQ compared to *O. mossambicus* ($p < 0.05$). In contrast, Cr THQ showed no significant difference between species ($p > 0.05$) (Table 5.3). The CR for *O. mossambicus* were $7.84 \times 10^{-1}$, $9.95 \times 10^{-1}$, $1.47 \times 10^{-1}$, and 0 for As, Cr, Pb, and Cd, respectively. For *C. rendalli*, the CR values were $8.89\times 10^{-1}$ for As, $3.87\times 10^{-3}$ for Cd, $1.55\times 10^{-1}$ for Cr, and $5.59 \times 10^{1}$ for Pb.

**Table 5.3**: Target Hazard Quotient (THQ) for trace metals found in muscle tissue of *Coptodon rendalli* and *Oreochromis mossambicus* from the Shongweni Dam in 2021.

| Metals | *C. rendalli* | *O. mossambicus* |
|---|---|---|
| **Sb** | 0.483±0.318 | 0.262±0.298 |
| **As** | 0.592±0.180 | 0.522±0.225 |
| **Cd** | 0 | 0 |
| **Cr** | 11.900±2.457 | 1.990±0.576 |
| **Fe** | 0.257±0.065 | 0.194±0.034 |
| **Mn** | 0.022±0.021 | 0.005±0.009 |
| **Pb** | 18.332±4.103 | 17.263±4.116 |
| **Se** | 0.076±0.042 | 0.142±0.114 |
| **Sr** | 0.009±0.005 | 0.011±0.002 |

## 5.4 Discussion

### 5.4.1 Metal concentrations in muscle tissue between species

The accumulation of metals in freshwater fish poses a major risk to both the environment and human health (Ali and Khan, 2018). Fish absorb these metals from water, sediments, and their diet, leading to accumulation in tissues, particularly muscle tissue, which is the primary portion consumed by humans. The dynamics of bioaccumulation are governed by factors such as habitat quality, the chemical properties of metals, and of particular interest, species and feeding habits (Javed and Usmani, 2011; Yi et al., 2017; Luo et al., 2020; Khan et al., 2021). In the present study, the two species occupy the same level of the food chain and their potential to accumulate metals is likely to not differ. The two species showed notable concentrations of Sb, Fe, Cr, As, Pb, Cd, Mn, Se, and Sr. Moreover, Cr, Fe, and Mn have even shown a significant difference between the two species. Some individuals showed undetectable concentrations for metals such as Cd, As, Sb, and Mn. Although all fish were exposed to the same water, other underlying factors can affect metal accumulation in aquatic ecosystems. Factors such as age, size, reproductive cycle, seasonality, pH, pollution levels, temperature, etc. were reported to influence metal accumulation in fish (Maurya and Malik, 2016; Karunanidhi et al., 2017; Kortei et al., 2020).

Antimony is one of the problematic metals in water bodies impacted by industrial and mining activities (Jooste et al., 2015). In the present study, Sb was below the FAO (1983) permissible limit of 1.5 mg/kg for both species. The Sb concentrations observed corroborate those reported by Lebepe et al. (2024), and lower than that reported by Jooste et al. (2015). Similar to Sb, As was within the MOHSAC (2006) permissible limit for human consumption for both species. The As concentrations observed corroborated those reported at Inanda and Nagle Dams (Misra et al., 2024), and significantly lower than those reported for freshwater fish from Flag Boshielo, Mohammadpur, and Loskop dams (Addo-Bediako et al., 2014; Lynch et al., 2016; Ullah et al. 2017; Lebepe et al., 2020; Naangmengele et al., 2021).

Cadmium concentration was below detection level for *O. mossambicus* whereas a notable concentration was recorded for *C. rendalli*. However, the concentrations observed for *C. rendalli* were below the FAO (1983) permissible limit of 0.17 mg/kg. The *C. rendalli* concentration was comparable to those observed in various studies on freshwater fish (Addo-Bediako et al., 2014; Lebepe et al., 2020; Adegbola et al., 2021; Mannzhi et al., 2021). In contrast, the Cd concentration in *C. rendalli* was lower than that observed for *C. gariepinus* and higher than that observed for *O. mossambicus* reported by Ullah et al. (2017). Similar to the Cd trend, Cr exhibited relatively higher concentrations for *C. rendalli* compared to *O. mossambicus.* However, both species exhibited concentrations exceeding the WHO (2005) and MOHSAC (2006) permissible limits of 1 mg/kg and 2 mg/kg, respectively. The Cr concentrations reported in the current study corroborated those reported for *L. rohita* in Buriganga River (Ahmed et al., 2016), and significantly higher than those reported at Albasini (0.001 mg/kg), Karwan Bazar (0.08 mg/kg), Loskop Dam (0.02 to 0.75 mg/kg), and the Livhuvhu River (0.00672mg/kg) (Nibumereke et al., 2015; Ullah et al., 2017; Lebepe et al., 2020; Mannzhi et al., 2021). In contrast, the current Cr concentrations were significantly lower than those reported by Addo-Bediako et al. (2014) and Naangmengele et al. (2021).

Iron is an important metal in a human body, yet toxic at very high concentrations. In the present study, Fe concentrations were within the permissible limit of 333.30 mg/kg (JECFA, 1983) for both species. The observed Fe levels were significantly higher than those reported for tilapia species in Flag Boshielo (Lynch et al., 2016), Albasini Dam (Nibamureke et al., 2016), and Livhuvhu River (Mannzhi et al., 2021), but relatively lower than those reported by Addo-Bediako et al. (2014) and Seanego (2014). Similar to Fe, is the Mn which is an essential metal but toxic at elevated concentration. Manganese concentrations were within the 3.52 mg/kg permissible limit (IAEA, 2003) and exceeded the FAO (2003) limit of 2 mg/kg for both species. Manganese concentrations similar to those observed in the present study were observed for *Cyprinus carpio* from Masinga Dam and *Sarotherodon melanotheron* from Ogun River (Nzeve and Kitur, 2019; Adegbola et al., 2021). Contrastingly, Mn concentrations were notably lower than those reported for other freshwater fish (Nibumereke et al., 2015; Lynch et al., 2016; Mannzhi et al. 2021).

Lead is a metal that has been regarded as a cause for concern in most freshwater bodies. In the present study, Pb exceeded a regulatory safe threshold of 0.2 mg/kg (FAO, 2003) and 0.5 mg/kg (MHSAC, 2006) for fish consumption for both tilapia species. However, Pb concentrations were lower than those reported by Addo-Bediako et al. (2014), Ullah et al. (2017), Lebepe et al. (2020), and Naangmengele et al. (2021) in fish from polluted water bodies. In contrast, Jooste et al. (2015) reported Pb concentrations ranging from 1 – 2.7 mg/kg in Sharptooth catfish from the Olifants River. In contrast, the observed Pb concentrations corroborated those reported for *O. mossambicus* in the moderately polluted Nagle Dam (Lebepe et al., 2024). Despite Pb exceeding the permissible limit for human consumption, Se was within the MOHSAC (2006) limit of 1 mg/kg for both species. Corroborating Se concentrations, Mannzhi et al. (2021) and Misra et al. (2024) reported Se concentration within the limit in polluted water bodies. Selenium has an antagonistic association with most metals

(Lebepe et al., 2020), therefore, its presence in aquatic ecosystems could have a positive influence on the overall condition of the system.

Strontium (Sr) has a close association with calcium. In most cases, the co-occurrence of high concentrations of Ca protects fish from Sr accumulation and toxicity (Klaczek et al., 2022). Although Ca was not within the scope of this study, it is likely that it might have occurred in this dam since wastewater is among the drivers of pollution. Strontium concentration observed for both species was greater than 2 mg/kg with *O. mossambicus* exhibiting a mean of 3.48 mg/kg. The observed concentrations were higher than those reported in other studies conducted in moderately and highly polluted water bodies (Ulla et al., 2017; Lebepe et al., 2020; Mannzhi et al., 2021). Unfortunately, there is no permissible limit found in the literature for Sr which could be explained by its low toxicity in aquatic environment (McPherson et al., 2014).

The variability in metal bioaccumulation among both tilapia species reflects intrinsic physiological difference and external environmental drivers. Tissue type, particularly muscle, influences metal storage due to its distinct metabolic role (Ghannam et al., 2015), while species' feeding habits and ecological positions affect metal accumulation (Maurya and Malik, 2018). In addition, physico-chemical parameters including temperature and pH are possible drivers of metal accumulation in fish organs thus should be considered significantly (Dhanakumar et al., 2015). The dam received water from wastewater works, industrial and agricultural activities and the dam is categorised as eutrophic with high abundance of algal blooms. These aforementioned activities and conditions have the potential to influence the pH and other physical properties of the water, hence, influence the bioavailability and toxicity of metals. Therefore, the condition at the Shongweni Dam seems to be a cause for concern as metal concentrations are high and there is a chance of pH fluctuation to enhance accumulation.

### 5.4.2 Metal concentration in muscle between seasons

Metal concentrations varied from wet to dry season in both tilapia species, with no significant seasonal variation observed, except for Pb and Se in the muscle tissue of *O. mossambicus*. This pattern mirrors trends reported by Lebepe et al. (2020) for *Labeo rosae* at Loskop Dam and Proshad et al. (2018), who also documented a variation in metal concentrations during the dry season in various freshwater fish species. The seasonal variation of As, Cr, Mn, Pb, Sb, Se, and Sr was observed for sediment as well, with higher levels observed during the dry season. This seasonal variation in sediment metal levels may have influenced the accumulation patterns in fish muscle. However, Lebepe et al. (2020) highlighted that such seasonal variations in sediment metal levels do not always translate into predictable trends in fish tissues. This discrepancy might be attributed to several factors, including biological differences in species, the metal-binding capacities of tissues, and the environmental conditions of the study area. The absence of a clear trend in fish muscle metal concentrations may also be explained by external factors, particularly climate change. The extremely low rainfall recorded in 2021 likely played a significant role, as reduced water flow during the dry season would have facilitated the accumulation of metals in sediments (Rakib et al., 2022).

### 5.4.3 Inter-metal and fish length-metal correlations

Fish size is a significant factor influencing the bioaccumulation of metals among individuals of the same fish species in a shared environment (Ge et al., 2020; Balzani et al., 2022; Tashi et al., 2022). Previous studies reported strong negative correlations between fish size and metal concentrations in tissues, while others found no correlations or inconsistent patterns (Ge et al., 2020; Varol et al., 2022). In the present study, weak correlations were observed between fish length and metal accumulation for *O. mossambicus*. However, *C. rendalli* exhibited a significant moderate correlation between length and levels of Pb and Sb, suggesting that this species increased metals accumulated with increasing size, though not to a significant extent. Corroborating the current study were those reported for the same species in Inanda and Nagle Dams, where metals showed poor correlations with fish length (Hlatshwayo et al., 2024; Misra et al., 2024). Additionally, *O. mossambicus* and *L. rosae* showed little to no correlation between length and metal concentrations (Marr et al., 2017; Lebepe et al., 2020). Conversely, Maurya et al. (2019) noted a significant strong Cd-Cu correlation ($r = 0.75$) for *C. catla* and *C. mrigala* species. Positive correlations were observed in *T. ilisha* (Saleem et al., 2022) and small cyprinids (Balzani et al., 2022).

The weak correlations between fish length-metal may be explained by variations in metal bioavailability in the aquatic environment, as water chemistry significantly influences metal accumulation (Luoma and Rainbow, 2008). Although both tilapia specie exhibit slightly different feeding behaviours due to their sizes, metal accumulation is likely to be the same.

However, the present study has shown that metal-length relationships are not always definite and other factors should be considered to understand this concept (Borgå et al., 2012). Association between fish length and metal concentrations are believed to be negative to dilution effect as fish grows (Balzani et al., 2022), however, most studies have proven otherwise with most showing no to poor relationships (Jooste et al., 2015; Lebepe et al., 2024).

#### 5.4.4 Bioaccumulation factor in muscle tissue-sediment (BAFs)

The BAF is a critical metric used to evaluate the degree to which metals and other contaminants transfer from environmental media, such as water and sediments, into biological tissues. This metric provides valuable information about the availability of metals in sediments and their subsequent absorption by aquatic organisms. The BAF is determined by the ratio of metal concentrations in biological tissues (e.g., fish muscle) to those in the surrounding sediment, offering a standardized approach to assess contamination dynamics in aquatic ecosystem. In the current study, BAF values differed across metals, reflecting variations in their detoxification mechanisms, uptake efficiency, and environmental factors affecting their availability.

Antimony (Sb), strontium (Sr), Pb and As exhibited higher BAF values (greater than 1), indicating their effective transfer from sediment to muscle tissue of both tilapia species. Similarly, to previous research, Pb has a high mobility in aquatic systems and a strong affinity for binding with biological molecules such as proteins and enzymes (Duan et al., 2017; Simumoko et al., 2021). Conversely, metals such as Mn and Fe had lower BAF values, suggesting limited bioavailability in sediments or efficient detoxification and excretion by fish.

#### 5.4.5 Non-carcinogenic and carcinogenic risk assessment

Global per capita fish consumption has risen markedly, from an average of 9.9 kg in the 1960s to 20.5 kg in 2017 (FAO, 2018), reflecting a notable increase in demand and resilience on fish as a primary source of nutrition. This surge underscores the significance of fish as a vital source of essential nutrients, like micronutrients, high quality protein, and omega-3 fatty acids. However, this trend is accompanied by growing concerns regarding the potential health risks posed by the accumulation of hazardous metals in fish tissue, a consequence of environmental pollution. Contaminated fish consumption is now recognized as a critical global public health concern, necessitating more robust methods for evaluating and mitigating associated risks (Ahmed et al., 2019).

Risk analysis approaches, particularly those employing THQ, are commonly utilized to assess the potential non-carcinogenic risks of consuming contaminated aquatic foods (Saleem et al., 2022). This methodology offers insight into the severity of metal pollution and its implications for both environmental quality and human health (Zhang et al., 2012; Zohra and Habib, 2016). Such assessments are particularly relevant for freshwater systems, where subsistence fishers often depend on species such as tilapia for food security. Previous studies have frequently identified metals such as Sb, Cd, As, Pb, and Se in fish muscle tissue at levels that pose health risks (Ekere et al., 2018; Lebepe et al., 2020; Simumoko et al., 2021; Hlatshwayo et al., 2024; Misra et al., 2024).

In this study, most metals analysed in the muscle tissue of *C. rendalli* and *O. mossambicus* did not exceed threshold associated with human health risks, except for Cr and Pb. These findings corroborate with studies by Jooste et al. (2015), who reported that muscle of Sharptooth catfish in the Olifants River contained concentrations exceeding regulatory thresholds for Pb and Cr, highlighting significant potential health risks. Similarly, elevated THQ values, exceeding threshold of 1 for Cr have been observed in freshwater fish across various systems, such as Benue-Niger River Confluence, Challawa River, Loskop, Inanda, and Nagle Dams (Ekere et al., 2018; Edogbo et al., 2021; Lebepe et al., 2020; Hlatshwayo et al., 2024).

The carcinogenic risk, as outlined by Javed and Usmani (2016), classify risk as follows: CR $\leq 10^{-6}$ is considered low, $10^{-4}$ —$10^{-3}$ moderate, $10^{-3}$ —$10^{-1}$ high, and $\geq 10^{-1}$ very high. In the present study, the carcinogenic risks associated with Pb, Cr, and As in both *C. rendalli* and *O. mossambicus* from Shongweni Dam were categorized as very high, indicating a greater than 1 in 100,000 probability of cancer development in humans consuming these species. The carcinogenic risks identified in this study were notably higher than those reported for tilapia species in Inanda and Nagle Dams (Hlatshwayo et al., 2024; Lebepe et al., 2024), Lake Manzala (Abd-Elghany et al., 2024), and Kwanar-Are Dam (Yaradau et al., 2022). These elevated risks are attributed to heavy metal contamination, primarily from human activities such as domestic waste, agricultural runoff, wastewater works, and industrial effluents.

Similarly, the CR values for As ($\geq 10^{-1}$) observed in this study were notably higher than those for trout barb and common carp from Atatürk, Karakaya, and Keban reservoirs, where aquaculture, agriculture, and industrial wastewater discharge are dominant sources of pollution (Varol and Sünbül, 2020). The CR values for Pb exceeded those reported for slinger and Cape horse mackerel ($<10^{-4}$) from the Durban Basin (Debipersadh et al., 2023). On the other hand, CR values for Cr in this study, though high ($> 10^{-3}$), were similar to those reported for various fish species (*Anabas testudineus, Catla catla, Heteropneustes fossilis*, two *Labeo* species, *Puntius sarana*, and *Sperata aor*) from the Perak River, a region significantly impacted by anthropogenic metal pollution (Salam et al., 2020). However, these values were still lower than those reported for *Clarias gariepinus* and *Saratherodon melanotheron* from the Eleyele and Ogun Rivers (Adegbola et al., 2021).

The findings underscore a greater cancer risk for consumers of *O. mossambicus* and *C. rendalli* from Shongweni Dam, with particular concern for children and individuals weighing less than 70 kg. These risks align with global observations of higher susceptibility among vulnerable populations (Abdel-Kader and Mourad, 2022; Ramish et al., 2024). This further highlights the urgent need for monitoring and mitigating heavy metal contamination to safeguard public health, especially the local communities of Ntshongweni.

# Chapter 6

## Summary and conclusions

The study aimed to assess the impact of water quality on the health of two tilapia species, as well as metal accumulation and human health risks that could be associated with the consumption of these tilapia species. Physico-chemical characteristics of the dam are compromised with nutrients exhibiting eutrophic status. Eutrophic status comes with its threats to aquatic ecosystems as it enhances algal bloom which will eventually influence the dynamics of the system with regard to oxygen content. Nevertheless, some metals were below detection level in surface water. The metals that were detected in surface water were relatively higher during the dry season compared to the wet season. A contrasting trend was observed for sediment which showed relatively higher metal concentrations during the wet season. The hypothesis that contaminants would exhibit higher concentrations during the dry season was not fully supported as no definite trend was observed for surface water and bottom sediment. However, it is evident that the Shongweni Dam is on the verge of becoming detrimental to its inhabitants. Elevated metal concentrations reported in sediment remain a cause for concern as this sediment can become a future source of metals should there be disturbances in the bottom. Moreover, changes in physico-chemical properties such as pH, temperature, salinity, etc. can also resuspend metals fixed in sediment back into the water column.

Despite surface water and sediment pollution, the general fish health was not severely affected. Condition factor, hepatosomatic index and gonadosomatic index were not conclusive in segregating the health condition between the two seasons. Moreover, these indices did not respond to water quality deterioration observed. In contrast, the health assessment index has shown that the health of fish is somehow compromised with liver and gills exhibiting some abnormalities. However, when applied alone, the health assessment index (HAI) can be misleading as most response starts at the biochemical and microscopic levels. Histopathology provides early signs of tissue damage due to pollutants and it was clearer compared to HAI with gills exhibiting prominent anomalies such as hyperplasia, aneurism, hemorrhage, edema, sinusoidal dilation, and epithelial damage that could even influence the functioning of the system. However, the overall gill index classified the condition as moderately affected. Similar class was observed for the liver which were dominated by steatosis, necrosis, hypertrophied cells, melanomacrophages, hepatocellular pleomorphism, and vacuolation. No severe anomalies were observed for gonads histopathology and this could be explained by the role of these organs in metabolism.

These metals pose significant risks to both aquatic life and human health, with potential effects like organ damage, impaired growth, and mortality in fish, as well as health issues in humans consuming contaminated fish.

It is evident that Shongweni Dam is characterized by elevated metal concentrations and these were also observed in fish muscle. Both tilapia species were found to have accumulated metals either through diet, gaseous exchange system or dermal contact with water. Populations showed greater variability within each group, however, the mean concentrations showed no significant difference between the two populations. Chromium and lead showed concentrations exceeding permissible limits for human consumption, rendering these fish unsafe for consumption. Additionally, the non-essential metal Sr, rarely reported in other studies and lacking established safety guidelines, was detected at notably high levels, raising further concerns. Moreover, for non-carcinogenic risk assessment, Cr and Pb exhibited THQ > 1 whereas As and Sb showed THQ of 0.5 which still is a cause for concern. The THQ was calculated based on the assumption that a 70 kg adult consumes 0.15 kg portion once a week. Therefore, should one of these variables change, the quotient will also increase, meaning these concentrations may be detrimental to a child or if the portion size per week increases. Nevertheless, carcinogenic risk exceeded the set range of $10^{-4}$ to $10^{-6}$ for most metals, indicating a pressing need for improved water management and pollution control in this dam.

It is evident that the anthropogenic activities in the uMlazi River catchment are having a significant impact on the water quality of the Shongweni Dam. These findings highlight the critical importance of implementing effective water management and pollution control measures to reduce human-induced impacts on the Shongweni Dam and the uMlazi River catchment. It is recommended that regular monitoring be implemented in this river system and that metal concentration studies be extended to other species targeted by local communities. Moreover, a fish advisory should be developed to guide those consuming fish to ensure safety and sustainability among communities.

# Reference list


1. Abalaka, S.E., 2017. Histopathological evaluation of *Oreochromis mossambicus* gills and liver as biomarkers of earthen pond water pollution. *Sokoto Journal of Veterinary Sciences*, *15*(1), pp.57—66. https://doi.org/10.4314/sokjvs.v15i1.8
2. Abbaspour, N., Hurrell, R. and Kelishadi, R., 2014. Review on iron and its importance for human health. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, *19*(2), p.164.
3. Abdel-Baki, A., Dkhil, M. and Al-Quraishy, S., 2013. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. *African Journal of Biotechnology*, 10(13), 2541—2547.
4. Abdel-Kader, H. and H Mourad, M., 2019. Bioaccumulation of heavy metals and physiological/histological changes in gonads of catfish (*Clarias gariepinus*) inhabiting Lake Maryout, Alexandria, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(2), pp.363—377. https://dx.doi.org/10.21608/ejabf.2019.28010
5. Abdel-Khalek, A.A., Elhaddad, E., Mamdouh, S. and Saed Marie, M.A., 2016. Assessment of metal pollution around Sabal drainage in River Nile and its impacts on bioaccumulation level, metals correlation and human risk hazard using *Oreochromis niloticus* as a bioindicator. *Turkish J Fish Aquat Sci 16*:227—239. https://doi.org/10.4194/1303-2712-v16_2_02
6. Adams, S.M., Brown, A.M. and Goede, R.W., 1993. A quantitative health assessment index for rapid evaluation of fish condition in the field. *Transactions of the American Fisheries Society*, *122*(1), pp.63—73. https://doi.org/10.1577/1548-8659(1993)122%3C0063:AQHAIF%3E2.3.CO;2
7. Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P. H. and Spry, D., 2020. Bioavailability assessment of metals in freshwater environments: A historical review. *Environmental Toxicology and Chemistry*, 39(1), 48—59.
8. Addo-Bediako, A., Marr, S.M., Jooste, A. and Luus-Powell, W.J., 2014, January. Are metals in the muscle tissue of Mozambique tilapia a threat to human health? A case study of two impoundments in the Olifants River, Limpopo province, South Africa.

In *Annales de Limnologie-International Journal of Limnology* (Vol. 50, No. 3, pp. 201—210). EDP Sciences. https://doi.org/10.1051/limn/2014091

9. Adentunde, L.A., Gover, R.L.K. and Oguntola, G.O., 2011. Assessment of groundwater quality in Ogbomoso township of Oyo state, Nigeria. *IJRRAS*, 8(1): 115—122.
10. Afzaal, M., Hameed, S., Liaqat, I., Ali Khan, A.A., abdul Manan, H., Shahid, R. and Altaf, M., 2022. Heavy metals contamination in water, sediments and fish of freshwater ecosystems in Pakistan. *Water Practice & Technology*, *17*(5), pp.1253—1272. https://doi.org/10.2166/wpt.2022.039
11. Ahmed, M.K., Habibullah-Al-Mamun, M., Parvin, E., Akter, M.S. and Khan, M.S., 2013. Arsenic induced toxicity and histopathological changes in gill and liver tissue of freshwater fish, tilapia (Oreochromis mossambicus). Experimental and Toxicologic Pathology, 65(6), pp.903—909. https://doi.org/10.1016/j.etp.2013.01.003
12. Ahmed, M.K., Shaheen, N., Islam, M.S., Habibullah-al-Mamun, M., Islam, S., Mohiduzzaman, M. and Bhattacharjee, L., 2015. Dietary intake of trace elements from highly consumed cultured fish (*Labeo rohita*, *Pangasius pangasius* and *Oreochromis mossambicus*) and human health risk implications in Bangladesh. *Chemosphere*, *128*, pp.284—292. https://doi.org/10.1016/j.chemosphere.2015.02.016
13. Ahmed, M.K., Baki, M.A., Kundu, G.K., Islam, M.S., Islam, M.M., Hossain, M.M., 2016. Human health risks from heavy metals in fish of Buriganga river, Bangladesh. *SpringerPlus*, 5(1), 1697—1709. https://doi.org/10.1186/s40064-016-3357-0
14. Ahmed, A.S., Sultana, S., Habib, A., Ullah, H., Musa, N., Hossain, M.B., Rahman, M.M. and Sarker, M.S., 2019. Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests higher potential health risk in children than adults. *Plos one*, 14(10), p.e0219336.
15. Ahmed, N.M., Ghannam, H.E., Tayel, S.I. and Mohamed, M.Y., 2020. Biochemical and Histopathological Alterations of *Oreochromis niloticus* Fish Related to Heavy Metals in Lake Nasser, Egypt. *Egyptian Journal of Histology*, *43*(4), pp.1059—1069.
16. Ahmed, S. F., Kumar, P. S., Kabir, M., Zuhara, F. T., Mehjabin, A., Tasannum, N., Hoang, A. T., Kabir, Z. and Mofijur, M., 2022. Threats, challenges and sustainable conservation strategies for freshwater biodiversity. *Environmental Research*, 214, 113808. https://doi.org/10.1016/j.envres.2022.113808

17. Akhtar, N., Syakir Ishak, M.I., Bhawani, S.A. and Umar, K., 2021. Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water*, *13*(19), p.2660. https://doi.org/10.3390/w13192660
18. Akinnawo, S.O., 2023. Eutrophication: Causes, consequences, physical, chemical and biological techniques for mitigation strategies. *Environmental Challenges*, *12*, p.100733. https://doi.org/10.1016/j.envc.2023.100733
19. Algül, F. and Beyhan, M., 2020. Concentrations and sources of heavy metals in shallow sediments in Lake Bafa, Turkey. *Scientific reports*, 10(1), 11782. https://doi.org/10.1038/s41598-020-68833-2
20. Ali, S., Barat, A., Kumar, P., Sati, J., Kumar, R. and Haldar, R.S., 2014. Study of length-weight relationship and condition factor for the golden mahseer, *Tor puttiora* from Himalayan rivers from India. *Journal of Environmental Biology*, *35*(1), pp.225—228.
21. Ali, M.M., Ali, M.L., Islam, M.S. and Rahman, M.Z., 2016. Preliminary assessment of heavy metals in water and sediment of Karnaphuli River, Bangladesh. *Environmental Nanotechnology, Monitoring & Management*, *5*, pp.27—35. https://doi.org/10.1016/j.enmm.2016.01.002
22. Ali, H. and Khan, E., 2018. Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. *Environmental chemistry letters*, *16*(3), pp.903—917.
23. Ali, H. and Khan, E., 2018b. What are heavy metals? Long-standing controversy over the scientific use of the term 'heavy metals'—proposal of a comprehensive definition. *Toxicol Environ Chem*, 100:6—19. https://doi.org/10.1080/02772248.2017.1413652
24. Ali, H., Khan, E. and Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, *2019*(1), p.6730305. https://doi.org/10.1155/2019/6730305
25. Ali, M.M., Ali, M.L., Proshad, R., Islam, S., Rahman, Z., Tusher, T.R., Kormoker, T., and Al, M.A., 2020. Heavy metal concentrations in commercially valuable fishes with health hazard inference from Karnaphuli river., Bangladesh. Human and Ecological Risk Assessment: An International Journal, 26(10), 2646—2662. http://doi.org/10.1080/10807039.2019.1676635

26. Alm-Eldeen, A.A., Donia, T. and Alzahaby, S., 2018. Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, *Oreochromis niloticus*, in the Middle Delta, Egypt. *Environmental science and pollution research*, *25*, pp.14636—14646.
27. Alquezar, R.; Markich, S.J. and Booth, D.J., 2006. Effects of metals on condition and reproductive output of the smooth toadfish in Sydney estuaries, southeastern Australia. Environ. Pollut., 142: 116—122.
28. Apostolaki, S., Akinsete, E., Koundouri, P. and Samartzis, P., 2020. Freshwater: The importance of freshwater for providing ecosystem services. *Encyclopedia of the World's biomes*, pp.71—79.
29. Aradpour, S., Noori, R., Tang, Q., Bhattarai, R., Hooshyaripor, F., Hosseinzadeh, M., Haghighi, A.T. and Klöve, B., 2020. Metal contamination assessment in water column and surface sediments of a warm monomictic man-made lake: Sabalan Dam Reservoir, Iran. *Hydrology Research*, *51*(4), pp.799—814. https://doi.org/10.2166/nh.2020.160
30. Asmamaw, B., Beyene, B., Tessema, M. and Assefa, A., 2019. Length-weight relationships and condition factor of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae) in Koka Reservoir, Ethiopia. *International Journal of Fisheries and Aquatic Research*, *4*(1), pp.47—51.
31. Authman, M.M., Zaki, M.S., Khallaf, E.A. and Abbas, H.H., 2015. Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development*, *6*(4), pp.1—13.
32. Avtar, R., Kumar, P., Singh, C.K. and Mukherjee, S., 2011. A comparative study on hydrogeochemistry of Ken and Betwa Rivers of Bundelkhand using statistical approach. *Water Quality, Exposure and Health*, *2*(3), pp.169—179.
33. Azadbakht, F., Shirali, S., —Ronagh, M.T. and Zamani, E., 2018. Effect of *Aeromonas hydrophila* in the tissue structure of liver and immune organs in *Acanthopagrus latus*. *Veterinary Research & Biological Products*, *31*(3), pp.88—95.
34. Bakare, B.F. and Adeyinka, G.C., 2022. Evaluating the potential health risks of selected heavy metals across four wastewater treatment water works in Durban, South Africa. *Toxics*, *10*(6), p.340. https://doi.org/10.3390/toxics10060340
35. Balzani, P., Kouba, A., Tricarico, E., Kourantidou, M. and Haubrock, P.J., 2022. Metal accumulation in relation to size and body condition in an all-alien species

community. *Environmental Science and Pollution Research*, *29*(17), pp.25848—25857. https://doi.org/10.1007/s11356-021-17621-0

36. Bansal, O.P., 2023. Effects of potentially toxic metals on fish physiology, histopathology and impact on human health. *J. Agri. Res. Adv.*, 05(01): 15—35.
37. Barišić, J., Marijić, V.F., Mijošek, T., Čož-Rakovac, R., Dragun, Z., Krasnići, N., Ivanković, D., Kružlicová, D. and Erk, M., 2018. Evaluation of architectural and histopathological biomarkers in the intestine of brown trout (*Salmo trutta* Linnaeus, 1758) challenged with environmental pollution. *Science of the total environment*, *642*, pp.656-664. https://doi.org/10.1016/j.scitotenv.2018.06.045
38. Barnhoorn, I.E., Van Dyk, J.C., Genthe, B., Harding, W.R., Wagenaar, G.M. and Bornman, M.S., 2015. Organochlorine pesticide levels in Clarias gariepinus from polluted freshwater impoundments in South Africa and associated human health risks. *Chemosphere*, *120*, pp.391—397. https://doi.org/10.1016/j.chemosphere.2014.08.030
39. Benham, B.L., Ling, E., Wright, B. and Haering, K., 2011. Virginia Household Water Quality Program: Total Dissolved Solids (TDS) in Household Water.
40. Benjamin, L.V. and Kutty, R., 2019. Sub-lethal effects of potassium dichromate on hematological and histological parameters in climbing perch, Anabas testudineus (Anabantidae). *International Journal of Aquatic Biology*, *7*(3), pp.140—145.
41. Bhat, S.U. and Qayoom, U., 2021. Implications of sewage discharge on freshwater ecosystems.
42. Bhat, R.A., Saoca, C., Maricchiolo, G. and Fazio, F., 2022. Analysis of fatty acids and sex steroid hormones in rainbow trout testes (*Oncorhynchus mykiss*) during the reproductive process. *Aquaculture Research*, 53(12), pp.4426—4436.
43. Bhat, R.A., Saoca, C., Cravana, C., Fazio, F., Guerrera, M.C., Labh, S.N. and Kesbiç, O.S, 2023. Effects of heavy pollution in different water bodies on male rainbow trout (*Oncorhynchus mykiss*) reproductive health. *Environment Science and Pollution Research*, 30(9), pp.23467—23479.
44. Bibi, S., Naz, S., Saeed, S. and Chatha, A.M., 2021. A review on histopathological alterations induced by heavy metals (Cd, Ni, Cr, Hg) in different fish species. *Punjab Univ J Zool*, *36*(1), pp.81—89. https://dx.doi.org/10.17582/journal.pujz/2021.36.1.81.89

45. Bills, R., 2019. *Oreochromis mossambicus*. The IUCN Red List of Threatened Species version 2019: e.T63338A3124798. https://dx.doi.org/10.2305/IUCN.UK.2019-3
46. Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., Beklioğlu, M. and Bondar-Kunze, E., 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nature Ecology & Evolution*, *4*(8), pp.1060—1068.
47. Borgå, K., Kidd, K.A., Muir, D.C., Berglund, O., Conder, J.M., Gobas, F.A., Kucklick, J., Malm, O. and Powell, D.E., 2012. Trophic magnification factors: considerations of ecology, ecosystems, and study design. *Integrated environmental assessment and management*, *8*(1), pp.64—84. https://doi.org/10.1002/ieam.244
48. Burger, J. and Gochfeld, M., 2021. Biomonitoring selenium, mercury, and selenium: mercury molar ratios in selected species in North eastern US estuaries: risk to biota and humans. *Environmental Science and Pollution Research*, *28*(15), pp.18392—18406.
49. CCME, 2001. Canadian sediment quality guidelines for the protection of aquatic life https://www.elaw.org/system/files/sediment_summary_table.pdf, accessed on the 25th January 2018.
50. CCME, 2012. Canadian water quality guidelines for the protection of aquatic life. Introduction. Canadian environmental quality guidelines, Canada. Accessed on the 09 September 2019. http://st-ts.ccme.ca/en/index.html.
51. Chapman, P. J., Kay, P., Mitchell, G. and Pitts, C. S., 2019. Surface water quality. In: Holden, J, (ed.) Water resources: an integrated approach. Routledge**:** 99—150.
52. Chikodzi, D., Dube, K. and Ngcobo, N., 2021. Rethinking Harbours, Beaches and Urban Estuaries Waste Management Under Climate-Induced Floods in South Africa. *The Increasing Risk of Floods and Tornadoes in Southern Africa,* p.127.
53. Christensen, K.Y., Thompson, B.A., Werner, M., Malecki, K., Imm, P. and Anderson, H.A., 2016. Levels of persistent contaminants in relation to fish consumption among older male anglers in Wisconsin. *International journal of hygiene and environmental health*, *219*(2), pp.184—194. https://doi.org/10.1016/j.ijheh.2015.11.001
54. Crafford, D. and Avenant-Oldewage, A., 2009. Application of a fish health assessment index and associated parasite index to *Clarias gariepinus* (Teleostei: Clariidae) in the

Vaal River system, South Africa. *African Journal of Aquatic Science*, *34*(3), pp.261—272. https://doi.org/10.2989/AJAS.2009.34.3.8.984

55. Crafford, D. and Avenant-Oldewage, A., 2010. Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal River System — Strontium, aluminium, lead and nickel. Water SA, 36, 621 —640.
56. Dallas, H. F. and Day, J. A., 2004. The effect of water quality variables on aquatic ecosystems: A review. WRC Report No. TT 224/04. Water Research Commission, Pretoria**:** 1—232.
57. De Graaf, Gertjan, and Luca Garibaldi. "The value of African fisheries." *FAO fisheries and aquaculture circular* C1093 (2015): I.
58. Department of Water and Sanitation, South Africa: National State of Water Report (January 2023).
59. Dietrich, G.J., Florek-Łuszczki, M., Wojciechowska, M., Wójcik, T., Bąk-Badowska, J., Wójtowicz, B., Zięba, E., Gworek, B. and Chmielewski, J., 2022. Fish as bio-indicator of environmental pollutants and associated health risks to the consumer. J. Elem., 27(4): 879—896. https://doi.org/10.5601/jelem.2022.27.3.2322
60. Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L. and Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81: 163 — 182.
61. Dudgeon, D., 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current Biology*, *29*(19), pp. R960—R967. https://doi.org/10.1016/j.cub.2019.08.002
62. DWAF, 1996. South African Water Quality Guideline, Volume 7, Aquatic Ecosystem, 2nd Edition, Pretoria.
63. Ebrahimi, M. and Taherianfard, M., 2011. The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. Iran. J. Fish. Sci., 10: 13—26.
64. Edokpayi, J.N., Odiyo, J.O., Msagati, T.A. and Potgieter, N., 2015. Temporal variations in physico-chemical and microbiological characteristics of Mvudi River, South Africa. International journal of environmental research and public health, 12(4), pp.4128—4140. https://doi.org/10.3390/ijerph120404128

65. Edokpayi, J.N., Rogawski, E.T., Kahler, D.M., Hill, C.L., Reynolds, C., Nyathi, E., Smith, J.A., Odiyo, J.O., Samie, A., Bessong, P. and Dillingham, R., 2018. Challenges to sustainable safe drinking water: A case study of water quality and use across seasons in rural communities in Limpopo Province, South Africa. *Water*, 10(2), p.159. https://doi.org/10.3390/w10020159

66. Edokpayi, J.N., Nkhumeleni, M., Enitan-Folami, A.M. and Olaniyi, F.C., 2022. Water quality assessment and potential ecological risk of trace metals in sediments of some selected rivers in Vhembe district, South Africa. *Physics and Chemistry of the Earth, Parts A/B/C*, *126*, p.103111. https://doi.org/10.1016/j.pce.2022.103111

67. Eker, Ç.S. and Kiliç, E.D., 2024. Pollution level, environmental risk, and source of potentially toxic elements in the sediment of Çoruh River (Bayburt, NE Turkey). *Arabian Journal of Geosciences*, *17*(1), p.52.

68. Ekere, N.R., Yakubu, N.M. and Ihedioha, J.N., 2018. Assessment of levels and potential health risk of heavy metals in water and selected fish species from the Benue-Niger River Confluence, Lokoja, Nigeria. *Journal of Aquatic Food Product Technology*, 27(7), pp.772—782.

69. El-Bouraie, M.M., El-Barbary, A.A., Yehia, M.M. and Motawea, E.A., 2010. Heavy metal concentrations in surface river water and bed sediments at Nile Delta in Egypt.

70. Eltholth, M., Fornace, K., Grace, D., Rushton, J. and Häsler, B., 2018. Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh fish markets. *Global food security*, *17*, pp.14—20. https://doi.org/10.1016/j.gfs.2018.03.003

71. FAO, 1983. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fisheries Circular No. 764. Rome.

72. FAO, 2003. Heavy metals regulations Legal Notice No. 66/2003. http://extwprlegs1.fao.org/docs/pdf/eri42405.pdf. Accessed 08 June 2017.

73. FAO. *State of World Fisheries and Aquaculture 2018-Meeting the Sustainable Goals*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2018.

74. Faysse, N. and Gumbo, J., 2004. *The transformation of irrigation boards into water user associations in South Africa: Case studies of the Umlaas, Komati, Lomati and Hereford Irrigation Boards. Volume 2* (Vol. 73). IWMI.

75. Fazio, F., Habib, S.S., Naz, S., Hashmi, M.A.H., Saoca, C. and Ullah, M., 2022. Cadmium sub-lethal concentration effect on growth, haematological and biochemical parameters of *Mystus seenghala* (Sykes, 1839). *Biol Trace Elem Res*, 200: 2432— 2438. https://doi.org/10.1007/s12011-021-02852-6
76. Ford, AG., Bullen, TR., Pang, L., Genner, MJ., Bills, R., Flouri, T., Ngatunga, BP., Rüber, L., Schliewen, UK., Seehausen, O., Shechonge, A., Stiassny, MLJ., Turner, GF., & Day, JJ. (2019). Molecular phylogeny of *Oreochromis* (Cichlidae: Oreochromini) reveals mito-nuclear discordance and multiple colonisation of adverse aquatic environments. Molecular Phylogenetic Evolution, 136:215–226. https://doi.org/10.1016/j.ympev.2019.04.008
77. Froese, R. and Pauly, D., 2020. Fish Base. World Wide Web Electronic Publication. Retrieved from http://www.fishbase.org/Search.php.
78. Gaine, M.A., Khan, M.I. and Parveen, M., 2012. Seasonal Variation In Physico-Chemical Charateristics of Pahuj Resrevoir, District Jhansi, Bundelkhand Region, Central India. *International Journal of Current Research*, *4*(12), pp.115—118.
79. Gárriz, Á., Del Fresno, P.S., Carriquiriborde, P. and Miranda, L.A., 2019. Effects of heavy metals identified in Chascomús shallow lake on the endocrine-reproductive axis of pejerrey fish (*Odontesthes bonariensis*). *General and Comparative Endocrinology*, *273*, pp.152—162. https://doi.org/10.1016/j.ygcen.2018.06.013
80. Ge, M., Liu, G., Liu, H. and Liu, Y., 2020. Levels of metals in fish tissues of *Liza haematocheila* and *Lateolabrax japonicus* from the Yellow River Delta of China and risk assessment for consumers. *Marine Pollution Bulletin*, *157*, p.111286. https://doi.org/10.1016/j.marpolbul.2020.111286
81. Gerbron, M., Geraudie, P., Fernandes, D., Rotchell, J.M., Porte, C. and Minier, C., 2014. Evidence of altered fertility in female roach (*Rutilus rutilus*) from the River Seine (France). *Environ. Pollut*. 191: 58—62.
82. Getnet, M.A., Mekonnen, M.Y., Yimam, H.M., Berihun, A.M. and Malede, B.A., 2024. Histopathology based study of Nile tilapia fish (*Oreochromis niloticus*) as a biomarker for water pollution evaluation in the southern gulf of Lake Tana, Ethiopia. *BMC Veterinary Research*, *20*(1), p.409.
83. Gilbert, B. and Avenant-Oldewage, A., 2014. Arsenic, chromium, copper, iron, manganese, lead, selenium and zinc in the tissues of the largemouth yellowfish,

*Labeobarbus kimberleyensis* (Gilchrist and Thompson, 1913), from the Vaal Dam, South Africa, and associated consumption risks. *Water Sa*, 40(4), 739—748.

84. Gilbert, B.M., Hussain, E., Jirsa, F. and Avenant-Oldewage, A., 2017. Evaluation of trace element and metal accumulation and edibility risk associated with consumption of *Labeo umbratus* from the Vaal Dam, South Africa. *International Journal of Environmental Research and Public Health*, *14*(7), p.678. https://doi.org/10.3390/ijerph14070678
85. Giri, A.K., Sahu, N.P. and Dash, G., 2021. Improvement in the growth status and carbohydrate utilization of *Labeo rohita* (Hamilton, 1822) fingerlings with dietary supplementation of chromium picolinate. *Fish Physiology Biochemistry*, 47: 599—616.
86. Guevarra, R.D., Paraso, M.G.V. and Lola, S.E.G., 2020. Biomarker Evaluation in Nile Tilapia (*Oreochromis niloticus*) to Assess the Health Status of Aquaculture Areas in the Seven Lakes of San Pablo. *Philippine Journal of Science*. p.149.
87. Gunes, M.U.H.A.R.R.E.M., Sokmen, T.E.O.M.A.N. and Kirici, M., 2019. Determination of some metal levels in water, sediment and fish species of Tercan Dam Lake, Turkey. *Applied Ecology and Environmental Research*, *17*(6). http://doi.org/10.15666/aeer/1706_1496114972
88. Hassan, K.T., Ferdoushi, Z., Rana, M.M. and Alam, M.S., 2024. Assessing the Seasonal Variability of Water Quality and Heavy Metals Concentration in Sediment, Water, and Fish Muscles of Korotoa River in Bangladesh. *Aquaculture Research*, *2024*(1), p.5343363. https://doi.org/10.1155/2024/5343363
89. Hassaninezhad, L., Sahahieh, A.R., Salamat, N., Savari, A., Majd, N.E., 2014. Assessment of gill pathological responses in the tropical fish yellowfin seabream of Persian Gulf under mercury exposure. Toxicology reports, 1: 621—628.
90. Hayati, A., Giarti, K., Winarsih, Y., 2017. The effect of cadmium on sperm quality and fertilization of *Cyprinus carpio* L. *J Trop Biodiv Biotech*, 2(2): 45—54. https://doi.org/10.22146/jtbb.26744
91. He, D., Li, F., Dominech, S., Wen, X. and Yang, S., 2019. Heavy metals of surface sediments in Changjiang (Yangtze River) Estuary: Distribution, speciation and environmental risks. *Journal* of *Geochemical Exploration*. 198, pp.18—28.
92. Higgins, J., Zablocki, J., Newsock, A., Krolopp, A., Tabas, P. and Salama, M., 2021. Durable freshwater protection: A framework for establishing and maintaining long-

term protection for freshwater ecosystems and the values they sustain. *Sustainability*, *13*(4), p.1950. https://doi.org/10.3390/su13041950

93. Hlatshwayo, S.S.B., Bissessur, A., Selala, M.C., Takai, Y. and Lebepe, J., 2024. Metal distribution in three organs and edibility assessment on *Coptodon rendalli* from the Umgeni River impacted by metallurgic industrial activities. *Environmental Monitoring and Assessment*, *196*(8), p.742.
94. Hou, X., Feng, L., Dai, Y., Hu, C., Gibson, L., Tang, J., Lee, Z., Wang, Y., Cai, X., Liu, J. and Zheng, Y., 2022. Global mapping reveals increase in lacustrine algal blooms over the past decade. *Nature Geoscience*, *15*(2), pp.130—134.
95. Huang, Y., Zhang, D., Xu, Z., Yuan, S., Li, Y. and Wang, L., 2017. Effect of overlying water pH, dissolved oxygen and temperature on heavy metal release from river sediments under laboratory conditions. *Arch*. *Environ*. *Protect*. 43(2), 28—36.
96. Huang, C., Chen, Y., Zhang, S. and Wu, J., 2018. Detecting, extracting, and monitoring surface water from space using optical sensors: a review. Rev, Geophys. 56, 333—360. https://doi.org/10.1029/2018RG000598
97. Huang, C.-W., Yen, P.-L., How, C. M., Chai, Z. Y. and Liao, V. H.-C., 2021. Levels of bioavailable manganese in river sediment may elevate reproductive risk in model organism Caenorhabditis elegans. *Aquatic Toxicology*, 239, 105958. https://doi.org/10.1016/j.aquatox.2021.105958
98. Huang, Y., Su, K., Lin, L., Chen, Y., Zhang, D., Li, X., Lin, Y., Ma, J., Xu, J., Ji, J. and Yao, Y., 2023. Trace elements in crayfish from China: tissue concentrations, spatial distribution and health risk assessment. *Journal of Food Composition Analysis, 123*, p105540.
99. IAEA, 2003. World-wide intercomparison exercise for the determination of trace elements and methylmercury in fish homogenate ınternational atomic energy agency 407 report no IAEA/ AL/144IAEA/MEL/72
100. Islam, M.S., Proshad, R. and Ahmed, S., 2018. Ecological risk of heavy metals in sediment of an urban river in Bangladesh. *Human and ecological risk assessment: an international journal*, *24*(3), pp.699—720. https://doi.org/10.1080/10807039.2017.1397499
101. Islam, M.A., Das, B., Quraishi, S.B., Khan, R., Naher, K., Hossain, S.M., Karmaker, S., Latif, S.A. and Hossen, M.B., 2020. Heavy metal contamination and ecological risk

assessment in water and sediments of the Halda river, Bangladesh: A natural fish breeding ground. *Marine Pollution Bulletin*, 160, p.111649.

102. Izah, S.C., Richard, G., Stanley, H.O., Sawyer, W.E., Ogwu, M.C., and Uwaeme, O.R., 2023. Integrating the one health approach and statistical analysis for sustainable aquatic ecosystem management and trace metal contamination mitigation. *ES Food & Agroforestry*, 14(2), p.1012. http://dx.doi.org/10.30919/esfaf1012

103. Jabeen, G., Manzoor, F., Javid, A., Azmat, H., Arshad, M., and Fatima, S. (2018). Evaluation of Fish Health Status and Histopathology in Gills and Liver Due to Metal Contaminated Sediments Exposure. *Bulletin of Environmental Contamination and Toxicology*, 100(4), 492—501. https://doi.org/10.1007/s00128-018-2295-7

104. Jackson, M.C., Loewen, C.J., Vinebrooke, R.D. and Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global change biology*, 22(1), pp. 180—189. https://doi.org/10.1111/gcb.13028

105. Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D. and Chimimba, C. T., 2020. Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global Change Biology*, 22(6), 1801—1810.

106. Jangu, S., Kumar, D., Brraich, O.S., Baek, K.H. and Mishra, A.K., 2021. Alterations in ultra-structures and elemental composition of ovaries of *Labeo rohita* (Hamilton-Buchanan) as a pollution indicator in Harike Wetland (Ramsar Site), India. *Journal of Histotechnology*, *44*(2), pp.62—69. https://doi.org/10.1080/01478885.2020.1840115

107. Javed, M. and Usmani, N., 2011. Accumulation of heavy metals in fishes: a human health concern. *Int. J. Environ. Sci.* 2(2), 659.

108. Javed, M. and Usmani, N., 2016. Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish *Mastacembelus armatus* inhabiting, thermal power plant effluent loaded canal. *SpringerPlus*, 5(776), pp.1—8. https://doi.org/10.1007/978-1-4020-4728-2_6

109. Javed, M., Ahmad, M.I., Usmani, N. and Ahmad, M., 2017. Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in *Channa punctatus* exposed to heavy metal loaded waste water. *Scientific reports*, *7*(1), p.1675. https://doi.org/10.1155/2014/435857

110. Javed, M. and Usmani, N., 2019. An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*, pp.389—403.

111. JECFA, 2002. Limit test for heavy metals in food additive specifications. FAO/WHO expert committee on food additives. http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/2002-09-10_Explanatory_note_Heavy_Metals.pdf

112. Jiang, X., Wang, J., Pan, B., Li, D., Wang, Y. and Liu, X., 2022. Assessment of heavy metal accumulation in freshwater fish of Dongting Lake, China: Effects of feeding habits, habitat preferences and body size. *Journal of Environmental Sciences*, *112*, pp.355—365. https://doi.org/10.1016/j.jes.2021.05.004

113. Jisr, N., Younes, G., Sukhn, C. and El-Dakdouki, M.H., 2018. Length-weight relationships and relative condition factor of fish inhabiting the marine area of the Eastern Mediterranean city, Tripoli-Lebanon. *The Egyptian Journal of Aquatic Research*, *44*(4), pp.299—305. https://doi.org/10.1016/j.ejar.2018.11.004

114. Jooste, A., Luus-Powell, W. and Polling, L., 2005. Bio-monitoring the impact of pollution by means of the fish health assessment index and fish parasites in the lower reach of the Ga-Selati River: a case study. University of Limpopo, Unpublished Foskor/NRF Report.

115. Jooste, A., Marr, S.M., Addo-Bediako, A. and Luus-Powell, W.J., 2015. Sharptooth catfish shows its metal: a case study of metal contamination at two impoundments in the Olifants River, Limpopo river system, South Africa. *Ecotoxicology and environmental safety*, *112*, pp.96—104. https://doi.org/10.1016/j.ecoenv.2014.10.033

116. Kaur, S., Singh Khera, K. and Kaur Kondal, J., 2018. Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeo rohita* (Hamilton). *Journal of Entomology and Zoology Studies*. 6(2): 2137—2144.

117. Keyombe, J. L., Malala, J. O., Waithaka, E., Lewo, R. M. and Obwanga, B. O., 2017. Seasonal changes in length-weight relationship and condition factor of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae) in Lake Naivasha, Kenya. Int. J. Aquat. Biol., 5(1), 7—11.

118. Khatri, N., and Tyagi, S., 2014. Influences of natural and anthropogenic factors on surface and groundwater quality in rural and urban areas. *Frontiers in Life Science*, *8(1)*, 23—39. https://doi.org/10.1080/21553769.2014.933716

119. Klaczek, C. E., Saari, G. N., Veilleux, H. D., Mielewczyk, D. A., Goss, G. G. and Glover, C. N., 2022. Acute waterborne strontium exposure to rainbow trout: Tissue accumulation, ionoregulatory effects, and the modifying influence of waterborne calcium. *Aquatic Toxicology*, 245, 106125. https://doi.org/10.1016/j.aquatox.2022.106125

120. Kormoker, T., Karmoker, R., Uddin, M., Kabir, M.H., Siddique, M.A.B., Khan, R., Islam, M.S., Al, M.A., Alam, M, Ustaoğlu, F., Islam, M., Idris, A.M., 2022. Toxic elemental abundances in the sediment of the Jamuna River, Bangladesh: pollution status, sources, toxicity, and ecological risks assessment. Int. J. Environ. Anal. Chem. http://doi.org/10.080/03067319.2022.2134781

121. Kormoker, T., Islam, M.S., Siddique, M.A.B., Kumar, S., Phoungthong, K., Kabir, M.H., Iqubal, K.F., Kumar, R., Ali, M.M. and Islam, A.R.M.T., 2023. Layer-wise physicochemical and elemental distribution in an urban river water, Bangladesh: potential pollution, sources, and human health risk assessment. *Environmental Science: Advances*, *2*(10), pp.1382-1398. https://doi.org/10.1039/D3VA00094J

122. Kumar, M., Gogoi, A., Kumari, D., Borah, R., Das, P., Mazumder, P. and Tyagi, V.K., 2017. Review of perspective, problems, challenges, and future scenario of metal contamination in the urban environment. *Journal of Hazardous, Toxic, and Radioactive Waste*, *21*(4), p.04017007.

123. Kumar, S., Raman, R. K., Talukder, A., Mahanty, A., Sarkar, D. J., Das, B. K., Bhowmick, S., Samanta, S., Manna, S. K. and Mohanty, B. P., 2022. Arsenic Bioaccumulation and Identification of Low-Arsenic-Accumulating Food Fishes for Aquaculture in Arsenic-Contaminated Ponds and Associated Aquatic Ecosystems. *Biological trace element research*, 200(6), 2923—2936. https://doi.org/10.1007/s12011-021-02858-0

124. Kuriakose, S., 2017. Estimation of length weight relationship in fishes. In: Course Manual Summer School on Advanced Methods for Fish Stock Assessment and Fisheries Management. Lecture Note Series No. 2/2017. CMFRI; Kochi, Kochi, 215—220.

125. Lebepe, J., Marr, S. and Luus-Powell, W., 2016. Metal contamination and human health risk associated with the consumption of *Labeo rosae* from the Olifants River system, South Africa. *African Journal of Aquatic Science*, 41(2), 161—170. http://dx.doi.org/10.2989/16085914.2016.1138100

126. Lebepe, J., Oberholster, P.J., Ncube, I., Smit, W. and Luus-Powell, W.J., 2020. Metal levels in two fish species from a waterbody impacted by metallurgic industries and acid mine drainage from coal mining in South Africa. *Journal of Environmental Science and Health, Part A*, *55*(4), pp.421—432. https://doi.org/10.1080/10934529.2019.1704604

127. Lebepe, J., Selala, M.C., Takai, Y., Maleka, N.M. and Hlatshwayo, S.B., 2024. Accumulation of Heavy Metals in Tissues of *Oreochromis mossambicus* from the uMgeni River and Human Health Risk Assessment. *Water, Air, & Soil Pollution*, *235*(11), p.726.

128. Lino, A. S., Kasper, D., Carvalho, G. O., Guida, Y. and Malm, O., 2020. Selenium in sediment and food webs of the Tapajós River basin (Brazilian Amazon) and its relation to mercury. *Journal of Trace Elements in Medicine and Biology*, 62, 126620. https://doi.org/10.1016/j.jtemb.2020.126620

129. Liu, J., Cao, L. and Dou, S., 2019. Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. *Science of the total environment*, *670*, pp.508—522. https://doi.org/10.1016/j.scitotenv.2019.03.140

130. Lynch, A.J., Cooke, S.J., Deines, A.M., Bower, S.D., Bunnell, D.B., Cowx, I.G., Nguyen, V.M., Nohner, J., Phouthavong, K., Riley, B. and Rogers, M.W., 2016. The social, economic, and environmental importance of inland fish and fisheries. *Environmental reviews*, *24*(2), pp.115—121. https://doi.org/10.1139/er-2015-0064

131. Mabika, N., Magqina, T. and Makoni, T., 2024. Length-weight relationship and condition factor for five common fish species in a recently constructed reservoir (Tugwi-Mukosi Dam)-Zimbabwe. *Aquatic Research*, *7*(2), pp.83—92. https://doi.org/10.3153/AR24008

132. Madanire-Moyo, G.N., Luus-Powell, W.J., Jooste, A. and Olivier, P.A.S., 2012. A comparative assessment of the health status of feral populations of *Clarias gariepinus*

from three dams in the Limpopo and Olifants river systems, Limpopo province, South Africa, using the fish health assessment index protocol. *African Journal of Aquatic Science*, *37*(1), pp.27—37. https://doi.org/10.2989/16085914.2012.665575

133. Malaj, E., 2015. Safeguarding freshwater organisms from chemicals: from the application of evolutionary concepts in ecotoxicology to large-scale risk assessment of chemicals.

134. Mannzhi, M.P., Edokpayi, J.N., Durowoju, O.S., Gumbo, J. and Odiyo, J.O., 2021. Assessment of selected trace metals in fish feeds, pond water and edible muscles of *Oreochromis mossambicus* and the evaluation of human health risk associated with its consumption in Vhembe district of Limpopo Province, South Africa. *Toxicology Reports*, *8*, pp.705—717. https://doi.org/10.1016/j.toxrep.2021.03.018

135. Marchand, M.J., Van Dyk, J.C., Barnhoorn, I.E. and Wagenaar, G.M., 2012. Histopathological changes in two potential indicator fish species from a hyper-eutrophic freshwater ecosystem in South Africa: a baseline study. *African Journal of Aquatic Science*, *37*(1), pp.39—48. https://doi.org/10.2989/16085914.2011.636902

136. Marr, S.M., Lebepe, J., Steyl, J.C.A., Smit, W.J. and Luus-Powell, W.J., 2017. Bioaccumulation of selected metals in the gill, liver and muscle tissue of rednose labeo *Labeo rosae* from two impoundments on the Olifants River, Limpopo river system, South Africa. *African Journal of Aquatic Science*, *42*(2), pp.123—130. https://doi.org/10.2989/16085914.2017.1351915

137. Matta, G. and Gjyli, L., 2016. Mercury, lead and arsenic: impact on environment and human health. *J Chem Pharm Sci*, *9*(2), pp.718—725.

138. Maurya, P.K. and Malik, D.S., 2016. Bioaccumulation of xenobiotics compound of pesticides in riverine system and its control technique: a critical review. *J. Ind. Pollut. Control*. 32 (2), 580—594.

139. Maurya, P.K. and Malik, D.S., 2018. Bioaccumulation of heavy metals in tissues of selected fish species from Ganga river, India, and risk assessment for human health, Hum. Ecol. Risk Assess. http://doi.org/10.1080/10807039.2018.1456897

140. Maurya, P.K., Malik, D.S., Yadav, K.K., Kumar, A., Kumar, S. and Kamyab, H., 2019. Bioaccumulation and potential sources of metal contamination in fish species in River Ganga basin: possible human health risks evaluation. *Toxicology Reports*. 6(1):472—481. https://doi.org/10.1016/j.toxrep.2019.05.012

141. McPherson, C. A., Lawrence, G. S., Elphick, J. R. and Chapman, P. M., 2014. Development of a strontium chronic effects benchmark for aquatic life in freshwater. *Environ Toxicol Chem*, 33(11), 2472—2478. 10.1002/etc.2696

142. Misra, A., Bissessur, A., Selala, M.C. and Lebepe, J., 2024. Accumulation and health implications of arsenic, mercury, and selenium in selected freshwater fish species in the uMgeni River, South Africa. *Environmental Pollutants and Bioavailability*, *36*(1), p.2296973. https://doi.org/10.1080/26395940.2023.2296973

143. Mitra, S., Chakraborty, A.J., Tareq, A.M., Emran, T.B., Nainu, F., Khusro, A., Idris, A.M., Khandaker, M.U., Osman, H., Alhumaydhi, F.A. and Simal-Gandara, J., 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. *Journal of King Saud University-Science*, *34*(3), p.101865. https://doi.org/10.1016/j.jksus.2022.101865

144. M'kandawire, E., Choongo, K., Yabe, J., Mwase, M., Saasa, N., Nakayama, S.M., Bortey-Sam, N. and Blindauer, C.A., 2017. Sediment metal contamination in the Kafue River of Zambia and ecological risk assessment. *Bulletin of environmental contamination and toxicology*, *99*, pp.108—116.

145. MOHSAC, 2006. China, peoples Republic of China's maximum levels of contaminants in foods. GAIN Report No. CH6064. USDA Foreign Agricultural Service: Beijing, China. p 11.

146. Mokhtar, M. B., Aris, A. Z., Munusamy, V. and Praveena, S. M., 2009. Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis* spp. in selected aquaculture ponds of high densities development area. *European Journal of Scientific Research*, 30(3), 348—360.

147. Molekoa, M.D., Kumar, P., Choudhary, B.K., Yunus, A.P., Kharrazi, A., Khedher, K.M., Alshayeb, M.J., Singh, B.P., Minh, H.V.T., Kurniawan, T.A. and Avtar, R., 2022. Spatio-temporal variations in the water quality of the Doorndraai Dam, South Africa: An assessment of sustainable water resource management. *Current Research in Environmental Sustainability*, *4*, p.100187. https://doi.org/10.1016/j.crsust.2022.100187

148. Monteiro, S.M., Mancera, J.M., Fontaínhas-Fernandes, A. and Sousa, M., 2011. Copper-induced alterations of biochemical parameters in the gills and plasma of

*Oreochromis niloticus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 154(1), 14—21. https://doi.org/10.1016/j.cbpc.2011.01.005

149. Moon, J.Y., Eom, S.Y., Seo, J.W., Lee, J.E., Choi, B.S., Kim, H., Hong, Y.S., Chang, J.Y., Jeon, M.J., Park, W.J. and Sakong, J., 2021. Effects of exposure to lead and cadmium on health of inhabitants of abandoned metal mine area in Korea. *Archives of Environmental Contamination and Toxicology*, *80*, pp.490—498.

150. Moore, J. W., Yeakel, J. D., Peard, D., Lough, J., and Beere, M., 2019. Life-history diversity and its importance to population stability and persistence of a migratory fish: steelhead in the SFS Basin, Alaska. *Ecological Monographs*, 89(3), e01335.

151. Murphy, C. A., Arnot, J. A., and Mackay, D., 2018. The toxicological impacts of pollutants on aquatic biota. *Environmental Science & Technology*, 52(11), 6792-6801.

152. Nasri, H., Abdellaoui, S., Omari, A., Kada, O., Chafi, A., Hammouti, B., and Chaabane, K., 2021. Length-weight Relationship and Condition Factor of *Trachurus Trachurus* Found in The Central-East Region of The Moroccan Mediterranean. *Indonesian Journal of Science & Technology*, 6(3): pp. 457 —468.

153. Naz, S., Hussain, R., Ullah, Q., Chatha, A.M.M., Shaheen, A. and Khan, R.U., 2021. Toxic effect of some heavy metals on hematology and histopathology of major carp (*Catla catla*). *Environmental science and pollution research*, *28*, pp.6533-6539.

154. Naz, M., Ghani, M.I., Sarraf, M., Liu, M. and Fan, X., 2022. Ecotoxicity of nickel and its possible remediation. In: Bhat, R.A., Tonelli, F.M.P., Dar, G.H., Hakeem, K. (Eds.), Phytoremediation. Academic Press, pp. 297—322.

155. Ndubi, D., Oyaro, N., Giathane, F. and Affulo, A., 2015. Determination of physico-chemical properties of sources of water in Narok, North sub country, Kenya, *Int. J. Env. Sciences*, 4(1). 47—51.

156. Nibamureke, U.M.C., Barnhoorn, I.E.J. and Wagenaar, G.M., 2016. Health assessment of freshwater fish species from Albasini Dam, outside a DDT-sprayed area in Limpopo province, South Africa: A preliminary study. *African Journal of Aquatic Science*, *41*(3), pp.297—308.

157. Nzeve, J.K. and Kitur, E., 2019. Bioaccumulation of Heavy Metals in Common carp (Cyprinus carpio) of Masinga Dam, Kenya. *IOSR J Environ Sci Toxicol Food Technol*, *13*(8), pp.23—28. https://doi.org/10.2989/16085914.2016.1172198

158. Oberholster, P.J. and Ashton, P.J., 2008. State of the nation report: An overview of the current status of water quality and eutrophication in South African rivers and reservoirs. *Parliamentary Grant Deliverable. Pretoria: Council for Scientific and Industrial Research (CSIR)*, p.2006.

159. Ojelabi, S.A., Agbede, O.A., Wahab, B.A., Aiyelokun, O.A. and Ojelabi, O.A., 2018. Water quality assessment of Eleyele Dam, Ibadan, South-Wetern, Nigeria. *Civil and Environmental Research*, 10(8): 52—59.

160. Okito, G.M., Agbohessi, P., Matunguru, J., Micha, J.C., Ntakimazi, G., Nshombo, V., Mbalassa, M., Jauniaux, T., Mandiki, R. and Kestemont, P., 2023. Histopathological disturbances in two fish species *Oreochromis niloticus* (Linnaeus 1758) and *Labeo barbus pellegrini* (Bertin et Estève 1948) from downstream mining sites in the upper Ulindi and Elila river basins, Eastern DR Congo. *Biolife International Quarterly Journal of Biology & Life Sciences*, *11*. https://hdl.handle.net/2268/307482

161. Pan, B., Wang, Y., Li, D., Wang, T. and Du, L., 2022. Tissue-specific distribution and bioaccumulation pattern of trace metals in fish species from the heavily sediment-laden Yellow River, China. *Journal of Hazardous Materials*, *425*, p.128050. https://doi.org/10.1016/j.jhazmat.2021.128050

162. Panda, P.K., Panda, R.B. and Dash, P.K., 2017. The study of PhysicoChemical and Bacteriological Parameters of River Salandi and Assessment of Water Quality from Hadagada dam to Akhandalmani, Bhadrak, Odisha, India, *IOSR J. of Environ Sci, Toxicology & Food Tech*., 11(4), Ver II, 31—52.

163. Panda, P.K., Panda, R.B. and Dash, P.K., 2018. The river water pollution in India and abroad: A critical review to study the relationship among different physico-chemical parameters. American Journal of Water Resources, 6(1), pp.25—38. https://doi.org/10.12691/ajwr-6-1-4

164. Panda, B.P., Mohanta, Y.K., Parida, S.P., Pradhan, A., Mohanta, T.K., Patowary, K., Mahari, W.A.W., Lam, S.S., Ghfar, A.A., Guerriero, G. and Verma, M., 2023. Metal pollution in freshwater fish: A key indicator of contamination and carcinogenic risk to public health. *Environmental Pollution*, *330*, p.121796. https://doi.org/10.1016/j.envpol.2023.121796

165. Pandit, D.N. and Gupta, M.L., 2019. Hepatosomatic index, gonadosomatic index and condition factor of *Anabas testudineus* as bio-monitoring tools of nickel and

chromium toxicity. *International Journal of Innovations in Engineering and Technology*, *12*(3), pp.25—28.

166. Plessl, C., Gilbert, B.M., Sigmund, M.F., Theiner, S., Avenant-Oldewage, A., Keppler, B.K. and Jirsa, F., 2019. Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. *Science of the Total Environment*, *659*, pp.1158—1167. https://doi.org/10.1016/j.scitotenv.2018.12.442

167. Plew, D.R., Zeldis, J.R., Shankar, U. and Elliott, A.H., 2018. Using simple dilution models to predict New Zealand estuarine water quality. *Estuaries and Coasts*, *41*(6), pp.1643—1659.

168. Prakash, S. and Verma, A.K., 2019. Length-weight relationship and Condition factors of freshwater fishes of Baghel Taal of Bahraich (U.P.), *India. J. Exp. Zool. India*. 22(1):343—345.

169. Prashanth, L., Kattapagari, K.K., Chitturi, R.T., Baddam, V.R.R. and Prasad, L.K., 2015. A review on role of essential trace elements in health and disease. *Journal of Dr. YSR University of Health Sciences*, *4*(2), pp.75—85.

170. Proshad, R., Islam, M.S., Kormoker, T., Masud, E.M. and Ali, M.M., 2018. Assessment of toxic metals contamination with ecological risk of surface water and sediment of Korotoa River in Bangladesh. *Int J Adv Geosc*, 6:214—221. https://doi.org/10.14419/ijag.v6i2.13742

171. Proshad, R., Kormoker, T., and Islam, S., 2019. Distribution, source identification, ecological and health risks of heavy metals in surface sediments of the Rupsa River. *Toxin reviews*, 40, 1—25.

172. Proshad R., Islam S., Tusher T. R., Zhang D., Khadka S., Gao J., and Kundu S., 2021. Appraisal of heavy metal toxicity in surface water with human health risk by a novel approach: a study on an urban river in vicinity to industrial areas of Bangladesh, *Toxin Reviews*. **40**, no. 4, 803—819.

173. Prudencio, A., Chabi, O., Edéya Orobiyi Rodrigue, P., Dzorgbe Agbeko Precious, M., Nicresse Léa, G., Ibrahim, I.T. and Antoine, C., 2023. Assessment of the health status of Lake Nokoué (Benin, West Africa) exposed to multiple pollutants during flood season. *Chemistry and Ecology*, *39*(7), pp.726—752. https://doi.org/10.1080/02757540.2023.2253225

174. Rahman, M. S., Molla A. H., Saha N. and Rahman, A., 2012. Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, *Food Chemistry*. **134**, no. 4, 1847—1854.

175. Rahman, M.A., Hasegawa, H., Rahman, M.M. and Miah, M.A., 2012. Accumulation of arsenic in tissues of freshwater fish and its relationship with arsenic levels in water. *Environmental Science and Pollution Research,* 19(4), 1343—1351.

176. Rahman, M.M., Asaduzzaman, M. and Naidu, R., 2013. Consumption of arsenic and other elements from vegetables and drinking water from an arsenic-contaminated area of Bangladesh. *Journal of hazardous materials*, 262, 1056—1063.

177. Raknuzzaman, M., Ahmed, M.K., Islam, M.S., Habibullah-Al-Mamun, M., Tokumura, M., Sekine, M. and Masunaga, S., 2016. Assessment of trace metals in surface water and sediment collected from polluted coastal areas of Bangladesh. *Journal of water and environment technology*, *14*(4), pp.247—259. https://doi.org/10.2965/jwet.15-038

178. Rakocevic, J., Sukovic, D. and Maric, D., 2018. Distribution and relationships of eleven trace elements in muscle of six fish species from Skadar Lake (Montenegro). *Turkish Journal of Fisheries and Aquatic Sciences*, *18*(5), pp.647—657.

179. Rana, A, and Kaur, R. (2023). Length-weight relationships and condition factors of three freshwater fish species – *Bangana dero* (Ham,), *Cyprinus carpioL*., and *Sperata seenghala* (Sykes) – from the Sutlej River, India. *Fisheries and Aquatic Life*, 31: 225 — 232.

180. Rasmussen, J.B. and Anderson, A., 2021. Ecotoxicology of freshwater ecosystems: assessing the impact of toxic substances on aquatic biota. *Environmental Pollution*, 271, 116253.

181. Ray, S. and Vashishth, R., 2024. From Water to Plate: Reviewing the Bioaccumulation of Heavy Metals in Fish and Unraveling Human Health Risks in the Food Chain. *Emerging Contaminants*, p.100358.

182. Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J. and Smol, J.P., 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological reviews*, *94*(3), pp.849—873. https://doi.org/10.1111/brv.12480

183. Roberts, R. (2012). Fish Pathology, 4th Edition, Blackwell Publishing Ltd, Oxford

184. Russell, D.J., Thuesen, P.A. and Thomson, F.E., 2012. A review of the biology, ecology, distribution and control of Mozambique tilapia, *Oreochromis mossambicus* (Peters 1852) (Pisces: Cichlidae) with particular emphasis on invasive Australian populations. Reviews in Fish Biology and Fisheries, 22(3), 533—554.

185. Saeed, F., Iqbal, K.J., Atique, U., Javid, A., Khan, N., Iqbal, S., Majeed, H., Azmat, H., Khan, B.Y.A., Baboo, I. and Shahid, M.T., 2020. Toxic trace metals assessment in selected organs of edible fish species, sediment and water in Head Punjnad, Punjab, Pakistan. *Punjab University Journal of Zoology*, *35*(1), pp.43—50.

186. Saha, N., Rahman, M.S., Ahmed, M.B., Zhou, J.L., Ngo, H.H. and Guo, W., 2017. Industrial metal pollution in water and probabilistic assessment of human health risk. *Journal of environmental management*, *185*, pp.70—78. https://doi.org/10.1016/j.jenvman.2016.10.023

187. Saleem, M., Iqbal, J. and Shah, M.H., 2019. Seasonal variations, risk assessment and multivariate analysis of trace metals in the freshwater reservoirs of Pakistan. *Chemosphere*, 216, p.715—724. https://doi.org/10.1016/j.chemosphere.2018.10.173

188. Saleem, M., Iqbal, J., Shi, Z., Garrett, S.H. and Shah, M.H., 2022. Distribution and bioaccumulation of essential and toxic metals in tissues of Thaila (*Catla catla*) from a Natural Lake, Pakistan and its possible health impact on consumers. *Journal of Marine Science and Engineering*, *10*(7), p.933. https://doi.org/10.3390/jmse10070933

189. Santos, D., Félix, L., Luzio, A., Parra, S., Bellas, J. and Monteiro, S.M., 2021. Single and combined acute and subchronic toxic effects of microplastics and copper in zebrafish (*Danio rerio*) early life stages. *Chemosphere*, 277: 13062. https://doi.org/1016/j.chemosphere.2021.130262

190. Shabalala, A.N., Combrinck, L. and McCrindle, R., 2013. Effect of farming activities on seasonal variation of water quality of Bonsma Dam, KwaZulu-Natal. *South African Journal of Science*, *109*(7), pp.1—7. https://hdl.handle.net/10520/EJC139387

191. Sharma, A., Grewal, A.S., Sharma, D., and Srivastav, A.L., 2023. Heavy metal contamination in water: consequences on human health and environment. *Metals in Water*. 39 —52.

192. Sharma, A.K., Sharma, M., Sharma, S., Malik, D.S., Sharma, M. and Sharma, A.K., 2024. A systematic review on assessment of heavy metals toxicity in freshwater

fish species: current scenario and remedial approaches. *Journal of Geochemical Exploration*, p.107472. https://doi.org/10.1016/j.gexplo.2024.107472

193. Singh, T.A., Meetei, N.S. and Meitei, L.B., 2013. Seasonal Variation of Some Physico-chemical Characteristics of Three Major Riversin Imphal, Manipur: A Comparative Evaluation. *Current world environment*, *8*(1), pp.93—102.

194. Singh, S. and Srivastava, A.K., 2015. Variations in hepatosomatic index (HSI) and gonadosomatic index (GSI) in fish *Heteropneustes fossilis* exposed to higher sub-lethal concentration to arsenic and copper. *Journal of Ecophysiology and Occupational Health*, pp.89—93. https://doi.org/10.15512/joeoh/2015/v15i3-4/121589

195. Singh, J., Yadav, P., Pal, A.K., 2020. Water pollutants: origin and status. In D. Pooja, P. Kumar, P. Singh, & S. Patil (Eds), Sensors in water pollutants monitoring: role of material. Advanced Functional Materials and Sensors, Gateway East, Springer. https://doi.org/10.1007/978-981- 15-0671-0_2

196. Skelton, P.H., 2001. A Complete Guide to the Freshwater Fishes of Southern Africa. Revised new edition. Struk Publishers, Cape Town, 395pp.

197. Sonarghare, P.C., Masram, S.C., Sonparote, U.R., Khaparde, K.P. and Kharkate, S.K., 2020. Causes and effects of eutrophication on aquatic life (a review). *International Journal for Environmental Rehabilitation and Conservation, XI*, pp.213—218.

198. Tadesse, H., Lakew, A., Gutema, B., Hailemichael, F. and Mehdi, M. (2024). Length-Weight Relationship and Condition of Indigenous Fish Species in the Upper Awash River, Ethiopia in relation to human activities. *Aquatic Science and Fish Resources (ASFR)*, *5*(1). https://doi.org/10.21608/asfr.2024.280782.1058

199. Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metals toxicity and environment, NIH-PA, EXS 101: 133—164. https://doi.org/10.1007/978-3-7643-8340-4_6

200. Tokatli, C., 2019. Sediment quality of Ergene River Basin: bio–ecological risk assessment of toxic metals. *Environmental monitoring and assessment*, *191*, pp.1—12. https://doi.org/10.1007/s10661-019-7885-2

201. Uddin, N. and Ghosh, S., 2021. Length-weight relation and condition factor of Coilia dussumieri (Valenciennes, 1848) in the trawl fishery off Digha, West Bengal, India. *Journal of Entomology and Zoology Studies*, *9*(2), pp.1—5.

202. Ujjania, N., Kohli, M. and Sharma, L. (2012). Length-weight relationship and condition factors of Indian major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) in Mahi Bajaj Sagar, India. *Res. J. Biol*., 2(1), 30—36.

203. US-EPA. Guidance for assessing chemical contaminant data for use in fish advisories. Washington DC: Diane Publishing; 2000. (Report No. EPA-823-B-00-008). p.156.

204. Vadde, K.K., Wang, J., Cao, L., Yuan, T., McCarthy, A.J. and Sekar, R., 2018. Assessment of water quality and identification of pollution risk locations in Tiaoxi River (Taihu Watershed), China. *Water*, *10*(2), p.183. https://doi.org/10.3390/w10020183

205. Van Dyk, J.C., Marchand, M.J., Pieterse, G.M., Barnhoorn, I.E. and Bornman, M.S., 2009. Histological changes in the gills of Clarias gariepinus (Teleostei: Clariidae) from a polluted South African urban aquatic system. *African Journal of Aquatic Science*, *34*(3), pp.283—291. https://doi.org/10.2989/AJAS.2009.34.3.10.986

206. Van Ginkel, C.E., 2011. Eutrophication: Present reality and future challenges for South Africa. *Water Sa*, *37*(5), pp.693—702. https://doi.org/10.4314/wsa.v37i5.6

207. Varol, M. and Sünbül, M.R., 2020. Macroelements and toxic trace elements in the muscle and liver of fish species from the largest three reservoirs in Turkey and human risk assessment based on the worst-case scenarios. *Environmental research*, 184, p.109298.

208. Varol, M., Kaçar, E., Sünbül, M.R. and Islam, A.R.M.T., 2022. Species, tissue and gender-related metal and element accumulation in fish species in a large reservoir (Turkey) and health risks and nutritional benefits for consumers. *Environ Toxicol Pharmacol*. 94: 103929.

209. Wagenaar, G.M. and Barnhoorn, I.E.J., 2018. Health and chemical burdens of fish species from polluted and hyper-eutrophic freshwater ecosystems in South Africa. *African Journal of Aquatic Science*, *43*(3), pp.271—280. https://doi.org/10.2989/16085914.2018.1490245

210. Waweru, K.P., Gichohi, M.P., Lilly, B., Wanjiru, N.L. and Njeru, N.P., 2024. Pathology of *Oreochromis niloticus* Due to Infections and Water Pollution in Kisumu and Homa Bay, Lake Victoria, Kenya.

211. Weber-Scannell, P.K. and Duffy, L.K., 2007. Effects of total dissolved solids on aquatic organisms: a review of literature and recommendation for salmonid species.

212. Weber, A.A., Sales, C.F., de Souza Faria, F., Melo, R.M.C., Bazzoli, N. and Rizzo, E., 2020. Effects of metal contamination on liver in two fish species from a highly impacted neotropical river: a case study of the Fundão dam, Brazil. *Ecotoxicology and environmental safety*, 190, p.110165.

213. World Health Organization (WHO), List of Maximum Levels of Heavy Metals Recommended for Fish by the Joint FOA/WHO Codex Alimentarius Commission, 2nd edition, 2003, pp. 1–8. Rome. Italy.

214. World Health Organization, 2022. *Guidelines for drinking-water quality: incorporating the first and second addenda*. World Health Organization.

215. Yan, W., Hamid, N., Deng, S., 2020. Individual and combined toxicogenetic effects of microplastics and heavy metals (Cd, Pb, and Zn) pertube gut microbiota homeostasis and gonadal development in marine medaka (*Oryzias melastigma*). *Journal of Hazardous Materials*, 397: 122795. https://doi.org/10.1016/j.jhazmat.2020.122795

216. Yancheva, V., Georgieva, E., Velcheva, I., Iliev, I., Stoyanova, S., Vasileva, T., Bivolarski, V., Todorova-Bambaldokova, D., Zulkipli, N., Antal, L. and Nyeste, K., 2022. Assessment of the exposure of two pesticides on common carp (Cyprinus carpio Linnaeus, 1758): Are the prolonged biomarker responses adaptive or destructive? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *261*, p.109446. https://doi.org/10.1016/j.cbpc.2022.109446

217. Yao, F., Livneh, B., Rajagopalan, B., Wang, J., Cretaux, J.F., Wada, Y., Berge-Nguyen, M. and Pitcher, L.H., 2021. Multi-decadal global lake volume variability impacted by climate and human activities. In *AGU Fall Meeting Abstracts* (Vol. 2021, pp. GC55K-0553).

218. Yilmaz, F., Özdemir, N., Demirak, A. and Tuna, A.L., 2007. Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbos*. *Food Chem*. 100(2), 830—835.

219. Yu, H.R., Li, L.Y., Shan, L.L., Gao, J., Ma, C.Y. and Li, X., 2021.Effect of supplemental dietary zinc on the growth, body composition and ant-oxidant enzymes of coho salmon (*Oncorhynchus kisutch*) alevins. *Aquaculture Reports*. 20, 100744. https://doi.org/10.1016/j.aqrep.2021.100744

220. Zahoor, I. and Mushtaq, A., 2023. Water pollution from agricultural activities: A critical global review. *Int. J. Chem. Biochem. Sci*, *23*(1), pp.164—176.

221. Zhong, W., Zhang, Y., Wu, Z., Yang, R., Chen, X., Yang, J., Zhu, L., 2018. Health risks assessment of heavy metals in freshwater fish in the Central and Eastern North China. *Ecotoxicology and Environmental Safety*. 157(1):343—347. https://doi.org/10.1016/j.ecoen.207.03.048

222. Zhong, W., Wang, S., Dong, Y., Ni, Z., Fan, Y. and Wu, D., 2022. Trends of the response-relationship between net anthropogenic nitrogen and phosphorus inputs (NANI/NAPI) and TN/TP export fluxes in Raohe basin, China. *Chemosphere*, *286*, p.131662.

223. Zhou, H., Yang, W.T., Zhou, X., Liu, L., Gu, J.F., Wang, W.L., Zou, J.L., Tian, T., Peng, P.Q., and Liao, B.H., 2016. Accumulation of heavy metals I n Vegetable Species planted in Contaminated Soils and the Health Risk Assessment, International Journal of Environmental Research and Public Health, 13(3): 289—300.

224. Zohra, B.S. and Habib, A., 2016. Assessment of heavy metal contamination levels and toxicity in sediments and fishes from the Mediterranean Sea (southern coast of Sfax, Tunisia). *Environmental science and pollution research*, *23*, pp.13954-13963.

225. Zulfahmi, I., Muliari, M., Akmal Y. and Batubara A.S., 2018. Reproductive performance and gonad histopathology of female Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) exposed to palm oil mill effluent, Egypt. *J. Aquat. Res*., Vol. 44, pp. 327—332.

# Appendix

**Table 4.6:** Biometric data of Coptodon rendalli and Oreochromis mossambicus sampled from the Shongweni Dam during October—December 2021.

| Species | Season | Gender | W(g) | TL (cm) | LW (g) | GW (g) | HAI | CF | HS | GSI |
|---|---|---|---|---|---|---|---|---|---|---|
| *Coptodon rendalli* | wet | F | 470 | 28 | 7.07 | 11.87 | 30 | 2.141 | 1.504 | 2.526 |
| | | M | 1310 | 40.7 | 13.75 | 6.42 | 0 | 1.943 | 1.049 | 0.490 |
| | | M | 1070 | 37.1 | 9.57 | 3.58 | 30 | 2.095 | 0.894 | 0.335 |
| | dry | M | 1070 | 37.5 | 14.47 | 1.42 | 30 | 2.029 | 1.352 | 0.133 |
| | | M | 390 | 27.6 | 2.3 | 0.01 | 30 | 1.855 | 0.589 | 0.003 |
| | | F | 960 | 40.7 | 20.5 | 19.95 | 0 | 2.671 | 2.135 | 2.078 |
| | | F | 790 | 35.5 | 12.85 | 14.66 | 60 | 1.766 | 1.627 | 1.856 |
| | | F | 630 | 31.6 | 13.47 | 14.62 | 120 | 1.997 | 2.138 | 2.321 |
| | | F | 890 | 34 | 15.69 | 14.85 | 60 | 2.264 | 1.763 | 1.669 |
| | | F | 1710 | 43.5 | 7.01 | 50 | 30 | 2.077 | 0.45 | 2.924 |
| *Oreochromis mossambicus* | wet | M | 1050 | 35 | 16.85 | 3.94 | 30 | 2.449 | 1.605 | 0.375 |
| | | M | 1460 | 41 | 15.38 | 8.31 | 30 | 2.118 | 1.053 | 0.569 |
| | | M | 1170 | 32 | 11.22 | 3.45 | 30 | 3.571 | 0.959 | 0.295 |
| | | M | 1270 | 41.2 | 15.77 | 8.3 | 30 | 1.816 | 1.242 | 0.654 |
| | | M | 1530 | 41.5 | 15.55 | 6.8 | 30 | 2.141 | 1.016 | 0.444 |
| | | F | 760 | 39 | 24.25 | 10.84 | 30 | 1.281 | 3.191 | 1.426 |
| | | F | 1000 | 38.5 | 18.71 | 36.16 | 30 | 1.752 | 1.871 | 3.616 |
| | | F | 950 | 37 | 13.34 | 19.95 | 60 | 1.876 | 1.404 | 2.100 |
| | | F | 1180 | 37 | 20.58 | 33.7 | 60 | 2.33 | 1.745 | 2.856 |
| | dry | M | 1040 | 33.7 | 14.09 | 7.87 | 30 | 2.717 | 1.355 | 0.757 |
| | | M | 1200 | 38.3 | 16.88 | 0.15 | 30 | 2.136 | 1.407 | 0.013 |
| | | M | 1220 | 40.5 | 13.26 | 3.8 | 30 | 1.837 | 1.087 | 0.312 |
| | | M | 1480 | 41.6 | 14.48 | 8.8 | 30 | 2.056 | 0.978 | 0.595 |
| | | M | 1150 | 39.5 | 14.75 | 3.7 | 30 | 1.866 | 1.283 | 0.322 |
| | | M | 1170 | 39.5 | 12.15 | 3.4 | 30 | 1.898 | 1.039 | 0.291 |
| | | M | 1370 | 40 | 13.2 | 4.62 | 30 | 2.141 | 0.964 | 0.337 |