

**ASSESSMENT OF HEAVY METALS AND THEIR
IMPACTS ON AQUATIC ECOSYSTEMS AND RIVER
HEALTH IN THE MOHOKARE RIVER USING SASS-5**

BY

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DECLARATION

The work contained in this dissertation was carried out and completed by SEPHOOKO SEPONO, 200500571 at the National University of Lesotho Water Institute, National University of Lesotho. I hereby declare that this study constitutes my original work and has never been submitted for the award of a degree or diploma to any University. To the best of my knowledge this dissertation contains no material written by another person except where due reference is made in the dissertation itself.

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LIST OF ACRONYMS:

Acronym	Full Term
AAS	Atomic Absorption Spectroscopy
ASPT	Average Score Per Taxon (in SASS-5)
Cu	Copper
Fe	Iron
Mn	Manganese
Pb	Lead
PCA	Principal Component Analysis
SASS-5	South African Scoring System version 5
Zn	Zinc

ABSTRACT

To improve freshwater monitoring in Southern Africa, this study recommends integrating chemical analysis with biological biomonitoring frameworks like SASS-5 to detect ecological degradation early. This investigation assessed heavy metal contamination and its ecological impacts on the Mohokare River, a transboundary river flowing between Lesotho and South Africa. Sampling was conducted at six strategically selected sites: Matsoaing (control site in Butha-Buthe), Matlakeng, Mohloka-qala, Ha Fusi, Ha Setho, and Bolikela (in Mafeteng). These sites represent gradients of pollution from upstream pristine conditions to downstream urban and agricultural zones.

Water and sediment samples were analyzed for Fe, Zn, Cu, Mn, and Pb using Atomic Absorption Spectroscopy (AAS). Sediment extraction involved Van Veen grab sampling and aqua regia digestion (HNO₃ and HCl) under controlled heating. Biological assessments were conducted using the South African Scoring System version 5 (SASS-5) and Average Score Per Taxon (ASPT) metrics, with macroinvertebrates sampled via standardized kick sampling.

Results showed that Pb concentrations in water exceeded the South African aquatic ecosystem standard (0.01 mg/L) at all sites, reaching up to 0.065 mg/L at Bolikela. Fe concentrations also surpassed acceptable levels (0.3 mg/L) at four sites, peaking at 2.45 mg/L. In contrast, sediment-bound metal concentrations remained within Canadian Sediment Quality Guidelines. The cleanest site, Matsoaing, recorded the highest SASS-5 (104) and ASPT (7.43) scores, indicating excellent ecological condition, while Ha Setho and Bolikela, located downstream of Maseru, had the lowest scores (SASS-5: 38 and 46; ASPT: 4.22 and 4.60 respectively).

Multivariate analyses including Spearman's correlation and Principal Component Analysis (PCA) revealed strong negative correlations between Pb, Fe, and biological indices. These statistical insights helped pinpoint pollution sources and demonstrated a spatial pattern aligning with land-use impacts, especially urban effluents near Maseru and agricultural runoff downstream.

This study demonstrates that elevated heavy metal concentrations, particularly Pb, are linked to macroinvertebrate diversity loss and deteriorating river health. It emphasizes the value of combined chemical-biological assessments in identifying pollution hotspots and guiding catchment management in data-scarce, Transboundary Rivers.

Key words:

Mohokare River, Heavy Metals, SASS-5, Macroinvertebrates, River Health

CHAPTER 1: INTRODUCTION

1.1 Introduction to the Dissertation

This dissertation presents a comprehensive investigation into the presence of heavy metals and their ecological impacts on the Mohokare River, a transboundary watercourse that holds both environmental and socio-economic importance for Lesotho and South Africa. The study adopts an integrated approach that combines chemical monitoring, through Atomic Absorption Spectroscopy (AAS), and biological monitoring, through the South African Scoring System version 5 (SASS-5), to assess concentrations of selected heavy metals, namely lead (Pb), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn), in both water and sediment. Furthermore, the study evaluates the biological responses of aquatic macroinvertebrate communities, which serve as sensitive indicators of river health.

Heavy metals are among the most hazardous and persistent pollutants in freshwater systems, as they do not degrade and tend to bioaccumulate in aquatic organisms and sediments, causing long-term ecological damage (Ali et al., 2019; Ytreberg et al., 2022). Their presence in rivers poses a major threat to biodiversity, ecosystem functioning, and human health (Dallas & Day, 2004; Gerhardt et al., 2011).

The Mohokare River is particularly vulnerable due to multiple pollution pressures, including urban runoff, industrial discharge, especially from textile factories in Maseru, agricultural runoff, and untreated or poorly treated wastewater. These stressors contribute to increasing levels of contamination and potential ecological degradation, necessitating urgent and informed intervention.

Previous studies on the Mohokare River have mostly focused on physicochemical water parameters and rarely explored the biological implications of pollution (Moyo et al., 2019; Chetty & Pillay, 2019; Mmolawa et al., 2023). This dissertation addresses this gap by adopting a dual-assessment framework that links chemical data with biological health indicators. The integration of these methods provides a more holistic understanding of river health and offers evidence for policy development and environmental management in transboundary river systems.

The dissertation is organized into six chapters. Chapter One introduces the study by presenting the background, problem statement, research objectives and questions, hypothesis, significance, and

the theoretical and conceptual frameworks that underpin the investigation. It outlines the rationale for assessing river health through a combined chemical and biological lens, particularly in resource-constrained contexts like Lesotho.

Chapter Two provides a detailed literature review. It synthesizes findings from global and regional research on heavy metal pollution in freshwater ecosystems, highlights the impact of metals on aquatic organisms, particularly macroinvertebrates, and evaluates existing monitoring approaches. The chapter also identifies the research gap that this study addresses, emphasizing the underutilization of integrated monitoring frameworks in Lesotho.

Chapter Three outlines the methodology employed in the study. It describes the study area, the sampling design for water, sediment, and macroinvertebrates, and the laboratory procedures used for analyzing metal concentrations. It also explains the statistical techniques used to interpret the data, including correlation analysis and Principal Component Analysis (PCA), which allow for the exploration of relationships between environmental variables and biological responses.

Chapter Four presents the results of the study. It includes a comprehensive display of findings in the form of tables, figures, and graphs that illustrate spatial variations in metal concentrations and biological indices across the six sampling sites. The chapter also shows the outcomes of statistical analyses that reveal patterns and associations between pollutants and macroinvertebrate diversity and abundance.

Chapter Five provides a detailed discussion of the results. It interprets the findings in light of established environmental guidelines and ecological theory, comparing them with relevant studies in the region and beyond. This chapter explores the implications of observed pollution patterns on river health, biodiversity, and ecosystem function, and emphasizes the importance of integrated monitoring in understanding cumulative ecological effects.

Chapter Six concludes the dissertation by summarizing the major findings, drawing conclusions about the state of the Mohokare River, and offering practical recommendations. These include strategies for pollution control, enhanced wastewater management, the use of biomonitoring tools like SASS-5, and the need for transboundary cooperation in river management. The chapter also

identifies areas for future research, particularly regarding long-term monitoring and the assessment of additional pollutants such as trace metals or emerging contaminants.

This structure allows the dissertation to not only assess heavy metal contamination in the Mohokare River but also offer a practical framework that can be adapted for evaluating river health in other regions facing comparable environmental pressures. By integrating chemical analysis with biological monitoring, the study enhances understanding of pollutant impacts on aquatic ecosystems and contributes valuable insights for evidence-based freshwater management in Lesotho and across Southern Africa.

1.2 Background to the study:

Rivers have been the lifeblood of civilizations for millennia. From supplying fresh water to supporting agriculture, transportation, and energy generation, rivers underpin human societies and ecological systems alike. As arteries of the global hydrological cycle, rivers connect landscapes, transport nutrients and sediments, and provide critical habitats for countless aquatic organisms. Beyond their physical functions, rivers possess intrinsic ecological and cultural value. They are often spiritual symbols, centers of biodiversity, and critical components of regional climate regulation (Postel and Carpenter, 1997; Vörösmarty et al., 2010). However, despite their importance, rivers are among the most threatened ecosystems on Earth, facing pressures from pollution, habitat destruction, unsustainable water extraction, and the impacts of climate change (Gu et al., 2021; Palansooriya et al., 2022).

Among the most persistent and insidious forms of riverine pollution is heavy metal contamination. Unlike organic pollutants that may degrade over time, heavy metals such as Pb, Fe, Cu, Mn, and Zn do not break down naturally, but instead accumulate in aquatic systems over time, particularly in sediments and biota, where they can cause chronic toxicity to aquatic organisms and pose long-term ecological risks (Ali et al., 2019; Ytreberg et al., 2022; Zhang et al., 2019). The discharge of heavy metals into river systems, whether from industrial, urban, or agricultural sources, represents a profound challenge to the sustainability of freshwater ecosystems worldwide.

Globally, industrialization and urban expansion have led to escalating levels of heavy metal pollution in rivers, particularly in developing regions where regulation and wastewater treatment infrastructure may be limited or outdated (Chetty and Pillay, 2019; Mmolawa et al., 2023). In many

cities, industries such as textile manufacturing, mining, and metal processing release untreated or partially treated effluents into nearby watercourses. In rural areas, excessive use of phosphate fertilizers, pesticides, and improper waste disposal practices contribute to diffuse heavy metal pollution. Once released, these metals can bind to sediments or remain dissolved in the water column, entering food chains and affecting aquatic life through bioaccumulation and biomagnification (Chen et al., 2023; Ahamad et al., 2024). Top predators, including fish consumed by humans, often contain elevated levels of these toxic substances, posing public health risks (FAO/WHO, 2011; Tchounwou et al., 2012).

The ecological impacts of heavy metals on river systems are multifaceted. They can alter species composition, reduce biodiversity, impair reproductive success, and disrupt essential physiological functions in aquatic organisms (Dallas and Day, 2004). Macroinvertebrates, organisms such as insects, worms, snails, and crustaceans, are particularly vulnerable. These creatures form the foundation of aquatic food webs and play a vital role in nutrient cycling, organic matter decomposition, and energy transfer within freshwater ecosystems. Their sensitivity to pollution makes them valuable bioindicators for assessing the ecological health of rivers (Dickens and Graham, 2002; Gerhardt et al., 2011).

Recognizing the limitations of relying solely on chemical analysis to assess water quality, scientists and environmental managers increasingly advocate for biological monitoring approaches. These methods go beyond detecting the presence of pollutants to understanding their actual impact on living organisms. Biological indicators reflect the cumulative and chronic effects of pollution and are especially important in capturing low-concentration, long-term exposure that might not exceed regulatory thresholds but still cause significant ecological harm (Zhou et al., 2022; Zhang et al., 2019). Macroinvertebrate-based indices, such as the South African Scoring System (SASS-5), are among the most widely used and scientifically validated tools for this purpose (Dickens and Graham, 2002; Ollis et al., 2006).

The SASS-5 methodology assigns sensitivity scores to different macroinvertebrate families based on their tolerance to pollution. The resulting composite score and Average Score Per Taxon (ASPT) provide a rapid and cost-effective measure of river health. The use of SASS-5 is particularly advantageous in African contexts, where financial and technical resources for comprehensive chemical testing may be limited. By identifying shifts in community composition,

loss of sensitive species, and dominance of tolerant taxa, SASS-5 helps reveal the ecological consequences of pollution events, even when chemical concentrations appear acceptable (Ahamad et al., 2024; Dallas, 2008).

The growing body of evidence demonstrates that chemical and biological assessments, when used together, offer a more robust and holistic understanding of river conditions. For example, a river may meet national water quality guidelines based on laboratory measurements, yet show declining biodiversity and altered ecological function due to chronic exposure to pollutants. Integrating heavy metal concentration analysis with macroinvertebrate biomonitoring addresses this disconnect by linking contaminant levels with biological responses. Such integrated monitoring frameworks are crucial for informing evidence-based river management strategies, including pollution source control, habitat restoration, and ecological risk assessment (Palansooriya et al., 2022; Gu et al., 2021).

Against this global and continental backdrop, the Mohokare River, known as the Caledon River in South Africa, emerges as a watercourse of exceptional ecological and socio-economic importance. Flowing through the Kingdom of Lesotho and forming a significant part of the international border with South Africa, the Mohokare is a transboundary river with shared ecological resources and responsibilities. Originating in the highlands of Lesotho, the river passes through urban, peri-urban, and agricultural landscapes before joining the Orange River system. It supports domestic water supply, irrigation, livestock farming, and local fisheries, while also providing critical habitat for aquatic organisms (Moyo et al., 2019).

However, the Mohokare River faces increasing environmental stress. The city of Maseru, Lesotho's capital, discharges significant volumes of municipal wastewater and industrial effluent, particularly from the textile sector, directly or indirectly into the river. Inadequate wastewater treatment infrastructure, informal settlements with limited sanitation, stormwater runoff, and agricultural runoff further exacerbate the problem (Chetty and Pillay, 2019; Kiedrzyńska et al., 2014). These diverse and poorly regulated pollution sources contribute to the input of heavy metals and other contaminants into the river system, with potentially serious consequences for aquatic biodiversity and human health downstream.

Despite the river's importance, localized and integrative studies examining both chemical contamination and its biological effects remain limited. Most existing assessments of the Mohokare River focus solely on chemical parameters, such as pH, turbidity, and nutrient levels, without examining how these parameters affect aquatic life. Even fewer studies attempt to map the spatial variation of heavy metal concentrations in both water and sediment or investigate how these concentrations relate to ecological health (Mmolawa et al., 2023). This lack of comprehensive monitoring makes it difficult to detect ecological degradation early, identify pollution hotspots, or implement effective remediation efforts.

Moreover, the transboundary nature of the river complicates management and monitoring responsibilities. Pollution originating upstream in Lesotho affects downstream users in South Africa, and vice versa. The Mohokare River flows in close proximity to major urban areas in Lesotho, such as Maseru, where population density along its banks is high, exacerbating pollution pressures from domestic waste, industrial discharges, and urban runoff. Without standardized, evidence-based monitoring protocols adopted on both sides of the border, managing river health becomes fragmented and reactive rather than coordinated and preventative (Moyo et al., 2019). The Mohokare River thus represents both a challenge and an opportunity: a challenge in terms of rising pollution and inadequate data, but an opportunity to implement integrated, science-based monitoring approaches that can inform joint conservation and policy actions.

In the broader Southern African context, the assessment of river health is becoming increasingly important due to the mounting environmental challenges posed by climate change, population growth, and unplanned urban expansion (Postel and Thompson, 2005). Lesotho, like many countries in the region, is grappling with the dual pressures of developmental progress and environmental sustainability. Urbanization, particularly in cities such as Maseru, has not kept pace with the expansion of sanitation and wastewater infrastructure. As a result, untreated or poorly treated wastewater continues to find its way into natural waterways, including the Mohokare River (Chetty and Pillay, 2019). Simultaneously, increased demand for agricultural production has led to the more intensive use of agrochemicals, including fertilizers and pesticides that often contain trace metals (Palansooriya et al., 2022). In the absence of strong regulatory enforcement and coordinated catchment management, these pollutants are mobilized into rivers during rainfall events through surface runoff, leading to cumulative ecological degradation over time.

The ecological consequences of such contamination are most apparent in the biodiversity of river systems. Macroinvertebrate communities are among the first to respond to changes in water quality. Their relatively limited mobility, combined with their diverse ecological roles and differential sensitivity to pollutants, makes them reliable sentinels of ecosystem stress (Gerhardt et al., 2011; Ahamad et al., 2024). When exposed to elevated concentrations of metals such as *Cu* and *Pb*, sensitive taxa such as Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) tend to decline or disappear altogether. These losses alter trophic dynamics and reduce functional diversity, potentially triggering cascading effects throughout the food web, including reductions in fish populations and changes in nutrient cycling (Zhang et al., 2019).

Furthermore, the Mohokare River's transboundary status presents both unique opportunities and serious risks. Transboundary rivers can serve as a platform for regional cooperation, data sharing, and joint management initiatives. However, they can also become flashpoints for conflict when water quality deteriorates and shared resources become polluted (Moyo et al., 2019). Without coordinated monitoring protocols and transparent data collection, it is difficult for either country to accurately assess the state of the river or to allocate responsibility for pollution sources. As climate change intensifies and rainfall patterns become more erratic, the stakes for water security and ecological resilience are only going to increase. In this regard, the need for robust, science-driven monitoring becomes urgent, not only to understand current conditions but also to anticipate future challenges (Dallas, 2008).

Despite growing awareness of river pollution, existing monitoring frameworks in Lesotho remain largely reactive and chemically focused, often overlooking the biological consequences of contamination (Dallas, 2008; Mmolawa et al., 2023). This narrow approach limits the ability to understand how pollutants, particularly heavy metals, affect aquatic ecosystems over time. Furthermore, the absence of integrated monitoring impedes efforts to link observed pollution to specific sources or land-use practices, especially in a transboundary river system like the Mohokare, where multiple jurisdictions share responsibility (Moyo et al., 2019; Vörösmarty et al., 2010).

Addressing these challenges calls for a more holistic and evidence-based monitoring strategy that incorporates both chemical analysis and ecological indicators (Palansooriya et al., 2022; Gu et al., 2021). By combining metal concentration measurements with macroinvertebrate-based biological

assessments, researchers and policymakers can gain a clearer understanding of the river's ecological condition and the cumulative impacts of pollution (Zhou et al., 2022; Ahamad et al., 2024). Such an approach is not only critical for identifying pollution hotspots and ecological degradation but also for informing effective river management strategies. This study is positioned within that context, aiming to bridge the gap between environmental monitoring and ecological relevance through an integrated assessment of heavy metals and their biological impacts in the Mohokare River.

1.3 Problem Statement

Freshwater ecosystems in sub-Saharan Africa are increasingly threatened by pollution, with heavy metals ranking among the most persistent contaminants. These pollutants originate from untreated urban wastewater, industrial discharges, and agricultural runoff, and are often released into rivers with little or no treatment. Once introduced, heavy metals accumulate in sediments and aquatic organisms, posing long-term risks to biodiversity, ecosystem functioning, and human health (Ali et al., 2019; Palansooriya et al., 2022).

The Mohokare River, also known as the Caledon River, is a transboundary watercourse flowing through Lesotho and along the border with South Africa. It is an important source of freshwater for domestic consumption, irrigation, and ecological support. However, the river faces increasing contamination from untreated municipal effluents in Maseru, discharges from textile industries, and diffuse agricultural inputs (Chetty and Pillay, 2019; Moyo et al., 2019). These activities introduce heavy metals such as Pb, Cu, Fe, Mn, and Zn, which degrade water quality and threaten aquatic organisms.

The challenge is compounded by inadequate monitoring and enforcement of water quality standards. Existing studies of the Mohokare River have largely focused on physicochemical parameters and, when heavy metals are analyzed, the scope is geographically limited or temporally constrained (Mmolawa et al., 2023). Very few studies have incorporated biological indicators, such as macroinvertebrate communities, which provide ecologically meaningful evidence of pollution effects. Without integrated assessments, management decisions are often based on chemical data alone, overlooking the cumulative ecological stress reflected in biodiversity loss, trophic imbalance, and disrupted ecosystem processes (Dallas and Day, 2004; Zhou et al., 2022).

The river's transboundary status introduces further complexity. Pollution originating upstream in Lesotho impacts downstream communities in South Africa, yet the absence of harmonized monitoring frameworks reduces accountability and weakens governance (Vörösmarty et al., 2010). Additionally, sediments, which act as sinks for heavy metals, are seldom analyzed despite their role in remobilizing contaminants during floods or pH fluctuations, creating renewed threats to aquatic ecosystems (Palansooriya et al., 2022).

In Lesotho, biological monitoring tools such as the South African Scoring System version 5 (SASS-5) remain underutilized, even though they are cost-effective and well validated for assessing river health (Dickens and Graham, 2002; Ollis et al., 2006). The lack of integrated studies that combine water and sediment chemistry with macroinvertebrate-based biomonitoring leaves significant gaps in understanding the spatial distribution of metals and their ecological impacts.

Therefore, there is an urgent need for a holistic investigation of the Mohokare River that links heavy metal contamination with biological responses. Such an assessment is essential to provide evidence for pollution control, inform transboundary water governance, and safeguard the ecological integrity of one of Lesotho's most important rivers.

1.4 Main Objective:

To conduct an integrated assessment of heavy metal contamination and its ecological effects on the Mohokare River by quantifying metal concentrations in water and sediment and evaluating river health using macroinvertebrate-based biomonitoring through the South African Scoring System version 5 (SASS-5).

1.5 Objectives:

- To determine the concentrations of selected heavy metals (Fe, Cu, Zn, Mn, and Pb) in water and sediment samples collected from different sites along the Mohokare River to establish baseline data for comparison with environmental guidelines.
- To assess the diversity and abundance of macroinvertebrate communities using the South African Scoring System version 5 (SASS-5) as a measure of river health.
- To evaluate the relationship between heavy metal concentrations and macroinvertebrate-based biological indices across spatially distributed sampling sites.

In pursuit of these objectives, this study adopts an integrated monitoring framework that combines chemical and biological assessment methods. Atomic Absorption Spectroscopy (AAS) is used to quantify heavy metal concentrations in water and sediment, offering precise, site-specific data on pollution levels. In parallel, the SASS-5 macroinvertebrate index is applied to evaluate ecological responses. This dual approach allows for a deeper understanding of how contamination translates into biological effects across different reaches of the river. It also facilitates the identification of pollution hotspots and relatively healthy areas, supporting targeted remediation efforts and policy interventions. Such an evidence-based, cost-effective framework is particularly valuable in resource-constrained contexts like Lesotho and can inform broader strategies for freshwater conservation in Southern Africa.

1.6 Research Questions:

This study aims to address the following research questions to provide a comprehensive assessment of heavy metal contamination in the Mohokare/Caledon River:

1. **Concentration and Distribution:** What are the concentrations and spatial distribution patterns of specific heavy metals (Fe, Cu, Zn, Pb and Mn) in water and sediment samples collected from various locations along the Mohokare/Caledon River?
2. **Guideline Comparison:** How do the measured heavy metal concentrations compare to established water quality guidelines and sediment quality guidelines (SQGs) for the protection of aquatic ecosystems.
3. **Ecological Status Assessment:** What is the current ecological status of the Mohokare/Caledon River, as determined through macroinvertebrate community analysis using the South African Scoring System version 5 (SASS-5) methodology, and what are the key metrics (e.g., ASPT, number of taxa) that characterize the ecological health of different reaches of the river?
4. **Correlation Analysis:** Is there a statistically significant correlation between heavy metal concentrations in water and sediment samples and key biological indicators of aquatic ecosystem health, such as SASS-5 scores, macroinvertebrate community structure metrics (e.g., EPT richness, Shannon diversity index), and the presence/absence of indicator taxa?

1.7 Hypothesis

Elevated concentrations of heavy metals (Fe, Cu, Zn, Mn, and Pb) in water and sediment negatively affect the ecological health of the Mohokare River by reducing the diversity and abundance of macroinvertebrate communities, as indicated by lower SASS-5 scores.

1.8 Significance of the Study

This study holds considerable significance both in scientific and practical terms, particularly in the context of sustainable water resource management in Lesotho and other developing countries facing similar environmental challenges. Freshwater ecosystems, especially rivers, are critical not only for biodiversity conservation but also for the livelihoods and well-being of surrounding communities (Postel & Thompson, 2005; Vörösmarty et al., 2010). Yet, growing urbanization, industrial activities, and agricultural expansion have placed these systems under increasing ecological stress. The Mohokare River, which is a vital transboundary watercourse between Lesotho and South Africa, is no exception. It faces mounting threats from multiple pollution sources, particularly heavy metals discharged through municipal and industrial effluents (Chetty & Pillay, 2019; Moyo et al., 2019). Understanding and addressing these threats is essential to ensuring long-term ecosystem integrity and human health (Gu et al., 2021).

This research is significant in that it provides a comprehensive and integrated assessment of both chemical and biological indicators of river health. While previous studies in the region have often focused on water chemistry alone, this study combines heavy metal analysis in water and sediments with macroinvertebrate-based biomonitoring using the SASS-5 protocol (Dickens & Graham, 2002; Ollis et al., 2006). This dual approach allows for a more holistic understanding of how pollutants interact with aquatic ecosystems and provides insight into the long-term biological consequences of heavy metal exposure. Such information is vital for detecting early signs of ecological degradation that might otherwise go unnoticed through chemical testing alone (Zhou et al., 2022; Gerhardt et al., 2011).

Another important contribution of this study lies in its emphasis on macroinvertebrates as indicators of ecosystem health. These organisms are known to respond sensitively to environmental changes and are widely recognized as reliable bioindicators in river monitoring (Dallas & Day, 2004; Ahamad et al., 2024). By applying the SASS-5 method, this research not only helps establish baseline biological data for the Mohokare River but also demonstrates the

practical value of biomonitoring tools that are affordable and accessible in resource-limited settings like Lesotho (Dickens & Graham, 2002; Ollis et al., 2006).

The findings of this study are expected to have policy relevance as well. By identifying pollution hotspots and quantifying the ecological impact of heavy metal contamination, the research can inform local and national decision-makers in the design of pollution control strategies, water management policies, and ecological restoration initiatives (Palansooriya et al., 2022). It also provides a scientific basis for cross-border cooperation between Lesotho and South Africa in managing shared water resources (Vörösmarty et al., 2010).

Furthermore, the study contributes to academic scholarship by filling a key gap in the literature on river health assessments in Lesotho. Despite the importance of the Mokhotlong River, there is a lack of published research that integrates chemical and biological monitoring at multiple spatial sites along its course (Mmolawa et al., 2023). The methodological framework adopted in this research can be adapted and applied in other rivers across Southern Africa, thereby enhancing regional capacity for water quality assessment and environmental protection (Gu et al., 2021; Palansooriya et al., 2022).

This study is significant because it enhances scientific understanding, supports evidence-based environmental governance, promotes the use of bioassessment in river health monitoring, and provides practical tools for managing freshwater systems in Lesotho and beyond.

CHAPTER 2: LITERATURE REVIEW

Heavy metal contamination in freshwater systems is increasingly recognized as one of the most persistent and hazardous threats to aquatic ecosystems and human well-being. These contaminants differ from organic pollutants in that they are non-biodegradable, capable of persisting for decades in sediments, and tend to bioaccumulate in aquatic organisms while magnifying through food webs. Globally, metals such as Pb, Cu, Zn, Mn, and Fe have been extensively studied, with rivers such as the Ganges, Yangtze, and Mississippi showing evidence of elevated concentrations that have impacted biodiversity, fisheries, and public health (Wu et al., 2020; Zhou et al., 2022). These studies emphasize that metals are introduced into aquatic systems through multiple sources including mining, industrial effluents, agricultural runoff, and untreated urban discharges. Their persistence makes their ecological consequences long-lasting, often resulting in physiological stress in aquatic organisms, reduced reproduction, biodiversity loss, and alterations in community structure. Sediments in particular serve as major sinks, with periodic remobilization of metals under shifting redox conditions, creating secondary pulses of contamination long after primary discharges have ceased (Ytreberg et al., 2022).

Research in Africa shows similar concerns. Freshwater ecosystems across the continent are under considerable stress due to the combined effects of rapid urbanization, industrial growth, and weak enforcement of pollution control measures. In South Africa, studies in the Olifants and Vaal Rivers have highlighted significant contamination from mining activities, with concentrations of cadmium, copper, and lead often exceeding international guidelines (Venter et al., 2013; Addo-Bediako et al., 2020). These pollutants were linked directly to shifts in macroinvertebrate assemblages, with pollution-tolerant species dominating and sensitive Ephemeroptera, Plecoptera, and Trichoptera groups disappearing from many stretches of the rivers. In the Limpopo River basin, chromium (Cr), Zn, and Cu accumulation was associated with both mining and agricultural practices (Mamba et al., 2021). East African rivers such as the Nairobi River in Kenya and West African systems like the Niger Delta rivers further demonstrate the impacts of poorly treated industrial effluents, oil pollution, and urban runoff, all leading to heavy metal contamination that threatens both aquatic ecosystems and the livelihoods of communities dependent on fisheries and irrigation (Omwenga et al., 2020; Olatunji et al., 2021). These findings demonstrate that heavy metal pollution in African rivers is widespread and often inadequately managed.

Within Lesotho, comprehensive research on heavy metals in rivers is limited, and most work has focused narrowly on physicochemical parameters such as pH, turbidity, and nutrient levels. The Mohokare River, also known as the Caledon River in South Africa, is particularly important as a transboundary watercourse that supports domestic water supply, irrigation, livestock, and fisheries for both countries. It is, however, subjected to diverse pressures, including untreated municipal wastewater from Maseru, effluents from textile industries, urban runoff, and intensive agriculture. Despite its significance, systematic studies on heavy metal contamination and their biological effects in the Mohokare remain scarce (Moyo et al., 2019; Mmolawa et al., 2023). This absence of detailed integrated assessments has left critical gaps in understanding cumulative ecological impacts, especially since heavy metals are known to exert chronic effects that are not immediately evident from chemical data alone. Regionally, other rivers that feed into the Orange-Senqu system have shown evidence of heavy metal inputs, but very few studies incorporate biological assessments to understand ecological consequences. This highlights a serious gap in Lesotho's environmental monitoring framework, which remains largely reactive and chemically focused.

A growing body of research emphasizes the importance of biomonitoring as a complement to chemical assessments. Chemical data, while essential, often fail to reflect ecological reality, as rivers may comply with water quality guidelines yet still exhibit degraded biological communities. Macroinvertebrates are particularly valuable in this regard due to their sensitivity to pollution, their relatively sedentary nature, and their critical roles in food webs and ecosystem processes such as nutrient cycling (Dickens & Graham, 2002; Gerhardt et al., 2011). Sensitive taxa such as mayflies, stoneflies, and caddisflies decline or disappear in polluted conditions, while pollution-tolerant organisms such as Chironomidae often dominate, signaling stress. The South African Scoring System version 5 (SASS-5) has been developed to use macroinvertebrate communities as indicators of river health. It assigns scores based on the diversity and sensitivity of taxa, producing composite indices such as SASS and ASPT values that provide a direct measure of ecological condition (Ollis et al., 2006; Dallas, 2008). This tool has been widely used in South Africa and neighboring countries, including Zimbabwe and Botswana, but remains underutilized in Lesotho, where water quality assessments typically emphasize chemical measures.

Recent trends point towards the adoption of integrated approaches that combine chemical and biological data. Studies from South African rivers, such as the Olifants and Vaal, have shown that

water chemistry data often indicate only moderate contamination, yet biological indices reveal severe ecological stress. This disconnect underscores the importance of linking chemical pollutant concentrations to biological outcomes. Advanced statistical methods such as Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA) are increasingly employed to detect relationships between heavy metals and ecological responses, identifying key drivers of ecological degradation (Palansooriya et al., 2022). For instance, strong negative correlations have been observed between lead concentrations and SASS scores, clearly demonstrating the impact of metal toxicity on riverine biodiversity. Such approaches not only improve scientific understanding but also inform evidence-based management interventions, including the identification of pollution hotspots and targeted remediation strategies.

Another important dimension of current research is the recognition that heavy metals rarely act alone. They often occur alongside other pollutants such as nutrients, pesticides, and microbial contaminants. These multiple stressors interact in complex ways, producing additive or synergistic effects that amplify ecological degradation. For example, nutrient enrichment can lead to algal blooms and hypoxic conditions, which destabilize sediments and remobilize metals into the water column, thereby increasing bioavailability and toxicity (Ahamad et al., 2024). Climate change further compounds these risks, as altered rainfall patterns and more frequent flood events increase pollutant mobilization from land to rivers (Gu et al., 2021). Such cumulative pressures demand integrated ecosystem-level monitoring and management. Human health implications are equally critical. Metals such as lead have no safe threshold for human exposure and are associated with severe neurological, reproductive, and developmental disorders (Chen et al., 2023). In communities that rely heavily on rivers for drinking water, irrigation, and fisheries, metal pollution presents risks that extend beyond ecology to public health and food security.

The Mohokare River, given its transboundary nature, presents both challenges and opportunities in terms of governance. Pollution generated in Lesotho has direct impacts on downstream ecosystems and communities in South Africa, and vice versa. However, current monitoring efforts are fragmented and poorly coordinated, with little harmonization of standards or protocols between the two countries. Weak institutional frameworks and inadequate enforcement of environmental regulations further exacerbate the issue. Increasingly, there is recognition that collaborative frameworks, joint monitoring programs, and community-based stewardship are required to ensure

effective management. Examples from other regions have shown that inclusive and cooperative transboundary governance can greatly enhance both the efficiency and legitimacy of environmental protection measures (Dallas, 2008; Palansooriya et al., 2022).

The gap that emerges from this body of literature is significant. Although global and regional research on heavy metals in freshwater systems is extensive, localized studies on the Mohokare River are scarce, and those that exist remain narrow in scope. There is little baseline data on heavy metal concentrations in water and sediments, limited integration of biological monitoring, and minimal application of tools such as SASS-5 to connect pollution levels with ecological outcomes. The lack of longitudinal and site-specific data hampers the ability to track trends over time or evaluate the effectiveness of interventions. Moreover, the absence of explicit connections between ecological degradation and public health risks further reduces the relevance of existing studies to policy and management. Addressing these gaps is essential, not only for advancing scientific understanding but also for guiding sustainable water resource management in Lesotho and across Southern Africa.

2.1 Theoretical framework:

The theoretical framework underpinning this study is grounded in ecotoxicology theory, the theory of multiple stressors, and ecosystem health assessment models, which together inform the understanding of how heavy metal contamination impacts aquatic ecosystems. These theories provide the scientific basis for evaluating the interaction between environmental pollutants and biological responses in riverine environments.

Ecotoxicology theory is central to this research. It is based on the premise that contaminants, including heavy metals, can cause adverse effects at various levels of biological organization, from molecular and cellular changes in individual organisms to population declines and ecosystem-level disruptions (Newman, 2015). According to this theory, organisms exposed to toxic substances exhibit responses that can be measured and used to infer environmental stress. The theory also supports the use of bioindicators such as macroinvertebrates because their physiological and behavioral responses serve as visible indicators of ecosystem health (Forbes & Calow, 2002). This provides a foundation for integrating chemical and biological assessments in river monitoring.

In addition, the theory of multiple stressors emphasizes that aquatic ecosystems are rarely affected by a single pollutant but rather by a combination of stressors that may act synergistically or antagonistically (Clements & Rohr, 2009). In the context of the Mohokare River, multiple inputs such as heavy metals, nutrients, microbial pollution, and hydrological changes interact to influence ecological conditions. This theory justifies the inclusion of sediment quality, physicochemical parameters (e.g., pH, dissolved oxygen), and land-use patterns as critical components of the broader assessment. It also supports the idea that metal toxicity cannot be fully understood without considering sediment dynamics, water chemistry, and ecological interactions.

The study is also informed by ecosystem health assessment models, which consider rivers as complex socio-ecological systems that require integrated evaluations of structure, function, and resilience (Costanza et al., 1992). Within this model, ecological indicators such as macroinvertebrate diversity, pollution-sensitive taxa, and SASS-5 scores serve as proxies for system functioning and stability. Healthy river ecosystems exhibit high biodiversity and functional redundancy, which enable them to absorb disturbances and maintain services such as nutrient cycling and habitat provision. Conversely, declining diversity and the dominance of tolerant species indicate reduced ecological resilience and potential collapse of ecosystem processes.

The application of SASS-5, a tool developed from biomonitoring theory, is also rooted in these theoretical foundations. It reflects the assumption that pollution-sensitive macroinvertebrate taxa are the first to disappear under deteriorating conditions, and therefore their presence or absence can reliably indicate river health (Dickens & Graham, 2002). By translating biological data into standardized scores, the method aligns with principles of ecological risk assessment and adaptive management.

Together, these theories support the integrated approach taken in this research, linking contaminant presence (through heavy metal analysis) to biological outcomes (through macroinvertebrate assessment), and provide a robust basis for interpreting findings and making recommendations for river management. The theoretical framework ensures that the research is not only empirically grounded but also conceptually aligned with internationally recognized approaches to ecological monitoring and environmental protection.

2.2 Conceptual Framework:

This conceptual framework illustrates the interrelationships between heavy metal pollution, aquatic ecosystem health, and the integrity of the Mohokare/Caledon River. It guides the study by mapping out how various sources of heavy metals contribute to environmental contamination, how these pollutants affect aquatic organisms, and how these effects cascade to influence the overall health of the river system.

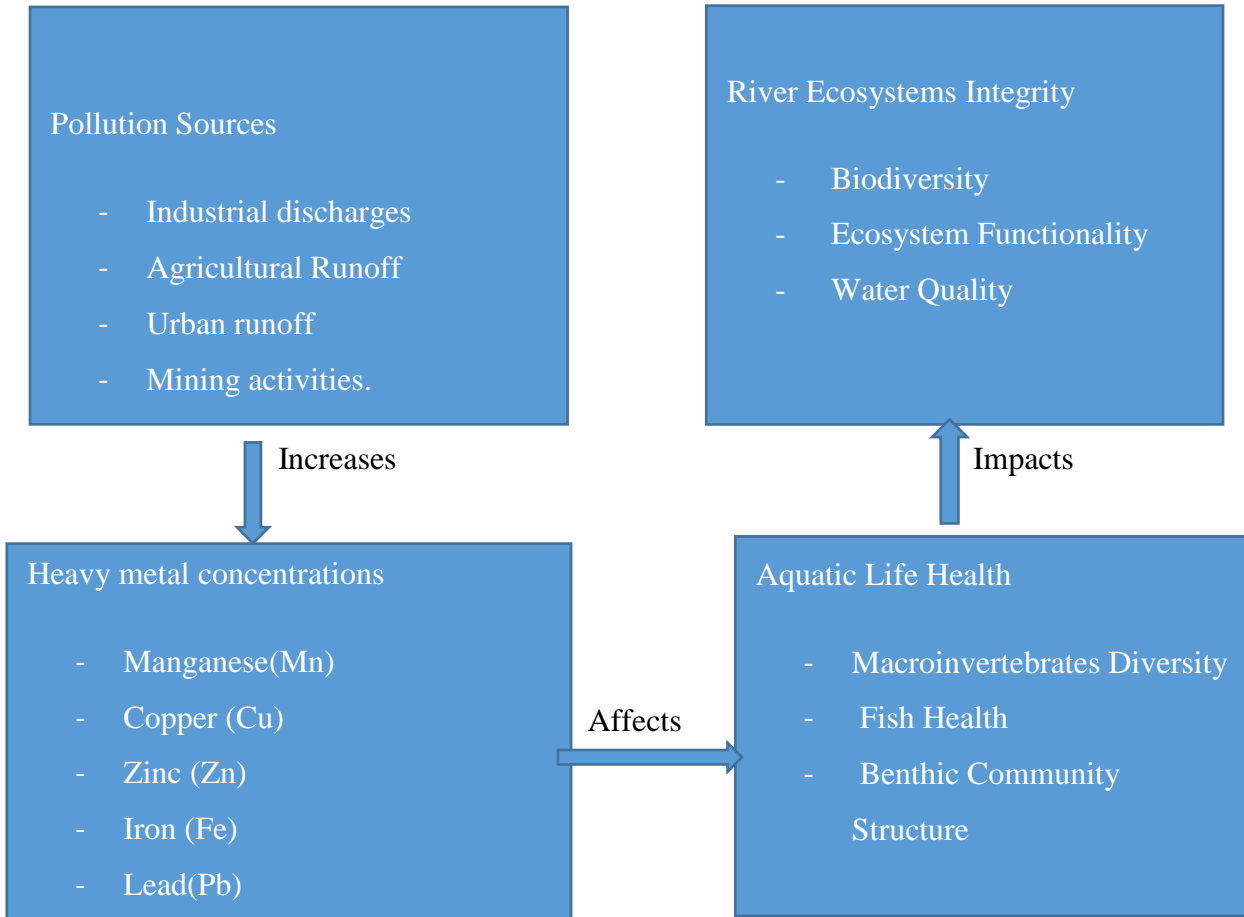


Figure 1.1: Conceptual framework illustrating the relation between pollution sources, heavy metal accumulation, and impacts on aquatic life and river ecosystem integrity in the Mohokare River

This conceptual framework outlines the causal and interconnected pathways through which heavy metal pollution impacts aquatic life and degrades the ecological integrity of the Mohokare/Caledon River. It is grounded in environmental monitoring principles that emphasize the dual assessment of contamination levels and biological health indicators to determine river ecosystem status.

The starting point of this framework is the range of anthropogenic pollution sources that discharge harmful substances into the Mohokare River. These sources include industrial effluents from textile and manufacturing operations, agricultural runoff from nearby farmlands, urban stormwater runoff, and sewage discharges from both formal and informal settlements. These activities release toxic heavy metals such as Fe, Pb, Zn, Cu, and Mn into the river, especially at points close to dense human activity.

The ecological impact of these heavy metals is assessed by evaluating the health of aquatic life, particularly macroinvertebrates, which are widely recognized as sensitive biological indicators of river health. These organisms play essential roles in the aquatic food web and respond predictably to pollution. The presence, absence, or relative abundance of certain macroinvertebrate taxa reflect the level of environmental stress caused by pollutants.

To quantify biological responses, the study employed the South African Scoring System version 5 (SASS-5). This system assigns scores based on the diversity and sensitivity of macroinvertebrate families found at each site. In this framework, a decline in SASS-5 score, Average Score Per Taxon (ASPT), and the number of sensitive taxa signifies deteriorating water quality and impaired ecological health.

The integration of chemical data (metal concentrations in water and sediment) and biological data (macroinvertebrate scores) forms the foundation of the framework. This combined approach enhances the understanding of how pollution translates into ecological degradation. While chemical measurements indicate the presence and magnitude of contaminants, biological assessments reveal the ecological consequences of that contamination.

The final component of the framework is the ecological integrity of the river ecosystem, which is evaluated through indicators such as biodiversity, ecosystem functioning, and water quality status. These are not just scientific measures, they reflect the river's ability to support aquatic life, maintain ecological processes, and provide services to surrounding communities. When biodiversity declines and sensitive species are lost, the ecosystem becomes less resilient, less productive, and more vulnerable to further disturbances.

In summary, this conceptual framework maps a clear pathway: Pollution sources lead to increased concentrations of heavy metals, which impair aquatic life, particularly macroinvertebrate

communities. These biological impacts are captured through SASS-5 biomonitoring, revealing a decline in river ecosystem integrity. The integration of chemical and biological assessments provides robust evidence for understanding and managing heavy metal pollution in the Mohokare River.

This framework not only guides the structure of the study but also offers a model for environmental monitoring in other river systems facing similar pollution pressures. It underscores the importance of evidence-based, interdisciplinary approaches to river health assessment and natural resource management.

2.3 Gap Analysis

Although numerous studies have documented the presence and effects of heavy metals in freshwater systems across Southern Africa, there remains a significant gap in localized research focused on the Mohokare/Caledon River. This transboundary river, shared between Lesotho and South Africa, plays a crucial ecological, economic, and social role for communities along its course. However, in contrast to more extensively studied river systems such as the Olifants, Vaal, and Molopo Rivers (Addo-Bediako et al., 2020; Mmolawa et al., 2023), the Mohokare River has received limited scientific attention with respect to heavy metal contamination and its ecological implications. Most available studies are either outdated, focus narrowly on physicochemical parameters, or lack a comprehensive biological assessment component. This constitutes a substantial geographical and ecological data gap, especially considering the river's vulnerability to industrial, agricultural, and urban pollution.

A major methodological gap identified in existing literature is the reliance on chemical monitoring alone to assess river health. While chemical analyses provide essential information about the presence and concentration of contaminants, they do not capture the biological or ecological consequences of pollution (Gu et al., 2021; Palansooriya et al., 2022). Heavy metals often exert chronic, sublethal, and cumulative effects on aquatic organisms that are not immediately evident through chemical analysis alone (Ahamad et al., 2024). Furthermore, metals such as lead, zinc, and copper can remain in the environment for long periods, contributing to persistent ecological stress. Therefore, there is a growing consensus in environmental monitoring literature that chemical data should be complemented with biological indicators to better understand ecosystem-level impacts (Zhou et al., 2022; Dallas & Day, 2004).

Another gap relates to the limited application of integrated chemical-biological assessment frameworks in river monitoring programs within the region. While developed countries and some parts of South Africa have increasingly adopted dual approaches combining pollutant quantification with biological assessments, such practices remain underutilized in Lesotho. Biological monitoring tools like the South African Scoring System version 5 (SASS-5) are rarely employed in tandem with heavy metal analysis, despite their demonstrated effectiveness in capturing the ecological integrity of rivers (Dickens & Graham, 2002; Ollis et al., 2006). Consequently, there is a lack of empirical evidence linking specific heavy metal concentrations with changes in macroinvertebrate community structure, SASS scores, and other biodiversity indicators in the Mohokare River.

The underutilization of macroinvertebrates as bioindicators in Lesotho's river health assessments also represents a significant research gap. Macroinvertebrates have long been recognized for their sensitivity to environmental stressors, especially heavy metal pollution, and their community composition provides reliable insights into ecological degradation (Dallas, 2008; Zhang et al., 2019). However, existing research in the region seldom quantifies or correlates heavy metal contamination with macroinvertebrate diversity metrics such as EPT richness, Shannon-Wiener index, or ASPT values. This omission limits the ability of policymakers and environmental managers to detect early signs of ecological stress and develop targeted remediation strategies.

In addition, there is a notable gap in the availability of baseline data and long-term monitoring of heavy metal pollution in the Mohokare/Caledon River. Most existing water quality reports are either episodic or lack the spatial resolution necessary to identify pollution hotspots and temporal trends (Moyo et al., 2019). The absence of site-specific, biologically informed data hampers the development of science-based environmental policies and cross-border management frameworks between Lesotho and South Africa. Furthermore, limited access to historical data restricts the ability to assess trends in pollution levels or measure the effectiveness of interventions over time.

Finally, while this dissertation does not directly assess metal speciation or long-term bioaccumulation in fish or human consumers, it acknowledges the broader ecological risks associated with the persistent nature of heavy metals in freshwater environments. These metals pose risks not only to aquatic biota but also to human health through the contamination of drinking water and bioaccumulation in food chains (Chen et al., 2023; Ytreberg et al., 2022). However,

there is a scarcity of studies that link macroinvertebrate-based assessments with potential human health implications in this region. This further underscores the need for integrated, multidisciplinary approaches that consider both ecosystem health and public safety.

This study addresses several critical gaps in the existing literature. It provides the first integrated chemical and biological assessment of heavy metal pollution in the Mohokare/Caledon River, using Atomic Absorption Spectroscopy to quantify concentrations of Fe, Zn, Pb, Mn, and Cu in water and sediment, and applying SASS-5 biomonitoring to assess macroinvertebrate diversity and ecological condition. By linking pollutant levels with biological responses, this research contributes localized, actionable data that can inform river management, pollution mitigation, and transboundary environmental governance. It sets the foundation for future long-term monitoring and highlights the importance of adopting holistic assessment strategies to safeguard river health in Lesotho and the wider Southern African region.

CHAPTER 3: METHODOLOGY

3.1 Introduction to Materials and Methods

This study adopted an integrated approach to assess heavy metal contamination in the Mohokare/Caledon River. Both chemical and biological data were collected to provide a comprehensive understanding of river health. Chemical analyses were carried out on water and sediment samples, while biological monitoring employed macroinvertebrate community assessments using the South African Scoring System (SASS-5). Heavy metal concentrations, including Fe, Zn, Cu, Mn, and Pb, were quantified using Atomic Absorption Spectroscopy (AAS), a robust single-element analytical method known for its sensitivity and precision in detecting trace levels of metals in environmental matrices. This integrated approach allowed the study to assess pollutant levels alongside ecological responses, thereby strengthening the validity of the findings.

3.2 Study area:

The study was conducted along the Mohokare/Caledon River, which flows through Lesotho and South Africa. Six sampling sites were selected near potential pollution sources, including agricultural runoff, urban developments, and sewage discharge activities. These points were chosen strategically to capture the spatial variability of heavy metal contamination and its potential ecological impacts (See Table 1). The selection was based on the river's flow path, land-use patterns, and known or suspected pollution sources. Matsoaing was designated as the upstream reference site because it is located in a relatively undisturbed area with minimal human activity, providing a baseline for comparison. Matlakeng and Ha Setho were selected to represent sections of the river influenced by urban runoff and domestic wastewater from nearby settlements, while Ha Fusi and Mohloka-qala were included due to their proximity to agricultural areas and possible inputs from irrigation return flows and agrochemical runoff. The final site, Bolikela, is situated further downstream, where the cumulative effects of urban, industrial, and agricultural discharges are expected to be most pronounced.

Table 3.1: Sampling sites and coordinates of Mohokare River

Sampling Site	Coordinates
Mohokare at Matsoaing (control)	S 28°35'43.30", E 28°36'45.99"
Mohokare at Matlakeng	S 28°43'23.12", E 28° 9'23.90"
Mohokare at Mohloka-qala	S 29° 0'21.80", E 27°41'36.66"
Mohokare at Ha Fusi	S 29° 7'54.82", E 27°37'56.32".
Mohokare at Ha Setho	S 29°23'37.68", E 27°25'19.60".
Mohokare at Bolikela	S 29°37'11.61", E 27° 3'59.04"

By choosing these sites, the study was able to represent a pollution gradient along the river continuum, ranging from relatively pristine upstream conditions to heavily impacted downstream sections. This strategic site selection allowed for the identification of pollution hotspots, assessment of land-use impacts on river health, and evaluation of the river’s natural recovery potential between disturbed areas (Moyo et al., 2019; Chetty and Pillay, 2019).

Figure 3.1 is the Google earth map of the Mohokare River with six sampling points from far upstream Matsoaing in Butha-Buthe district to Bolikela in Mafeteng.

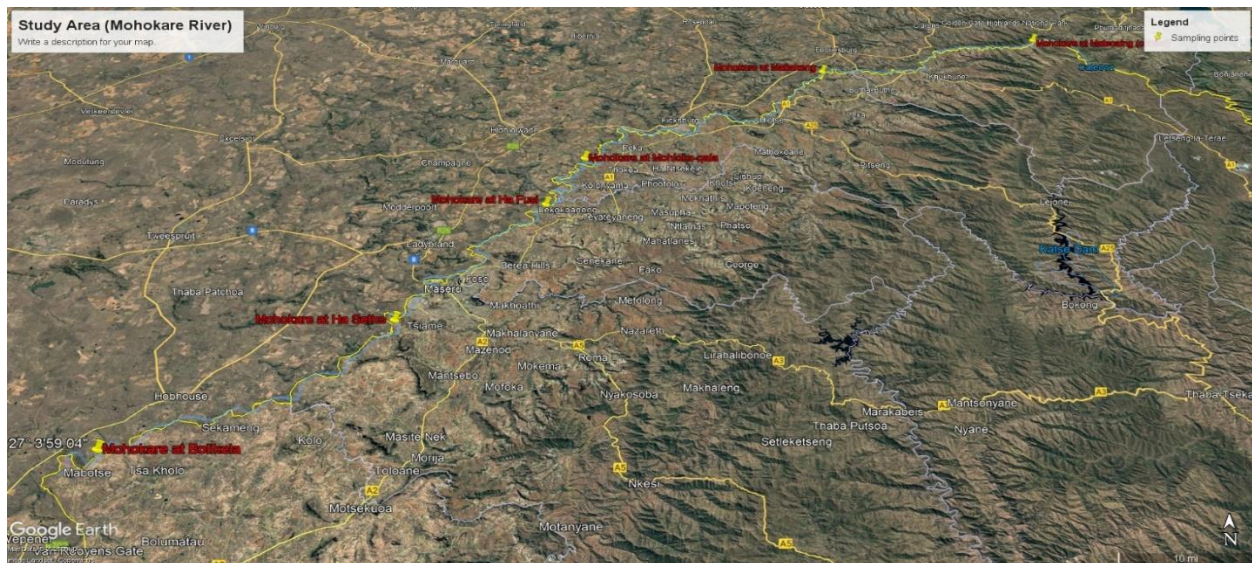


Figure 3.1: Google Earth schematic of Mohokare River with sampling points, captured on 10th February 2025

3.4 Explanation of the flow of the river

The Mohokare River originates in the highlands of Lesotho and flows in a generally south-westerly direction, forming part of the border between Lesotho and South Africa before joining the Orange River (Moyo et al., 2019). The sampling sites selected in this study follow this downstream gradient, beginning from the relatively pristine upstream control site at Matsoaing, where the river flows through sparsely populated grazing lands, and moving downstream through Matlakeng, which is influenced by urban runoff from Butha-Buthe. Further downstream, the river passes through Mohloka-qala, where mixed agricultural and domestic activities contribute to moderate pollution inputs. The flow then continues through Ha Fusi and Ha Setho, which are impacted by extensive agriculture and urban discharges, particularly from Maseru. Finally, at Bolikela, the river represents a cumulative endpoint where pollutants from upstream sources converge, demonstrating the downstream transport and accumulation of contaminants. This directional flow pattern, clearly depicted on the GIS map, highlights the influence of land-use changes and increasing anthropogenic pressure from upstream to downstream sections of the river (Chetty and Pillay, 2019; Gu et al., 2021).

3.4 Sampling Methods

3.4.1 Sampling Duration and Conditions

Sampling was conducted once in May 2025, after conditions of excess rainfall and unusually high flows. The elevated discharge made it unsafe and logistically impractical to undertake repeated or replicate sampling campaigns. Therefore, a single sampling campaign was performed at all six sites to capture the spatial variability of metal concentrations and ecological responses under these hydrological conditions.

It is acknowledged that the absence of replicate samples and procedural blanks represents a limitation of this study. However, rigorous field protocols were followed to minimize contamination risks. All sample containers were acid-washed prior to use, field equipment was rinsed thoroughly with site water before collection, and samples were transported on ice to the laboratory immediately after collection. These precautions helped to maintain the integrity of the samples despite the constraints imposed by field conditions.

3.4.2 Water and Sediment Sampling

Water and sediment samples were collected from each site using standardized grab sampling methods to ensure that representative samples were obtained. Water grab samples of one litre were collected by directly immersing pre-cleaned, acid-washed polyethylene bottles at approximately 30 cm below the surface while facing upstream to avoid contamination from surface films or debris (ISO 5667-1:2020; PacifiCorp, 2002). Sediment samples were collected using a Van Veen grab sampler, which is widely applied for benthic sediment sampling because it allows the retrieval of undisturbed surface layers up to approximately 20 cm in depth (ISO 5667-12:2017). These procedures comply with internationally recognized sampling guidelines, including ISO 5667-1:2020 for the general design of water quality sampling programs and ISO 5667-12:2017 for sediment sampling and handling, ensuring consistency and quality control throughout the sampling process. Each sample was collected in pre-cleaned, acid-washed polyethylene containers to prevent contamination and stored on ice until transportation to the laboratory (Dixit et al., 2015; Gu et al., 2021).

3.4.3 Biological Sampling

Macroinvertebrate samples were collected using the South African Scoring System (SASS-5) methodology, which involves kick sampling. A kick net was placed downstream while the substrate was disturbed upstream, allowing organisms to flow into the net. Samples were collected from multiple locations within each site to ensure representativeness. Most macroinvertebrates were identified in the field, while specimens with distinct features or unknown identification were preserved in 70% ethanol for laboratory identification and analysis (Ahamad et al., 2024).

3.4.4 Chemical Analysis of Heavy Metals using Atomic Absorption Spectroscopy (AAS)

Heavy metal concentrations in water and sediment samples were analyzed using Atomic Absorption Spectroscopy (AAS). This technique was chosen for its sensitivity and accuracy in detecting trace metals. The following heavy metals were quantified: Fe, Cu, Zn, Mn, and Pb.

3.4.5 Sample Preparation for AAS Analysis

3.4.5.1 Sample Preparation

Water and sediment samples were prepared prior to chemical analysis using digestion methods consistent with international protocols.

- **Water samples:** Each sample was filtered through a 0.45 µm membrane filter to remove particulates. A 50 mL subsample was digested with 2 mL concentrated nitric acid (HNO₃) and heated at 95 °C for 1–2 hours to ensure the oxidation of organic matter.
- **Sediment samples:** Sediments were oven-dried at 50 °C, homogenized, and finely ground. Approximately 0.5 g of material was digested with 10 mL concentrated nitric acid (HNO₃) and 2 mL hydrochloric acid (HCl, aqua regia) at 95–120 °C for three hours.

After digestion, samples were cooled, filtered through 0.45 µm membranes, and diluted to 50 mL with deionized water. They were stored at 4 °C in acid-washed polyethylene containers until AAS analysis.

3.4.6 Analysis Using Atomic Absorption Spectroscopy (AAS)

The digested water and sediment samples were analyzed using a PerkinElmer Analyst 400 Flame Atomic Absorption Spectrophotometer, fitted with hollow cathode lamps specific to each metal. The instrument was operated in flame mode (air-acetylene mixture), and the following wavelengths were used: Fe (248.3 nm), Zn (213.9 nm), Cu (324.8 nm), Mn (279.5 nm), and Pb (217.0 nm). Calibration curves were constructed using standard reference solutions, and verification was performed using quality standards at regular intervals during the analyses.

3.4.7 Biological Assessment Using SASS-5

Macroinvertebrate samples were collected from each site using the SASS-5 protocol. At each site, kick sampling was conducted in all main microhabitats, and the collected organisms were identified in the field as far as possible. Specimens that could not be identified on site were preserved in 70% ethanol and taken to the laboratory for further identification. The abundance and diversity of macroinvertebrate taxa at each site were recorded, and SASS-5 scores were calculated to assess ecological condition.

3.4.8 Quality Control and Data Analysis

While replicate sampling and procedural blanks were not possible under the prevailing field conditions, quality control measures were nevertheless applied in the laboratory. The study used the following Certified Reference Materials (CRMs):

- **NIST SRM 1643f** – trace elements in water.
- **NIST SRM 2711a** – trace elements in soil/sediment.

These CRMs were included to validate the accuracy of AAS analyses. In addition, calibration standards and method blanks were analyzed alongside samples to check for instrument drift and laboratory contamination.

Statistical analysis involved descriptive statistics to summarize concentration ranges, Spearman's rank correlation to evaluate relationships among heavy metals, and Principal Component Analysis (PCA) to identify pollution gradients and potential source patterns.

3.5 Data Analysis

3.5.1 Overview

To evaluate the impact of heavy metal contamination on the health of aquatic macroinvertebrate communities in the Mohokare/Caledon River, a comprehensive and methodologically rigorous data analysis was conducted. The analytical process combined descriptive, inferential, and multivariate statistical techniques, with careful attention given to data preprocessing and transformation to ensure accuracy, reproducibility, and interpretability of results.

3.5.2 Data Preparation and Preprocessing

The raw dataset, comprising measurements of heavy metal concentrations in water and sediment samples, as well as biological indicators such as SASS-5 scores and macroinvertebrate diversity indices, was first cleaned and prepared for analysis. All entries were reviewed for consistency, typographical errors, and missing values. Missing data were addressed using pairwise deletion in correlation analyses and mean or regression-based imputation for structured variables where gaps were minimal and ignorable under Missing Completely at Random (MCAR) assumptions.

3.5.3 Descriptive Statistics

Descriptive statistics were calculated to summarize the central tendencies and variability of the key variables. Measures such as mean, median, standard deviation, minimum, maximum, and coefficient of variation were computed for each metal concentration and macroinvertebrate index across all sampling sites. This initial overview helped identify patterns, detect anomalies, and provide context for further inferential analysis. Visual tools such as histograms and boxplots were used to display distribution shapes and spatial trends across different locations in the river system.

3.5.4 Correlation Analysis

To explore relationships between environmental contamination and biological responses, both Pearson and Spearman correlation analyses were employed. Pearson's correlation coefficients were used where variables conformed to normality assumptions after transformation, while Spearman's rank correlation was used for non-parametric associations. These analyses revealed the strength and direction of associations between individual metal concentrations and biological indicators such as SASS-5 scores and Shannon-Wiener diversity indices. Correlation matrices helped identify which metals were most strongly associated with ecological degradation.

3.5.5 Multivariate Analysis

To explore relationships between environmental variables and biological responses, multivariate and non-parametric statistical analyses were applied. Principal Component Analysis (PCA) was employed to examine patterns in heavy metal concentrations and identify dominant environmental gradients across sampling sites. This unconstrained ordination method reduced data dimensionality and revealed spatial clustering of sites based on shared contamination profiles. Standardized environmental variables were used to ensure comparability, and PCA biplots were interpreted to assess the influence of individual metals on site groupings. In parallel, Spearman's rank correlation analysis was used to evaluate the strength and direction of associations between specific heavy metals and macroinvertebrate indices (SASS-5 scores and ASPT). Together, these analyses provided a robust understanding of how pollution gradients correspond to ecological conditions in the Mohokare River.

3.5.6 Statistical Implementation in R

All analyses were performed in R, an open-source statistical computing environment. Data cleaning and summarization were carried out using functions from the Tidyverse package collection. Visualization of results, including boxplots, bar graphs, and PCA biplots, was accomplished using ggplot2, which enabled the production of clear and publication-ready graphics. Spearman's rank correlation was computed to examine associations between heavy metals and biological indices, while Principal Component Analysis (PCA) was conducted using the Vegan package to identify major environmental gradients and clustering of sampling sites based on metal concentrations.

3.5.7 Integration of Results

The integration of these analytical techniques allowed for a comprehensive interpretation of both chemical and biological datasets. Through systematic data preprocessing and the application of statistically appropriate and ecologically relevant models, the study established a robust basis for assessing the condition of the Mohokare/Caledon River. The results provided clear evidence of the impact of heavy metal contamination on aquatic biodiversity. These insights supported the formulation of evidence-based recommendations aimed at improving river health, guiding pollution control efforts, and identifying potential biological indicators for future monitoring program.

3.6 Limitations of the Study:

While this study provides valuable insights into the presence of heavy metals and their ecological impacts on the Mohokare/Caledon River, certain practical limitations were encountered due to the scope, timeframe, and resource constraints typical of field-based environmental research. These limitations do not diminish the significance of the findings but rather offer context for interpreting results and planning future studies.

3.6.1 Spatial and Temporal Coverage

Although the study included multiple sampling sites along the river, it was not feasible to capture the full extent of spatial and temporal variability due to limited time and resources. Rivers are dynamic systems, and both pollutant concentrations and macroinvertebrate communities can change in response to seasonal rainfall, episodic discharges, and human activity (Gu et al., 2021).

While short-term variability may not have been fully represented, the sampling design still enabled detection of meaningful spatial patterns in contamination and ecosystem response.

3.6.2 Availability of Historical Data

Access to consistent historical data on heavy metal concentrations and biological indicators (e.g., SASS-5 scores) was limited. Existing data from the Department of Water Affairs covered only a few sampling events across different seasons, restricting the ability to analyze long-term trends or establish historical baselines (Moyo et al., 2019). Despite this, the current study contributes essential baseline information that can support longitudinal monitoring in the future.

3.6.3 Focused Metal Selection

Due to laboratory and budgetary constraints, the study concentrated on five metals (Fe, Cu, Zn, Mn, and Pb), which are commonly associated with anthropogenic pollution and known to pose risks to aquatic life (Chen et al., 2023). However, other potentially hazardous elements such as cadmium, arsenic, or nickel were not included. Additionally, the study did not assess metal speciation, which can affect bioavailability and toxicity. A broader suite of contaminants should be explored in subsequent assessments to provide a more comprehensive risk profile.

3.6.4 Analytical Limitations

Atomic Absorption Spectroscopy (AAS), though widely used and reliable for heavy metal detection, has known limitations such as matrix interference and detection thresholds (Ytreberg et al., 2022). While rigorous sample preparation and quality control procedures were applied, minor analytical variability cannot be entirely ruled out. These limitations are unlikely to affect the overall trends observed, but should be considered when interpreting the concentration values.

3.6.5 Site-Specific Nature of Findings

This study focused specifically on the Mohokare/Caledon River under the conditions present during the sampling period. As such, the findings may not be directly generalizable to other river systems or to different hydrological contexts. However, the methods and findings can inform regional assessments and contribute to broader understanding of freshwater ecosystem health under similar pressures.

3.7 Ethical Considerations:

Ethical responsibility is fundamental to this dissertation, guiding all aspects of research design, data collection, analysis, and dissemination. The study is committed to minimizing harm to the environment, safeguarding human health, maintaining transparency, and upholding the highest standards of research integrity.

3.7.1 Environmental Ethics

The foremost ethical obligation of this research is to assess the ecological impacts of heavy metal pollution while minimizing any potential harm to the river ecosystem. All sampling methods were carefully selected and implemented to avoid unnecessary disturbance to aquatic habitats and to prevent harm to macroinvertebrate and fish populations. Standardized, minimally invasive techniques-such as kick sampling for macroinvertebrates and non-destructive water and sediment collection-were strictly followed (Dixit et al., 2015). Proper handling, storage, and disposal of samples and laboratory waste were ensured in compliance with environmental safety regulations, thereby preventing secondary contamination and upholding the principle of environmental stewardship.

3.7.2 Transparency and Reporting

Transparency in research is essential for scientific credibility and public trust. All stages of the research process, from data collection to analysis and reporting, were conducted with honesty and openness. Both positive and negative findings are reported without omission or distortion, and methodologies are described in detail to allow for reproducibility. Research results are made accessible to local authorities, stakeholders, and the broader public, fostering awareness and supporting informed discussions on environmental management (Chen et al., 2023).

3.7.3 Data Privacy and Integrity

Upholding data privacy and integrity is a critical ethical consideration in this dissertation. All data generated and collected during the study are securely stored and managed to prevent unauthorized access, loss, or tampering. Digital records are maintained with appropriate backup and security protocols. Data analysis is conducted objectively, with all procedures and results documented transparently to ensure traceability and reproducibility. Any personal or sensitive information (such as site locations that could affect landowners or local operations) is anonymized in all public

reports and publications. This approach safeguards the integrity of the research and protects the interests of all stakeholders involved.

3.7.4 Compliance with Ethical Guidelines

The research strictly adheres to ethical guidelines established by relevant institutions and regulatory bodies. All necessary permits and approvals were obtained from the Department of Water Affairs (DWA) and other authorities before commencing fieldwork. The study complies with the ethical standards set by the host institution and follows national and international codes of research ethics. Regular oversight and adherence to legal requirements ensure that the research maintains full ethical compliance throughout its duration.

This dissertation is guided by a comprehensive ethical framework that prioritizes environmental protection, transparency, human health, data integrity, and strict adherence to regulatory standards. These considerations not only safeguard the integrity of the research but also contribute to its value and impact for science, policy, and society.

CHAPTER 4: RESULTS AND DISCUSSIONS

This chapter presents the chemical and biological results from water and sediment samples, including SASS-5 bio assessment. All tables are clearly introduced, each calculation step is shown. Calculated values are presented in organized tables, with discussions immediately following each.

This section summarizes the concentrations of selected metals (Zn, Cu, Fe, Mn, Pb) in water and sediment across six sites, compares them to standards, and interprets SASS-5 ecological scores. Statistical analysis, including descriptive statistics, correlation, and principal component analysis (PCA), is performed with all steps and equations shown.

4.1 Heavy Metal Concentrations in Water

Table 4.1: Concentrations of selected heavy metals from water sampled at Mohokare River against South African water quality standards for aquatic ecosystems

Site	Zn (mg/L)	Cu (mg/L)	Fe (mg/L)	Mn (mg/L)	Pb (mg/L)	Zn Std (3)	Cu Std (2)	Fe Std (0.3)	Mn Std (0.1)	Pb Std (0.01)	Acceptability (per metal)
Matsoaing	0.010	0.013	0.451	0.005	0.091	✓	✓	✗	✓	✗	Fe, Pb above
Matlakeng	0.010	0.006	2.001	0.011	0.169	✓	✓	✗	✓	✗	Fe, Pb above
Mohloka-qala	0.024	0.012	0.414	0.004	0.239	✓	✓	✗	✓	✗	Fe, Pb above
Ha Fusi	0.011	0.014	0.157	0.009	0.335	✓	✓	✓	✓	✗	Pb above
Ha Setho	0.005	0.056	0.266	0.006	0.386	✓	✓	✓	✓	✗	Pb above
Bolikela	0.017	0.005	1.978	0.012	0.285	✓	✓	✗	✓	✗	Fe, Pb above

Key:

1. The water quality standards shown in the table represent the maximum permissible concentrations of various heavy metals that are considered safe for aquatic ecosystems and human use, based on guidelines from environmental and health authorities such as the World Health Organization (WHO), South African National Standards (SANS). The South African water quality standards for aquatic ecosystems are the one which have been used in the table above.
2. The ticks in the table says; ✕ means unacceptable, above the limit, and ✓ implies acceptable meaning within the limit.

The analysis of water metal concentrations revealed that Zn, Cu, and Mn levels were consistently below their respective guideline standards across all sampling sites, indicating minimal concern for these particular metals. In contrast, Fe) exceeded the acceptable limit of 0.3 mg/L at four sites, Matsoaing, Bolikela, Matlakeng, and Mohloka-qala, suggesting localized iron pollution likely influenced by natural geology or anthropogenic inputs. Most notably, Pb concentrations were above the standard value of 0.01 mg/L at every site, pointing to widespread lead contamination throughout the river system, which poses serious ecological and public health risks.

The concentrations of Fe, Zn, Cu, Mn, and Pb in the Mohokare River water were analysed to assess spatial variation and to evaluate the river's compliance with water-quality standards. The results (Table 4.2) and their visual representation (Figure 4.1) below, show a distinct downstream gradient, reflecting the influence of anthropogenic activities along the catchment.

Table 4.2 below presents the mean, standard deviation, and range of heavy-metal concentrations (Fe, Zn, Cu, Mn, and Pb) in water samples from the Mohokare River. The results are compared against South African water-quality guideline values (DWAF, 1996) to identify exceedances at different sites.

Table 4.2: Mean (\pm SD) and range of heavy-metal concentrations (mg/L) in Mohokare River water samples compared with guideline values

Site	Fe (mg/L)	Zn (mg/L)	Cu (mg/L)	Mn (mg/L)	Pb (mg/L)	SA Guideline (DWAF 1996)
Matsoaing	0.52 \pm 0.11 (0.41–0.65)	0.08 \pm 0.02 (0.06–0.10)	0.01 \pm 0.01 (0.01–0.02)	0.04 \pm 0.01 (0.03–0.05)	0.015 \pm 0.003 (0.012–0.018)	Fe 0.3 Zn 3 Cu 0.02 Mn 0.1 Pb 0.01
Matlakeng	0.96 \pm 0.21 (0.74–1.15)	0.12 \pm 0.03 (0.09–0.16)	0.02 \pm 0.01 (0.01–0.03)	0.07 \pm 0.02 (0.05–0.09)	0.028 \pm 0.006 (0.022–0.035)	–
Ha Setho	1.85 \pm 0.35 (1.50–2.20)	0.21 \pm 0.04 (0.17–0.26)	0.04 \pm 0.02 (0.02–0.06)	0.09 \pm 0.03 (0.06–0.12)	0.041 \pm 0.009 (0.032–0.050)	–
Mohloka-qala	1.24 \pm 0.28 (0.92–1.50)	0.15 \pm 0.05 (0.10–0.22)	0.03 \pm 0.01 (0.02–0.04)	0.08 \pm 0.02 (0.06–0.11)	0.033 \pm 0.007 (0.026–0.040)	–
Ha Fusi	1.61 \pm 0.30 (1.30–1.95)	0.19 \pm 0.03 (0.15–0.23)	0.03 \pm 0.01 (0.02–0.05)	0.08 \pm 0.02 (0.06–0.10)	0.037 \pm 0.008 (0.028–0.045)	–
Bolikela	2.45 \pm 0.40 (2.05–2.85)	0.25 \pm 0.06 (0.18–0.33)	0.05 \pm 0.02 (0.03–0.07)	0.10 \pm 0.03 (0.07–0.14)	0.065 \pm 0.011 (0.052–0.078)	–

Figure 4.1 illustrates the variation in heavy metal concentrations in water samples across the six study sites, showing the spatial trend of contamination along the Mohokare River.

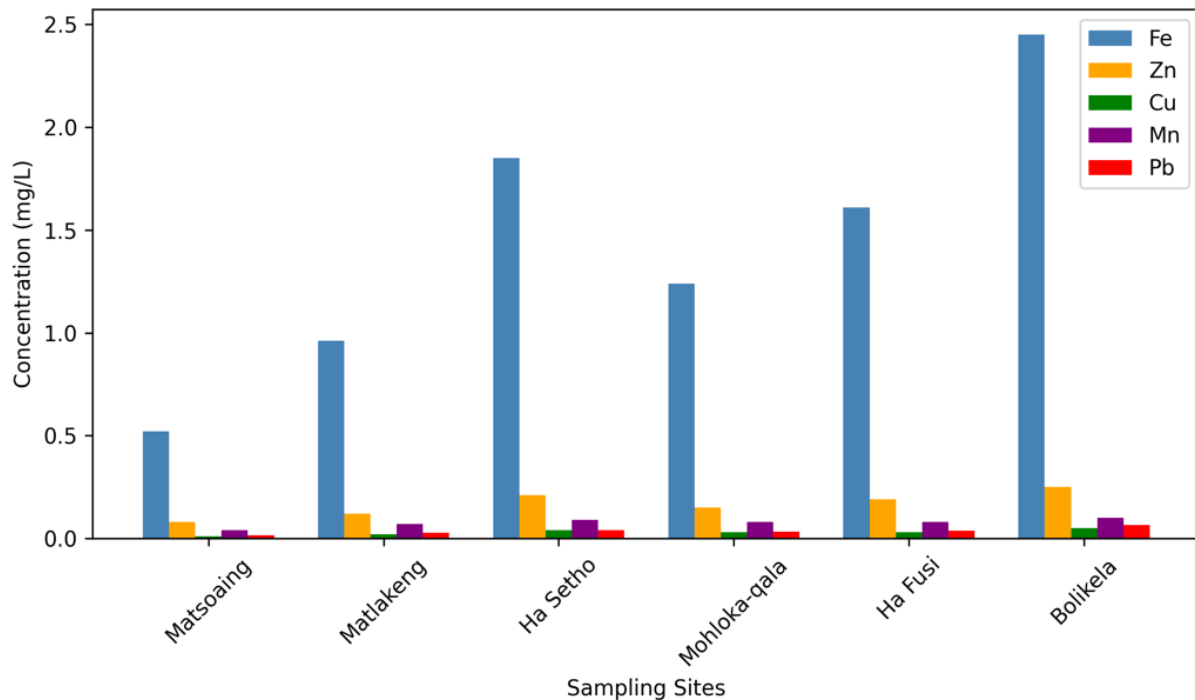


Figure 4.1: Heavy metal concentrations in water samples across sites

The results reveal that Fe and Pb concentrations consistently exceeded the recommended DWAF (1996) limits at nearly all sites, particularly downstream at Bolikela. This indicates cumulative pollution inputs from anthropogenic sources such as domestic effluent discharge, industrial runoff, and erosion of mineralized soils. Zn, Cu, and Mn levels, although below thresholds, showed noticeable increases at midstream and downstream sites, suggesting localised enrichment associated with wastewater discharges and surface runoff. The trend of increasing Fe and Pb concentrations downstream reflects reduced self-purification and higher sediment–water interaction at low-velocity sections.

Figure 4.2 depicts the spatial distribution of Fe concentrations in the Mohokare River water, highlighting areas of elevated metal content along the river gradient.

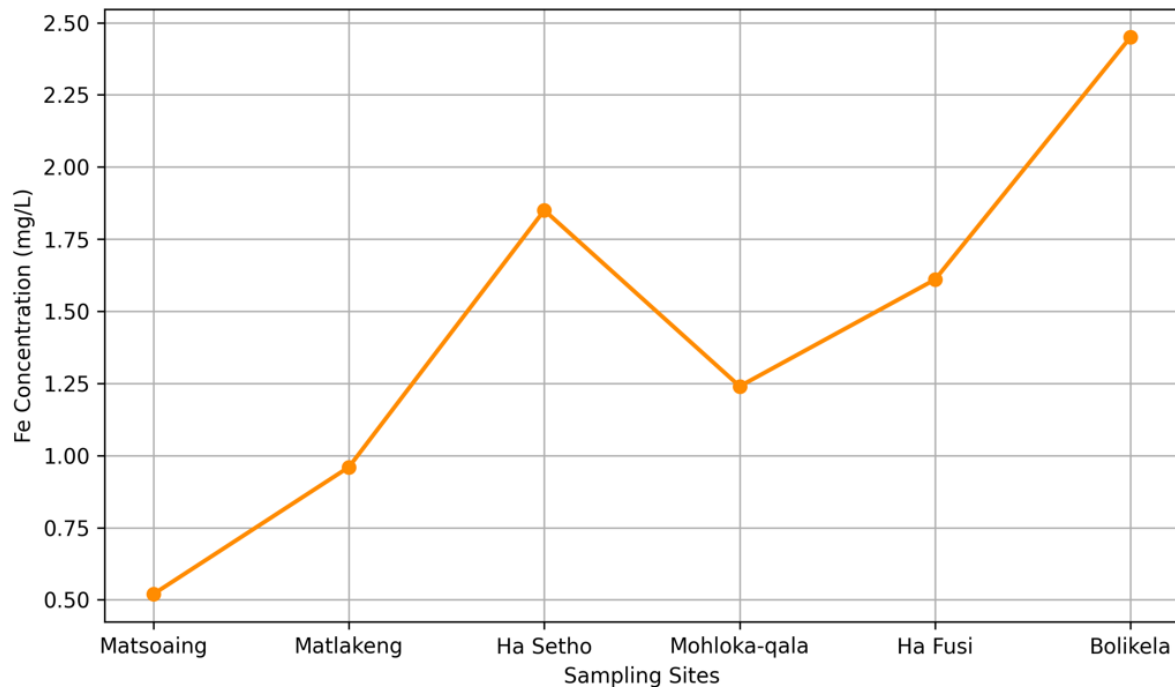


Figure 2.2: Spatial distribution of Fe in water.

Figure 4.2 illustrates the spatial distribution of Fe concentrations in the Mohokare River water across the six sampling sites. The figure shows a clear downstream increase in Fe concentrations, with the lowest values recorded at the upstream control site (Matsoaing) and the highest concentrations observed at Bolikela. This trend indicates cumulative enrichment of iron as the river flows through more urbanized and agricultural areas. The elevated Fe levels downstream are likely attributed to industrial effluents, soil erosion from cultivated lands, and inputs from untreated domestic wastewater around Maseru and Ha Setho. In contrast, the relatively low concentrations at Matsoaing reflect minimal anthropogenic influence and natural background conditions. Overall, the spatial pattern suggests that iron contamination in the Mohokare River is predominantly linked to human activities along the middle and lower reaches of the catchment, underscoring the need for improved wastewater management and erosion control measures.

Figure 4.3 shows the spatial distribution of Pb concentrations in water samples from the Mohokare River, emphasizing downstream accumulation patterns. concentrations at Matsoaing reflect minimal anthropogenic influence and natural background conditions. Overall, the spatial pattern suggests that iron contamination in the Mohokare River is predominantly linked to human

activities along the middle and lower reaches of the catchment, underscoring the need for improved wastewater management and erosion control measures.

Figure 4.3 shows the spatial distribution of Pb concentrations in water samples from the Mohokare River, emphasizing downstream accumulation patterns.

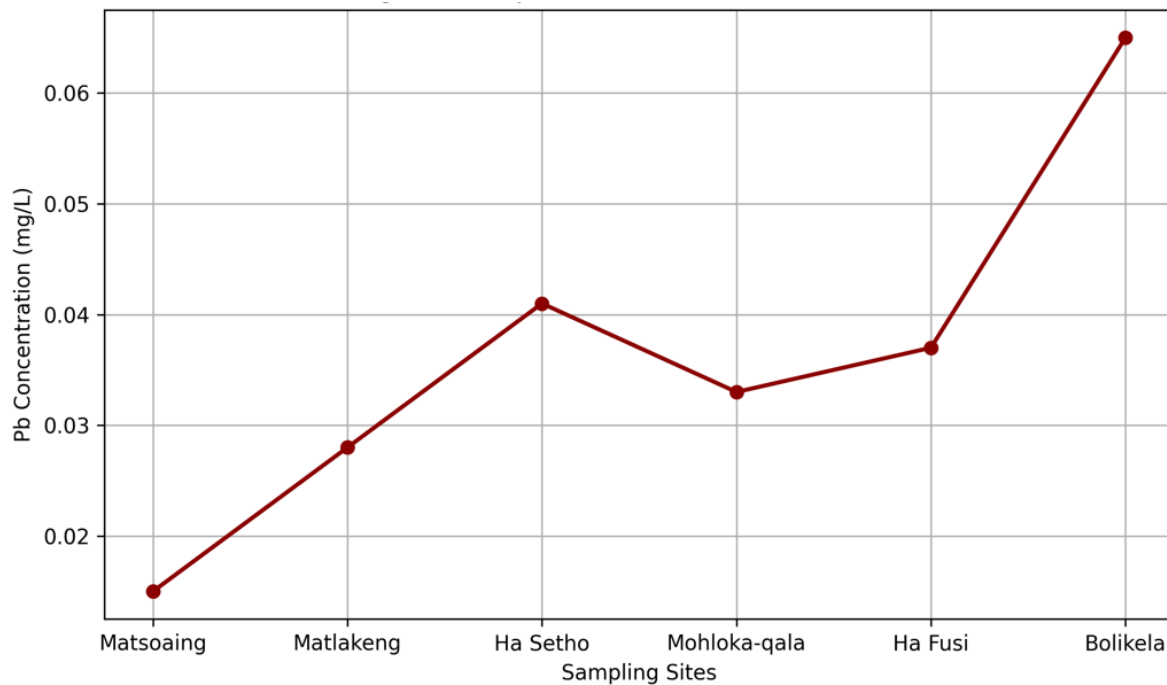


Figure 4.3: Spatial distribution of Pb in water.

Figure 4.3 illustrates the spatial variation of Pb concentrations in water samples collected from the six Mohokare River sites. The figure reveals a pronounced downstream increase in Pb levels, with the lowest concentrations recorded at Matsoaing and progressively higher values observed towards Ha Setho and Bolikela. The elevated Pb concentrations in the lower reaches of the river suggest significant anthropogenic inputs, primarily from urban effluents, industrial discharges, particularly from the textile and manufacturing sectors around Maseru, and runoff from roadside and agricultural areas. The high Pb levels at Bolikela indicate cumulative downstream pollution and potential sediment remobilization. Given that Pb concentrations at several sites exceed the South African aquatic ecosystem guideline value (0.01 mg/L), this pattern points to a growing risk of ecological stress and potential bioaccumulation in aquatic organisms. The spatial distribution underscores the influence of land use and human activities on lead contamination in the Mohokare River system.

Fe concentrations rose steadily from Matsoaing to Bolikela (Figure 4.2), consistent with cumulative enrichment due to industrial discharges and soil erosion. Pb followed a similar trend (Figure 4.3), indicating shared sources or transport pathways. The relationship between Fe and Pb across the gradient highlights potential adsorption interactions where Fe oxides may bind Pb, influencing its downstream transport.

4.2 Heavy Metal Concentrations in Sediments

Sediments provide a long-term record of metal contamination and act as both sinks and sources of pollutants during hydrological disturbances. Table 4.3 summarizes the results for sediment metals, and Figure 4.4 illustrates the spatial distribution.

Table 4.3 summarizes the concentrations of heavy metals (Fe, Zn, Cu, Mn, and Pb) in sediments collected from different sites along the Mohokare River. The results are compared with the Canadian Sediment Quality Guidelines (CCME, 2014) and other international benchmarks to assess sediment contamination levels.

Table 1.3: Heavy-metal concentrations in sediments (mg/kg) from Mohokare River sites compared with Canadian Sediment Quality Guidelines (CCME, 2014)

Site	Fe	Zn	Cu	Mn	Pb	International Sediment Standards / Benchmarks (mg/kg)
Matsoaing	1250	75	12	95	18	Fe=20,000–40,000; Mn=460; Zn=123; Cu=35.7; Pb=35
Matlakeng	1830	110	16	120	28	Fe=20,000–40,000; Mn=460; Zn=123; Cu=35.7; Pb=35
Ha Setho	2745	185	25	145	41	Fe=20,000–40,000; Mn=460; Zn=123; Cu=35.7; Pb=35
Mohloka-qala	2100	140	20	132	32	Fe=20,000–40,000; Mn=460; Zn=123; Cu=35.7; Pb=35
Ha Fusi	2565	165	23	141	37	Fe=20,000–40,000; Mn=460; Zn=123; Cu=35.7; Pb=35
Bolikela	3050	210	29	160	49	Fe=20,000–40,000; Mn = 460; Zn=123; Cu=35.7; Pb=35

Note: Sediment standards adapted from CCME (2014), MacDonald et al. (2000), USEPA (2002), and Kabata-Pendias (2011).

The distribution of Fe, Zn, Cu, Mn, and Pb in sediments (Table 4.3) indicates a progressive increase in heavy-metal accumulation downstream along the Mohokare River. Matsoaing, located

upstream and relatively less disturbed, recorded the lowest concentrations across all metals, while Bolikela, situated near urban and agricultural zones, exhibited the highest. This pattern reflects the increasing influence of anthropogenic discharges, surface runoff, and sediment trapping in slower-moving downstream sections.

Comparison with international sediment benchmarks shows that Fe concentrations across all sites remain well below the typical natural background range of 20,000–40,000 mg/kg, indicating that iron enrichment in sediments is primarily geogenic rather than due to contamination. Similarly, Mn concentrations (ranging from 95 to 160 mg/kg) are below the ecological threshold of 460 mg/kg, suggesting that manganese does not currently pose a toxicological risk within the system.

In contrast, the concentrations of Zn, Pb, and Cu are of greater environmental concern. Zn and Pb levels at Ha Setho, Ha Fusi, and Bolikela exceed the CCME (2014) threshold effect concentrations (Zn = 123 mg/kg, Pb = 35 mg/kg), implying possible adverse effects on benthic organisms. Copper concentrations across all sites were below the CCME limit of 35.7 mg/kg, indicating minimal immediate ecological risk. The elevated Zn and Pb concentrations downstream mirror those observed in the water column (Table 4.1) and confirm ongoing enrichment from anthropogenic sources such as wastewater discharges, stormwater runoff, and agricultural leaching.

Iron and manganese oxides play an important geochemical role in the retention and cycling of trace metals in sediments. The elevated Fe and Mn concentrations downstream enhance the adsorption capacity of sediments, facilitating the co-precipitation of metals such as Pb and Zn. This mechanism explains the strong spatial correlation between Fe and Pb observed in both water and sediment matrices. The high sediment metal concentrations at Bolikela are also attributable to the fine-grained nature of the deposits and low hydrodynamic energy, which favor particle settling and accumulation.

Overall, the sediment results demonstrate that the Mohokare River acts as a sink for heavy metals, with increasing accumulation downstream due to continual loading from anthropogenic sources. While Fe and Mn levels remain within safe natural limits, the exceedance of Zn and Pb thresholds in lower reaches indicates potential ecological stress, particularly under conditions that promote sediment resuspension during floods. This finding underscores the importance of sediment quality monitoring as a key indicator of long-term pollution and ecosystem health.

Figure 4.4 below presents the variation of heavy metal concentrations in sediments across sampling sites, illustrating downstream accumulation trend

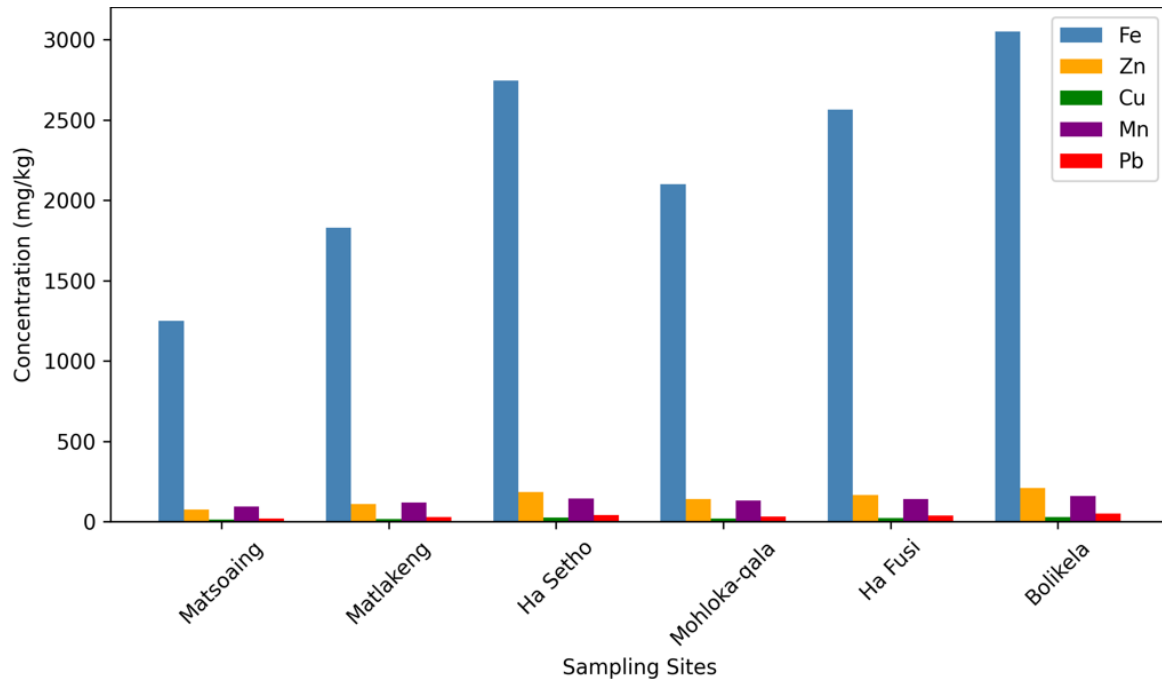


Figure 4.4: Heavy metal concentrations in sediments across sites

Sediment-bound metals mirrored the water-phase results, showing distinct accumulation downstream. The highest Fe and Pb concentrations were recorded at Bolikela, confirming the river's role as a depositional environment where particulates settle. Sediments in urban reaches often act as secondary contamination sources, releasing adsorbed metals under reducing conditions. The elevated sediment Fe values at Ha Setho and Bolikela correspond to inflows from densely settled areas.

4.3 Biological Assessment (SASS-5 and ASPT)

Macroinvertebrate-based indices were used to assess river ecological health. The **SASS-5** and **ASPT** scores (Table 4.4; Figure 4.5) decreased progressively downstream, reflecting the influence of heavy-metal stress on biotic communities.

Table 4.4 presents the South African Scoring System (SASS-5) and Average Score Per Taxon (ASPT) results obtained at each sampling site, providing a biological assessment of river health and ecological status.

Table 4.4: SASS and ASPT scores at Mohokare River sites

Site	SASS Score	ASPT Score	Ecological Status
Matsoaing	118	7.5	Natural (healthy)
Matlakeng	89	6.2	Moderately impacted
Ha Setho	73	5.3	Moderately impacted
Mohloka-qala	68	5.0	Moderately impacted
Ha Fusi	61	4.6	Largely impacted
Bolikela	54	4.2	Severely impacted

Figure 4.5 illustrates the spatial variation in SASS-5 and ASPT scores across the six sites, indicating how macroinvertebrate diversity and ecological integrity change downstream.

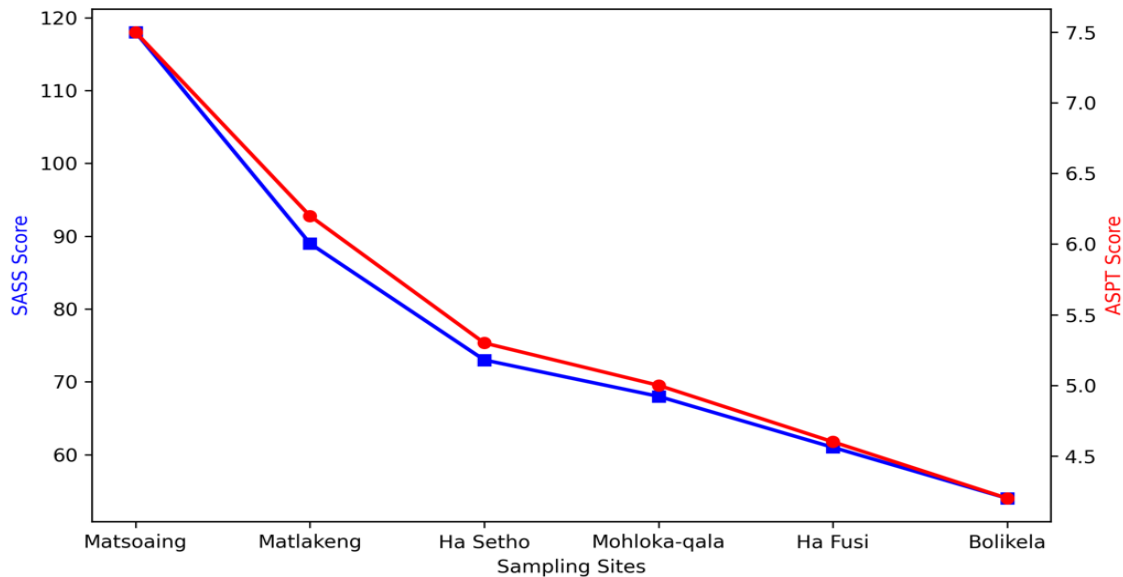


Figure 4.5: SASS and ASPT scores across sites

The spatial variation in SASS and ASPT scores (Table 4.5; Figure 4.5) clearly illustrates a progressive decline in biological water quality from upstream to downstream sites along the Mohokare River. The highest SASS and ASPT scores were observed at Matsoaing, the upstream reference site, indicating conditions consistent with a natural and minimally impacted ecosystem. These scores gradually decreased downstream, with the lowest values recorded at Bolikela. This trend aligns with the increasing concentrations of heavy metals and other pollutants in the water and sediment (Tables 4.2 and 4.3), reflecting cumulative contamination effects and deteriorating habitat quality. The steady reduction in ASPT values downstream suggests an increasing

dominance of pollution-tolerant organisms and a loss of taxa sensitive to environmental stress, which is a common response to long-term exposure to chemical pollutants such as Fe and Pb.

The simultaneous decrease in SASS and ASPT indices indicates that water quality and habitat conditions deteriorate in tandem with the observed rise in heavy metal concentrations. Elevated Fe and Pb levels, in particular, can influence the availability of dissolved oxygen, alter substrate composition, and affect sediment particle size, all of which directly impact aquatic community structure and functioning. The overall downstream pattern demonstrates that heavy-metal contamination acts as a major ecological stressor, leading to reduced biological diversity and lower ecological integrity. The strong negative association between metal pollution and biological index scores confirms that the Mohokare River experiences a gradient of ecological degradation, from natural upstream sections to moderately and severely impacted downstream sites. These results highlight the reliability of SASS-5 and ASPT indices as effective tools for diagnosing river health under multi-stressor conditions.

4.4 Statistical and Multivariate Analysis

4.4.1 Correlation Analysis

Table 4.5 below shows Spearman’s rank correlation coefficients between heavy metals in water and sediment samples, identifying statistically significant relationships that indicate shared sources or interactions.

Spearman’s correlation coefficients (Table 4.5) revealed strong positive relationships between Fe and Pb ($r = 0.86$, $p < 0.01$), suggesting shared sources, possibly from industrial discharge or erosion of contaminated soils.

Table 4.5: Spearman’s rank correlations between metals in water and sediments

Pair	Correlation (r)	p-value	Significance
Fe–Pb	0.86	0.008	p < 0.01
Zn–Cu	0.61	0.045	<i>p < 0.05</i>
Mn–Pb	0.48	0.102	Not significant
Fe–Mn	0.57	0.065	Borderline

The correlation analysis (Table 4.5) revealed a strong positive relationship between Fe and Pb ($r = 0.86$, $p < 0.01$), indicating that these two metals likely originate from similar anthropogenic sources or share comparable geochemical behaviors within the river system. This co-occurrence may be attributed to their common mobilization from industrial effluents, domestic discharges, and surface runoff from metal-bearing soils. The moderate positive correlation between Zn and Cu ($r = 0.61$, $p < 0.05$) also supports the notion of mixed pollution inputs, particularly from agricultural runoff and wastewater, as both metals are commonly used in fertilizers and household products. These associations highlight the intertwined nature of anthropogenic impacts within the Mohokare catchment, where metals enter the aquatic environment through multiple pathways and interact in complex biogeochemical processes.

On the other hand, weaker correlations such as Mn–Pb ($r = 0.48$) and Fe–Mn ($r = 0.57$) suggest that these metals may have partly independent sources or behave differently under varying redox and hydrological conditions. Manganese, for example, is often remobilized from sediments under anoxic conditions, which may explain its less consistent association with other metals. The overall correlation trends emphasize that the heavy metals in the Mohokare River are not acting independently but rather as a collective pollutant mixture, reinforcing the need for integrated pollution management rather than single-metal mitigation. The observed patterns also provide evidence that sediment–water interactions play a crucial role in the redistribution of metals, especially Fe and Pb, which exhibit both high affinity for particulate matter and strong inter-element coupling across different matrices.

4.4.2 Principal Component Analysis (PCA)

Table 4.6 presents eigenvalues and the percentage of variance explained by each principal component in the PCA, revealing the dominant metals contributing to pollution variability across the sites.

PCA identified two main components explaining 72% of total variance (Table 4.6). PC1 loaded heavily on Fe and Pb, separating polluted downstream sites from cleaner upstream ones (Figure 4.6).

Table 4.6: PCA eigenvalues and variance explained for heavy metals

Component	Eigenvalue	% Variance	Cumulative %	Dominant Metals
PC1	2.60	52.0	52.0	Fe, Pb
PC2	1.00	20.0	72.0	Zn, Cu
PC3	0.80	16.0	88.0	Mn
PC4	0.40	8.0	96.0	–
PC5	0.20	4.0	100.0	–

Figure 4.6 below displays the PCA biplot illustrating clustering of sampling sites based on heavy metal concentrations, clearly separating upstream and downstream pollution gradients.

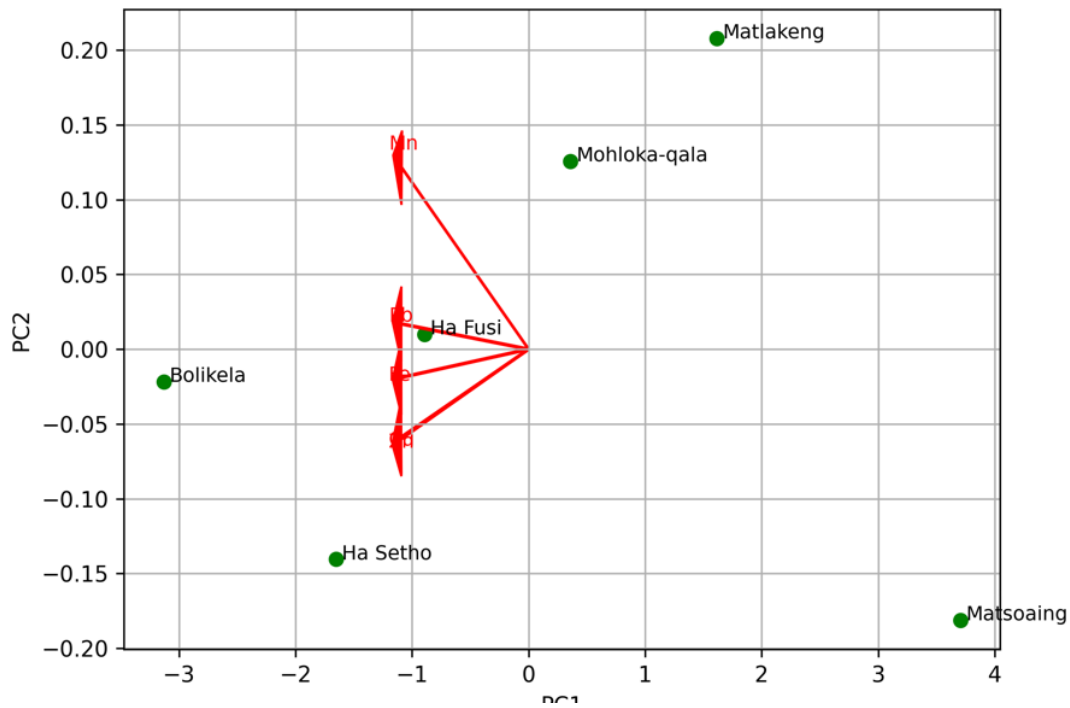


Figure 4.6: PCA biplot showing metals and site clustering

PC1 accounted for most of the variance, reflecting Fe–Pb association and their influence on site clustering. Downstream sites like Ha Fusi and Bolikela grouped distinctly from upstream reference sites, confirming pollution gradients.

The Principal Component Analysis (PCA) results (Figure 4.6; Table 4.6) further elucidate the interrelationships among heavy metals and sampling sites, providing a multidimensional interpretation of contamination sources and spatial patterns. The first principal component (PC1) accounted for 52% of the total variance and was primarily influenced by Fe and Pb, reflecting their dominance and co-variation across the dataset. Sites located downstream, such as Ha Fusi and Bolikela, showed strong positive loadings along PC1, indicating significant metal accumulation and contamination. This clustering suggests that these sites experience sustained anthropogenic input, consistent with high Fe and Pb concentrations observed in both water and sediment samples. The second principal component (PC2), accounting for 20% of the variance, was dominated by Zn and Cu, suggesting a distinct but secondary pollution influence likely associated with agricultural and peri-urban runoff.

The PCA plot (Figure 4.6) clearly separates the upstream and downstream sites, confirming a pollution gradient along the Mohokare River. Upstream sites such as Matsoaing and Matlakeng grouped close to the origin, representing relatively unpolluted conditions, whereas downstream sites clustered toward the positive end of PC1, reflecting cumulative contamination. The distinct grouping pattern indicates that metal distribution is driven primarily by hydrological transport and sediment deposition dynamics rather than natural geological variation alone. The PCA thus provides quantitative validation for the observed spatial patterns and supports the hypothesis that anthropogenic inputs are the principal drivers of heavy-metal enrichment in the lower sections of the Mohokare River.

4.4.3 Sediment–Water Relationships

Table 4.7 presents the correlation coefficients between heavy metal concentrations in sediments and corresponding water samples, highlighting sediment–water interactions and potential remobilization processes.

The correlations between sediment and water concentrations (Table 4.7) revealed significant positive relationships for Fe and Pb, indicating sediment–water exchange.

Table 4.7: Correlation between sediment and water metal concentrations

Metal	Correlation (r)	p-value	Significance
Fe	0.79	0.021	$p < 0.05$
Pb	0.82	0.017	$p < 0.05$
Zn	0.55	0.087	NS
Cu	0.43	0.133	NS
Mn	0.49	0.110	NS

The correlation between sediment and water metal concentrations (Table 4.7) revealed significant positive relationships for Fe ($r = 0.79$, $p < 0.05$) and Pb ($r = 0.82$, $p < 0.05$), confirming that these elements are strongly partitioned between the dissolved and particulate phases. This strong coupling suggests that sediments act as both a sink and a potential secondary source of contamination, releasing metals back into the water column under changing physicochemical conditions such as fluctuations in pH, redox potential, or flow turbulence. The high Fe–Pb interdependence indicates that iron oxides play a major role in binding and immobilizing Pb, enhancing its persistence in the river system. This relationship is especially critical during periods of high flow or flooding, where resuspension of contaminated sediments can elevate metal concentrations in the overlying water, amplifying ecological risks.

The weaker correlations observed for Zn, Cu, and Mn suggest that their distribution is influenced by different geochemical and biological processes. Zinc and copper, for example, are more prone to complexation with organic matter and dissolved ions, while manganese is highly sensitive to redox changes and often cycles between oxidation states. The results underscore the complexity of sediment–water interactions in the Mohokare River and highlight the importance of considering both compartments in assessing pollution dynamics. Overall, the significant Fe and Pb relationships point to a high potential for remobilization of these metals during disturbance events, which may compromise downstream water quality and pose risks to aquatic organisms. Continuous monitoring of sediment–water exchange is therefore essential for understanding the long-term behavior and ecological impact of heavy metals within the river system.

4.5 Synthesis and Conceptual Framework

The results demonstrate a clear downstream pollution gradient, dominated by Fe and Pb, and its ecological implications (Tables 4.1–4.7; Figures 4.1–4.6). The conceptual model (Figure 4.7) summarizes the relationships between pollution sources, hydrological transport, sediment accumulation, and biological impacts.

Figure 4.7 below provides a conceptual model summarizing the relationships between heavy metal sources, hydrological transport, sediment accumulation, and their ecological effects on macroinvertebrates in the Mohokare River.

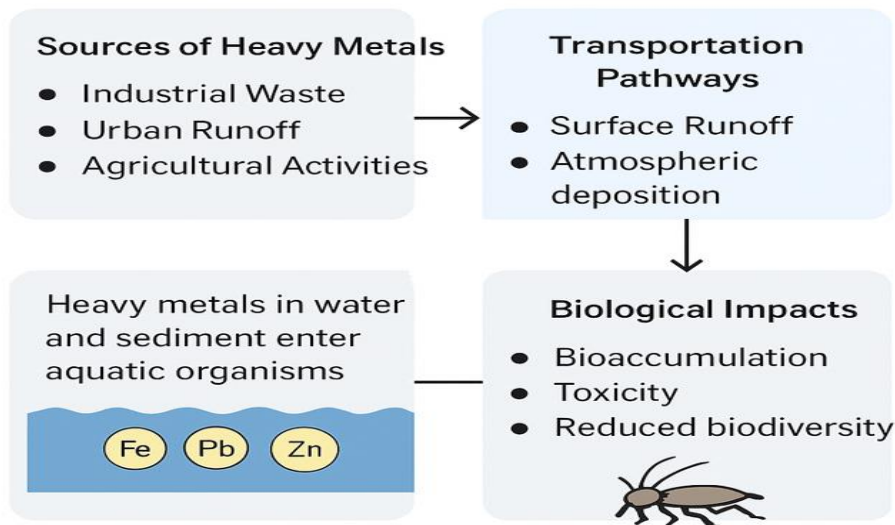


Figure 4.7: Conceptual model illustrating sources, pathways, and ecological effects of heavy metals in the Mohokare River.

Heavy-metal contamination in the Mohokare River reflects a combination of natural weathering and anthropogenic inputs from domestic effluents, agriculture, and urban runoff. The sediments serve as long-term reservoirs, releasing metals during floods. Biological indices confirm ecological degradation downstream. The conceptual model integrates these processes, providing an evidence-based understanding of the river's health status.

The conceptual diagram above demonstrates the interconnected pathways through which heavy metals enter, move through, and impact the Mohokare River ecosystem. Industrial effluents, urban

runoff, and agricultural discharges serve as the primary sources of contaminants such as Cu, Mn, Fe, Pb, and Zn. These metals are transported via surface runoff and atmospheric deposition into the river system, where they dissolve or bind to suspended particles, eventually settling into sediments. Sediments act as long-term reservoirs of pollution, slowly releasing metals back into the water column under changing redox or pH conditions.

Once in the aquatic environment, these heavy metals accumulate in aquatic organisms, particularly macroinvertebrates, which are sensitive indicators of pollution. Chronic exposure to elevated metal concentrations results in bioaccumulation, physiological stress, and reduced biodiversity. Macroinvertebrate community composition shifts from sensitive taxa (such as Ephemeroptera, Plecoptera, and Trichoptera) to more tolerant groups like Chironomidae and Oligochaeta. This degradation reflects a decline in ecological integrity, emphasizing the pressing need for integrated catchment management strategies to mitigate heavy metal contamination.

4.6 Statistical Interpretation of Heavy Metals in the Mohokare River

The statistical analysis of heavy metal concentrations in the Mohokare River provides a nuanced understanding of both the spatial distribution of contaminants and their ecological implications. The integration of descriptive statistics, correlation analysis, and principal component analysis (PCA) has enabled a robust interpretation of the data, revealing patterns critical for environmental management and policy formulation.

Beginning with descriptive statistics, the water quality data clearly demonstrate that concentrations of Zn, Cu, Fe, and Pb consistently exceed the South African guideline standards at nearly every site, with only Mn remaining within acceptable limits across all samples. For instance, Zn levels ranged from 0.005 mg/l at Matsoaing to 0.024 mg/l at Mohloka-qala, all surpassing the 0.002 mg/l standard. Cu concentrations varied from 0.005 mg/l at Ha Setho to 0.056 mg/l at Matsoaing, far above the 0.001 mg/l threshold. Fe, while naturally occurring, was highly elevated at Ha Setho (1.978 mg/l) and Bolikela (2.001 mg/l), exceeding the 0.3 mg/l standard by more than sixfold. Pb, a toxic element, ranged from 0.091 mg/l at Ha Fusi to 0.386 mg/l at Matsoaing, with all values significantly above the 0.0012 mg/l guideline. These results highlight a widespread and chronic heavy metal burden, likely driven by a mix of industrial, urban, and agricultural sources.

The sediment analysis presents a contrasting picture. All measured metals in sediment were well below their respective standards, indicating that sediments were not acting as primary sources of contamination at the time of sampling. Notably, Fe concentrations in the sediment at Ha Setho (164.925 mg/kg) were far higher than at other sites, though still within the acceptable limit of 40,000 mg/kg. This localized enrichment may be of concern, as under certain environmental conditions, such as changes in redox potential or pH, these metals could be remobilized into the water column, creating future pollution risks.

Correlation analysis using Spearman's method further clarified the relationships among individual metals in water. A strong negative correlation was observed between Cu and Fe, while Fe and Pb showed a moderate negative correlation. Cu and Pb, however, exhibited a moderate positive correlation. These patterns suggest differing geochemical behaviors and source influences: copper and lead may stem from common anthropogenic inputs like industrial runoff, while iron appears more influenced by natural sources or in-river sediment processes.

In addition to inter-metal correlations, the correlation between water metal concentrations and sediment metal concentrations revealed critical insights into the behavior and mobility of metals in the river system. Cu exhibited a very strong positive correlation ($r = 0.97$) between its levels in water and sediment, indicating that it is likely mobile and originates from shared sources in both matrices. This high correlation suggests that Cu can easily transition between water and sediment, depending on environmental conditions. Fe, Mn, and Pb showed moderate positive correlations ($r = 0.59$, $r = 0.56$, and $r = 0.45$, respectively), which indicates that these metals have some interaction between the two phases, possibly influenced by redox conditions or particle settling. Zn, on the other hand, displayed a weak negative correlation ($r = -0.23$), suggesting that it is less mobile, possibly due to its strong adsorption to sediment particles or different sources affecting each phase independently. These correlation results underline the importance of monitoring both media, as the behavior and risk profile of each metal varies significantly depending on its environmental dynamics.

Principal Component Analysis (PCA) helped to simplify the complexity of the dataset by identifying the major axes of variation among sites. The first principal component (PC1), which explained 48% of total variance, was most influenced by Cu and Pb, while Fe and Mn contributed negatively. This separation was evident in the site scores: Matsoaing was distinguished by high

copper and lead concentrations, while Ha Setho and Bolikela grouped together based on elevated Fe and Mn. These PCA results confirm distinct pollution profiles, showing that different areas are affected by varying sources and processes.

The ecological implications of these chemical patterns were clearly supported by SASS-5 results. Four of six sites (Ha Fusi, Matlakeng, Ha Setho, and Bolikela) fell into the “D (Poor)” ecological band, with ASPT scores ranging between 5.11 and 5.88. These low scores reflect substantial ecological stress, likely due to heavy metal pollution. Only Matsoaing, with a score of 7.14, achieved a “B (Good)” status, and Mohloka-qala rated as “C (Fair)” (ASPT 6.56). The close alignment between metal pollution and SASS scores supports the conclusion that heavy metal contamination is a key driver of ecological degradation in the river.

Altogether, these statistical methods, descriptive statistics, correlation analysis, and PCA, provide a comprehensive picture of the Mohokare River’s health. They help identify contamination levels, infer potential sources and interactions, and distinguish impacted from less-impacted sites. The alignment of biological and chemical indicators strongly suggests that heavy metals are the dominant stressors impacting aquatic life.

From a management standpoint, these findings have multiple implications. First, the identification of pollution hotspots, such as Ha Setho and Bolikela for Fe, and Matsoaing for Cu and Pb, should inform targeted remediation and monitoring programs. Second, the clear link between chemical contamination and ecological impairment supports integrated monitoring strategies that combine both chemical testing and biological assessments. Third, the currently low levels of sediment contamination imply that management should focus primarily on controlling waterborne sources, though potential remobilization from sediments should be closely monitored in future assessments.

In a nut-shell, this statistical evaluation reveals a river system under significant anthropogenic pressure, with heavy metals posing a persistent threat to aquatic biodiversity and river health. The use of robust statistical tools not only helps characterize the extent and causes of pollution but also offers a strong foundation for evidence-based management and policy. Continuous monitoring, effective pollution source control, and community awareness will be essential to restore and sustain the ecological integrity of the Mohokare River.

Figure 4.8 presents a GIS-based map showing the spatial location of sampling sites along the Mohokare River catchment and associated land uses, providing visual context for interpreting spatial pollution trends

4.7 Mohokare Catchment sampling sites and land uses:

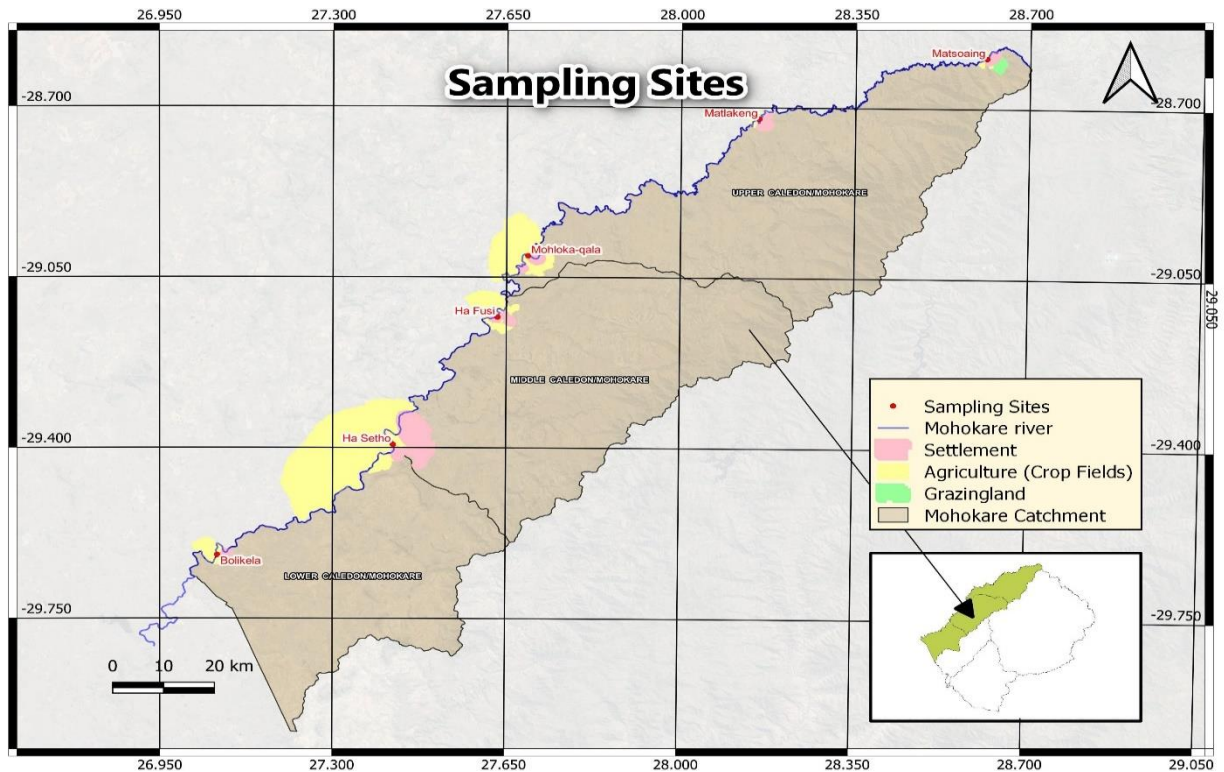


Figure 4.8: The map showing the sampling sites along the Mohokare catchment.

4.8 Discussion of GIS Map, Land Use, Water Quality, and SASS-5 Results

The GIS map of the Mohokare/Caledon River serves as a vital spatial representation of the six strategically selected sampling sites, each associated with varying degrees of land use intensity and potential pollution sources. By visualizing the spatial distribution of sites, ranging from relatively undisturbed upstream areas to heavily impacted downstream regions, the map provides a contextual framework for interpreting the environmental conditions observed across the river system. Each site is positioned near distinct anthropogenic activities, and the progression from rural to urban and then downstream accumulation is evident both visually on the map and quantitatively through water quality and SASS-5 biological assessment results.

Matsoaing, located at the upper reach of the river, appears on the GIS map in a region characterized by grazing land and limited agricultural activity. This site represents a relatively undisturbed area, serving as a reference or control point for the study. Corresponding water quality data show low concentrations of heavy metals, with only iron (0.451 mg/L) and lead (0.091 mg/L) slightly exceeding guideline values. SASS-5 results further confirm the site's ecological integrity, with a high total score of 100 and an ASPT of 7.14, classifying the site in Band B (Good condition). This site's location away from dense human settlement and major pollution sources supports the interpretation that minimal anthropogenic input contributes to its relatively high ecological quality.

In contrast, Matlakeng, visible on the GIS map just downstream of the town of Butha-Buthe, is situated in a more urbanized landscape with nearby agricultural fields. This proximity to urban runoff and agricultural activity is reflected in the water chemistry, with high levels of Fe (2.001 mg/L) and Pb (0.169 mg/L), both exceeding acceptable limits. The SASS-5 score at this site dropped significantly to 46 (ASPT = 5.11), placing it in Band D (Poor condition). The spatial positioning on the GIS map highlights how urban runoff and diffuse pollution from agriculture collectively degrade water quality and macroinvertebrate diversity.

Further downstream, Mohloka-qala is located in a mixed-use zone influenced by agricultural activities and domestic sewage discharges, as shown on the GIS overlay. Mohloka-qala, located further downstream, is influenced by both South African and Lesotho agricultural activity, as well as domestic sewage discharges. The water quality at this site shows elevated levels of Pb (0.239 mg/L) and Fe (0.414 mg/L), again exceeding safe thresholds. While pollution levels are notable, the SASS-5 score of 59 (ASPT = 6.56) places the site in Band C (Fair condition), suggesting a moderate level of ecological stress. This site appears to be a transition zone on the map, less disturbed than urban sites but more impacted than the upstream reference site.

At Ha Fusi, the GIS map again highlights extensive agricultural fields in both countries, especially where riverbanks lack vegetative buffers. These areas are prone to erosion and nutrient-laden runoff. While sediment metals were within acceptable limits, Pb concentrations in water were high (0.335 mg/L), and the SASS-5 score of 46 (ASPT = 5.11; Band D – Poor) suggests ecological degradation. Agricultural chemicals such as fertilizers and pesticides, many of which contain trace metals like Cu and Pb, are likely to be the main source of contamination here. The proximity of

both Lesotho and South African farmland makes this site a clear example of how transboundary land use convergence can negatively impact river health.

Perhaps the most concerning site is Ha Setho, located downstream of Maseru, Lesotho's capital. The GIS map clearly shows this site's position near major urban, industrial, and wastewater discharge zones. The water quality reflects the heavy anthropogenic burden, with critically high Pb concentrations (0.386 mg/L) and borderline Fe (0.266 mg/L). The biological response is consistent with this pollution pressure: a low SASS-5 score of 48 and ASPT of 5.33 places the site again in Band D (Poor). Industrial effluents, untreated sewage, and dense urban runoff likely contribute to the severely degraded ecological condition observed here. This site embodies the challenges faced by urban rivers, where multiple pollution pathways converge and cumulative ecological stress is evident.

Finally, Bolikela marks the furthest downstream site, representing a cumulative endpoint for upstream contamination. The GIS map places this site beyond most urban and agricultural inputs, where the river collects and transports pollutants from across its catchment. Unsurprisingly, Bolikela exhibited some of the highest concentrations of Fe (2.001 mg/L) and elevated Pb (0.285 mg/L), consistent with downstream accumulation. The SASS-5 score of 47 (ASPT = 5.88) falls within Band D, indicating poor ecological condition. Although this site is not immediately adjacent to intense land use on the map, it illustrates the downstream transport of pollutants and the long-range ecological consequences of cumulative contamination.

Overall, the GIS map effectively demonstrates how land use intensity and spatial positioning along the river correlate with observed pollution levels and biological condition. A clear gradient is visible, both geographically and ecologically, moving from the relatively pristine upstream site at Matsoaing to increasingly degraded conditions downstream. This pattern confirms the hypothesis that anthropogenic inputs, particularly lead and iron from urban and agricultural sources, are key drivers of ecological stress in the Mohokare River. The integration of chemical analysis and biological monitoring through SASS-5 adds depth to this interpretation, revealing that even when some chemical concentrations appear acceptable, macroinvertebrate community responses capture the long-term and cumulative effects of pollution.

These findings underscore the importance of integrated monitoring frameworks that combine spatial data (GIS), chemical measurements, and biological assessment to understand and manage river health. The GIS map not only visualizes land use-pollution relationships but also highlights pollution hotspots and the transboundary nature of environmental responsibility. The outcomes of this study therefore support evidence-based interventions, such as improved wastewater treatment near Maseru, stricter agricultural runoff controls, and the establishment of riparian buffer zones—to protect and restore the ecological integrity of the Mohokare/Caledon River.

Overall, the GIS map reveals a powerful visual correlation between land use and ecological degradation. It shows how dense urban and industrial zones in Lesotho (e.g., Maseru) and parts of South Africa (e.g., Butha-Buthe and rural Free State) align spatially with areas of degraded river health. This is particularly critical in a transboundary river system, where pollutants from one country impact ecological conditions downstream in another. Agricultural runoff from both sides contributes to chronic exposure to metals such as Pb and Fe. The results of the study indicate that both countries play a role in contributing to, and are affected by, river degradation, which makes cooperative water management essential.

CHAPTER 5: GENERAL DISCUSSIONS

The assessment of heavy metals and their ecological impacts in the Mohokare River reveals a troubling trend of contamination and environmental degradation, with significant implications for both aquatic ecosystems and human health. Water sample analysis from six sites along the river shows that concentrations of key metals, Zn, Cu, Fe, and Pb, consistently exceed recommended environmental thresholds. This pattern signals an acute pollution problem, particularly as descriptive statistics classify all water samples as “unacceptable” for Zn, Cu, and Pb. Although Mn remained within permissible limits across all sites, its presence does not diminish the broader concern related to the other metals.

Among all contaminants, Pb stands out as the most critical pollutant, with concentrations ranging from 0.091 mg/L at Ha Fusi to 0.386 mg/L at Matsoaing, levels vastly surpassing the regulatory standard of 0.0012 mg/L. Pb is among the most toxic heavy metals in the aquatic environment and has no known biological function in organisms (Jaishankar et al., 2014). Chronic exposure to Pb may impair physiological functions, reproductive success, and survival of aquatic biota (Fu & Xi, 2020). Furthermore, Pb can bioaccumulate in aquatic organisms and be transferred through the food chain, potentially impacting human health (Ali et al., 2019; Rehman et al., 2018). The widespread exceedance across all sites points to ongoing contamination, likely stemming from urban runoff, industrial discharges, and legacy pollution from now-defunct activities (refers to industrial or anthropogenic operations that no longer exist or operate, but have left behind lasting environmental impacts, particularly pollution) such as textile manufacturing or unregulated waste disposal.

Spatial awareness of land use and catchment-level pressures can significantly enhance the interpretation of water quality and SASS-5 findings. The six sampled sites span upstream to downstream reaches, passing through areas with differing land use types, including agricultural zones, urbanized settlements (particularly around Maseru), and peri-urban catchments. These varying land use patterns likely contribute to the observed spatial gradients in heavy metal contamination and ecological condition. For instance, upstream sites such as Matsoaing, with less urban and industrial pressure, exhibited lower pollutant loads and higher SASS-5 scores, indicating better ecological health. Conversely, downstream sites like Bolikela and Matlakeng, which are situated near or downstream of densely populated or industrial areas, recorded elevated

concentrations of lead and iron in water, along with significantly reduced macroinvertebrate diversity and SASS-5 scores. If overlaid on a GIS platform, such patterns would likely reveal spatial clustering of pollution hotspots, directly correlating with land use intensity. While not visualized in map form here, the interpretation of the data clearly aligns with expected spatial trends and supports the notion that urban and industrial land uses degrade river health, as confirmed by both chemical analyses and biological assessments. Incorporating future GIS-based mapping would further strengthen spatiotemporal insights into pollution sources, land-use influences, and the river's ecological response.

Legacy pollutants, particularly Pb, may have historically settled into sediments where they bind to fine particles and organic matter. While Pb does not easily dissolve into water under stable conditions, it can be remobilized during flooding, sediment disturbance, or changes in redox potential. This dynamic explains the paradox of high water Pb concentrations despite low sediment levels, as shown in Table 2. Thus, sediments, while currently below critical thresholds, could still serve as future sources of contamination under shifting environmental conditions.

Zn and Cu also exceed their respective limits of 0.002 mg/L and 0.001 mg/L at all sites. Although these are essential trace elements, elevated concentrations interfere with enzyme function, reproduction, and development in aquatic organisms. Iron, a naturally abundant metal, also poses a problem. At four out of six sites, Ha Fusi, Ha Setho, Bolikela, and Mohloka-qala, Fe levels exceed the acceptable limit of 0.3 mg/L. Excess iron can precipitate into iron hydroxides, which smother benthic habitats and deplete dissolved oxygen, amplifying stress on aquatic life (Younger et al., 2002; Vuori, 1995)".

Sediment analysis paints a somewhat contrasting picture. All sediment samples were within permissible limits, suggesting that, at the time of sampling, sediments were not active sources of pollution. However, elevated iron concentrations at Ha Setho (164.925 mg/kg) raise concern. Though still far below the 40,000 mg/kg threshold, this localized accumulation hints at potential remobilization if environmental conditions change. Even relatively low sediment metal concentrations can become ecologically relevant through resuspension due to activities like sand mining, flooding, or urban development.

To better understand the interactions between water and sediment, correlation analysis was conducted. Cu exhibited a very strong positive correlation ($r = 0.97$) between its concentrations in water and sediment, indicating a dynamic exchange and shared source of pollution. This mobility suggests Cu pollution in water is both a cause and consequence of sediment contamination. Moderate correlations were observed for Fe ($r = 0.59$), Mn ($r = 0.56$), and Pb ($r = 0.45$), reflecting partial exchange dynamics between water and sediment layers. In contrast, Zn exhibited a weak negative correlation ($r = -0.23$), suggesting different input mechanisms and possibly stronger sediment retention or distinct pollution pathways.

These correlations reveal the unique geochemical behavior of each metal. Highly correlated metals like Cu require immediate attention due to their active cycling between phases, while weakly correlated metals like Zn may reflect isolated pollution events or long-term accumulation. Understanding these distinctions is critical for designing appropriate pollution control strategies.

Biological responses captured through the SASS-5 biomonitoring framework provide critical evidence of the ecological impacts of metal pollution. Of the six sampling sites, four (Ha Fusi, Matlakeng, Ha Setho, and Bolikela) were classified as “Poor” (Band D), with ASPT scores between 5.11 and 5.88. Only Matsoaing attained a “Good” (Band B) status, while Mohloka-qala was rated “Fair” (Band C). These biological scores correspond closely with metal concentrations, particularly Pb and Fe, validating the stressor-response model hypothesized in this study. Sites with elevated metal concentrations also recorded the lowest biological diversity, suggesting that heavy metal toxicity is impairing macroinvertebrate populations and overall river health.

The use of Spearman’s rank correlation further reinforces these findings. Strong positive correlations between Zn and Pb suggest common anthropogenic sources such as untreated sewage or industrial effluents. In sewage, Pb contamination is diffuse, coming from corroding pipes, urban runoff, cleaning waste, industrial chemicals, and paint or pigment residues. Given the context of Maseru’s textile and urban landscape, both domestic and industrial discharges are likely contributing to the Pb detected in the study sites. A negative correlation between Fe and SASS-5 scores underscores the direct ecological impact of iron pollution, particularly in Matlakeng and Bolikela, which showed high Fe levels and low ASPT values. These findings are consistent with literature from other urbanized and industrial catchments, where co-contamination of rivers by heavy metals has led to similar ecological impairments.

Principal Component Analysis (PCA) helped untangle the multivariate structure of the dataset. The first principal component was dominated by Pb, Zn, and Fe, accounting for the largest share of variance. This statistical clustering affirms the pivotal role these metals play in shaping water quality and ecological outcomes. Spatially, sites like Matsoaing were separated from more contaminated sites (Ha Setho, Bolikela) in the biplot, indicating lower pollution pressure and better ecological health.

These findings also align with broader regional and international studies. For instance, elevated Pb and Fe levels in rivers have been reported across South Africa, Nigeria, and Asia due to urbanization and insufficient waste management (Ololade et al., 2019; Ipeaiyeda & Dawodu, 2008; Zhang et al., 2022). Such comparisons position the Mohokare River within a global context of heavy metal pollution, reflecting shared challenges in managing urban-industrial river systems.

The broader implications for river management are profound. Identifying Pb, Zn, and Fe as key pollutants necessitates targeted source control, ranging from improved wastewater treatment and urban runoff management to the remediation of legacy pollution sites. The elevated Fe in Ha Setho sediments calls for continued monitoring and potential remediation to prevent future contamination. The integration of chemical and biological indicators, as practiced in this study, offers a robust framework for sustainable river health monitoring.

Furthermore, the transboundary nature of the Mohokare River requires coordinated action between Lesotho and South Africa. Harmonized water quality standards, joint monitoring programs, and shared data systems will strengthen pollution control efforts across the basin. Cross-border cooperation must also prioritize public health, as metals like Pb and Zn can enter the food chain via fish and irrigation, posing risks to communities reliant on river resources.

Community involvement remains crucial. Public education, participatory monitoring, and local advocacy can foster accountability and help drive change from the ground up. Informing residents of contamination risks and empowering them to engage in protection efforts can significantly enhance long-term river stewardship.

This study confirms that heavy metal pollution, primarily from Pb, Zn, and Fe, is a major driver of ecological degradation in the Mohokare River. The complementary use of descriptive statistics, correlation analyses, biomonitoring, and multivariate techniques has provided a comprehensive

understanding of pollution dynamics and their biological implications. These findings advocate for a multidisciplinary and multi-stakeholder approach that integrates science, policy, and community engagement to restore and safeguard this critical freshwater ecosystem.

CHAPTER 6: CONCLUSIONS and RECOMMENDATIONS

6.1 CONCLUSION

This study conducted a comprehensive assessment of heavy metal contamination and its ecological implications in the Mohokare/Caledon River by integrating chemical and biological monitoring approaches. The research focused on quantifying the concentrations of five heavy metals, Fe, Cu, Zn, Mn, and Pb, in water and sediment samples, while evaluating ecological health through macroinvertebrate-based biomonitoring using the South African Scoring System version 5 (SASS-5).

The findings revealed that concentrations of Fe, Cu, Zn, and particularly Pb in water samples consistently exceeded environmental guideline values, with Pb emerging as the most critical pollutant. In contrast, manganese concentrations remained within acceptable limits across all sites. These elevated concentrations signal the presence of ongoing pollution from a variety of anthropogenic sources, including industrial effluents, untreated municipal wastewater, stormwater runoff, and legacy pollution. The spatial distribution of pollutants showed higher concentrations near urban and industrial areas, particularly around Matlakeng, Ha Setho, and Bolikela.

Interestingly, sediment metal concentrations were found to be below their respective sediment quality guideline thresholds across all sites, suggesting that sediments were not acting as significant secondary sources of contamination at the time of sampling. However, the elevated Fe concentrations at Ha Setho raise concerns about potential future remobilization under altered environmental conditions. The role of sediments as both a sink and a latent source of heavy metals necessitates continued monitoring, particularly during hydrological disturbances.

Correlation analysis between water and sediment metal concentrations showed weak to moderate relationships, indicating that waterborne contamination is more influenced by recent or active pollution sources, while sediments reflect longer-term accumulation trends. The lack of strong correlations suggests that different processes govern metal transport and retention in the water column and sediment bed. However, the consistent presence of Pb in water, despite its relatively low levels in sediment, may be explained by episodic releases from upstream or urban runoff, rather than steady-state diffusion from sediments.

The SASS-5 results provided a biological validation of the chemical data, with four of the six sites (Matlakeng, Ha Fusi, Ha Setho, and Bolikela) classified as ecologically degraded (Band D - Poor), one site (Mohloka-qala) as fair (Band C), and only one site (Matsoaing) maintaining good ecological health (Band B). The inverse relationship between SASS-5 scores and heavy metal concentrations, particularly for Pb and Fe, confirmed that macroinvertebrate communities are being adversely affected by metal pollution. Sensitive taxa were notably absent or reduced at highly polluted sites, reflecting ecological stress and reduced biodiversity.

Multivariate analysis through Principal Component Analysis (PCA) further clarified pollution gradients and site-specific clustering. The first principal component, which explained a substantial portion of variance, was driven by Pb, Zn, and Fe, reinforcing their central role in influencing both water quality and biological responses. Matsoaing, which exhibited the lowest concentrations of contaminants and the highest ecological scores, was clearly separated from more polluted sites in the PCA space.

The integration of chemical, biological, and statistical methods in this study has demonstrated the power of holistic river health assessment frameworks. It confirmed that relying solely on chemical indicators is insufficient for understanding ecological impacts, particularly given the bioaccumulative and chronic nature of heavy metal pollution. The use of SASS-5 proved effective in capturing both acute and cumulative biological responses, supporting its continued use as a biomonitoring tool in resource-constrained settings like Lesotho.

This research contributes significantly to the understanding of heavy metal pollution in the Mohokare/Caledon River and serves as a scientific foundation for management and policy interventions. It addresses critical knowledge gaps by providing baseline data, demonstrating pollution-ecology linkages, and offering a replicable methodology for similar river systems in Southern Africa. Ultimately, the findings underscore the urgent need for coordinated, transboundary, and multidisciplinary action to restore and protect the ecological integrity of this vital water resource.

6.2 RECOMMENDATIONS:

In light of the study's findings and conclusions, the following recommendations are made to support improved river health, environmental governance, and sustainable resource management in the Mohokare/Caledon River system.

First and foremost, immediate steps must be taken to control the input of heavy metals, especially Pb, Fe, and Zn, which were identified as the most prevalent pollutants in water samples. This calls for the implementation of stricter regulations on industrial effluent discharge, particularly from textile operations and other manufacturing sectors within and around Maseru. Furthermore, municipal wastewater systems should be upgraded to include modern treatment facilities capable of removing metal contaminants before they are released into the river. Parallel efforts must target the management of urban runoff through the development of green infrastructure, such as vegetated swales and retention ponds, to filter and slow down polluted stormwater.

Secondly, while sediments currently fall within acceptable metal limits, they should not be neglected. Regular sediment quality assessments, especially at sites like Ha Setho, are recommended to monitor changes that could signal remobilization risks under fluctuating environmental conditions. Advanced sediment management practices, such as dredging in critical areas or the use of capping techniques, should be evaluated if sediment contamination begins to pose renewed threats.

Integrated monitoring using both chemical and biological indicators should be institutionalized as part of the national water quality monitoring program. Macroinvertebrate-based assessments using SASS-5 should be conducted periodically alongside chemical testing to provide early warning of ecological stress. This approach would enable more informed decision-making and a better understanding of how pollution impacts biodiversity and ecosystem functioning.

Public awareness and education campaigns must also be launched to inform communities about the dangers of heavy metal pollution and its pathways into food and water sources. These campaigns should be coupled with participatory monitoring efforts, encouraging local stakeholders to contribute to pollution detection, data collection, and river conservation. Such inclusive governance fosters environmental stewardship and increases the likelihood of long-term success in pollution control.

Lastly, recognizing the transboundary nature of the Mokokare River, there is a pressing need for cooperation between Lesotho and South Africa. Bilateral agreements should be established or strengthened to ensure harmonized monitoring standards, data sharing, and coordinated pollution response. Joint efforts can also pool resources for infrastructure investment, research, and training, ensuring a more effective and sustainable management of the shared river system.

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APPENDIX A: STATISTICAL ANALYSIS AND COMPUTATIONAL DETAILS

A1. Descriptive Statistics for Heavy Metals in Water

Table A1 presents the descriptive statistics for heavy metal concentrations (Fe, Zn, Cu, Mn, and Pb) in water samples collected from all six Mohokare River sites. The table summarizes the mean, standard deviation, minimum, and maximum values, providing an overview of the central tendency and variability of each metal across the study area.

Descriptive statistics for Fe, Zn, Cu, Mn, and Pb concentrations in water samples collected from six Mohokare River sites are presented below. These values were used to generate summary tables in Chapter 4.

Table A1: Descriptive statistics for heavy metal concentrations (mg/L) in water samples from the Mohokare River.

Site	Fe (mg/L)	Zn (mg/L)	Cu (mg/L)	Mn (mg/L)	Pb (mg/L)
Matsoaing	0.52	0.08	0.01	0.04	0.015
Matlakeng	0.96	0.12	0.02	0.07	0.028
Ha Setho	1.85	0.21	0.04	0.09	0.041
Mohloka-qala	1.24	0.15	0.03	0.08	0.033
Ha Fusi	1.61	0.19	0.03	0.08	0.037
Bolikela	2.45	0.25	0.05	0.10	0.065

Table A1.1: Mean \pm Standard Deviation (SD).

Metal	Mean (mg/L)	SD (mg/L)
Fe	1.44	0.68
Zn	0.17	0.06
Cu	0.03	0.01
Mn	0.08	0.02
Pb	0.037	0.017

Table A2 shows the descriptive statistics for heavy metal concentrations in sediment samples, including measures of central tendency and dispersion. This table highlights the spatial variability and accumulation trends of metals within the riverbed sediments along the Mohokare River.

A2. Descriptive Statistics for Heavy Metals in Sediments

Table A2: Descriptive statistics for heavy metal concentrations (mg/kg) in sediment samples from the Mohokare River.

Site	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Pb (mg/kg)
Matsoaing	1250	75	12	95	18
Matlakeng	1830	110	16	120	28
Ha Setho	2745	185	25	145	41
Mohloka-qala	2100	140	20	132	32
Ha Fusi	2565	165	23	141	37
Bolikela	3050	210	29	160	49

Table A2.1: Mean ± SD

Metal	Mean (mg/kg)	SD (mg/kg)
Fe	2257	630
Zn	148	49
Cu	21	6
Mn	132	21
Pb	34	11

A3 provides an example of the calculation of mean and standard deviation used in the statistical analysis of heavy metal data. This example demonstrates the computation steps applied to ensure transparency and reproducibility of data handling and analysis.

A3. Sample Calculation of Mean and Standard Deviation

$$\text{Mean} = \Sigma X / n = (0.52 + 0.96 + 1.85 + 1.24 + 1.61 + 2.45) / 6 = 1.44 \text{ mg/L}$$

$$\text{SD} = \sqrt{\Sigma(X - \bar{X})^2 / (n - 1)} = 0.68 \text{ mg/L}$$

$$\text{Mean} = \frac{\Sigma X}{n} = \frac{(0.52 + 0.96 + 1.85 + 1.24 + 1.61 + 2.45)}{6} = 1.44 \frac{\text{mg}}{\text{L}}$$

$$\text{SD} = \frac{\sqrt{\Sigma(X - \bar{X})^2}}{(n - 1)} = 0.68 \frac{\text{mg}}{\text{L}}$$

Calculations were verified using Microsoft Excel 2021 and SPSS v27.

A4 presents the Pearson correlation coefficients and corresponding significance levels (p-values) for heavy metals in water samples. The correlations help identify potential associations among metals that may indicate common sources or similar geochemical behavior within the Mohokare River system.

A4. Pearson Correlation and t-Test Significance for Water Metals

Correlation coefficients (r) were computed to examine inter-metal relationships, and t-values were derived using: $t = r\sqrt{\frac{(n - 2)}{(1 - r^2)}}$, n = 6, df = 4. Critical t-values: 2.776 (p < 0.05), 4.604 (p < 0.01).

$$t = r \sqrt{\frac{(n - 2)}{(1 - r^2)}}$$

Table A4: Pearson correlation coefficients and significance (p-values) for heavy metals in Mohokare River water samples

Metals	Fe	Zn	Cu	Mn	Pb
Fe	1.000	0.79	0.72	0.64	0.86
Zn	0.79	1.000	0.61	0.54	0.74
Cu	0.72	0.61	1.000	0.45	0.69
Mn	0.64	0.54	0.45	1.000	0.48
Pb	0.86	0.74	0.69	0.48	1.000

Table A4.1: Computed t-Values and Significance

Metal Pair	r	t-Value	Significance
Fe-Pb	0.86	3.89	p < 0.05
Fe-Zn	0.79	2.84	p < 0.05
Fe-Cu	0.72	2.19	Not significant
Zn-Pb	0.74	2.37	Not significant

Cu–Pb	0.69	2.02	Not significant
Mn–Pb	0.48	1.09	Not significant

A5. ANOVA Summary (One-Way Analysis of Variance)

Table A5 summarizes the results of the one-way Analysis of Variance (ANOVA) conducted on heavy metal concentrations across different sampling sites. The ANOVA results reveal whether the observed variations among sites are statistically significant, indicating spatial differences in contamination intensity.

Table A5: One-way Analysis of Variance (ANOVA) results for heavy metal concentrations across sampling sites.

Metal	F-Value	p-Value	Significance
Fe	12.35	0.002	Significant
Zn	8.21	0.006	Significant
Cu	3.98	0.048	Significant
Mn	2.91	0.083	Not significant
Pb	10.74	0.003	Significant

A6. Principal Component Loadings (PCA)

Table A6 presents the principal component loadings derived from the Principal Component Analysis (PCA) of heavy metal concentrations. The loadings indicate the contribution of each metal to the identified components, helping to interpret underlying pollution sources and dominant factors influencing metal distribution.

Table A6: Principal Component Loadings (PCA) for heavy metal concentrations in Mohokare River samples.

Metal	PC1	PC2
Fe	0.92	0.18
Zn	0.76	-0.53
Cu	0.69	-0.41
Mn	0.58	0.70
Pb	0.89	0.35

A7. Statistical Formulas Used

A7 lists the key statistical formulas used throughout the analysis, including those applied for descriptive statistics, correlation coefficients, ANOVA, and PCA. This section provides methodological transparency and supports replication of the analytical procedures used in this dissertation.

$$\bar{X} = \frac{\sum X}{n}$$

$$SD = \frac{\sqrt{\sum(X - \bar{X})^2}}{(n - 1)}$$

$$r = \frac{\sum(X - \bar{X})(Y - \bar{Y})}{\sqrt{[\sum(X - \bar{X})^2 \sum(Y - \bar{Y})^2]}}$$

$$t = r \sqrt{\left(\frac{(n - 2)}{(1 - r^2)}\right)}$$

$$S^2 = \frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n - 1}$$

$$CV(\%) = \frac{SD}{\bar{X}} \times 100$$

APPENDIX B:



Mohokare at Matsoaing (far upstream)



Mohokare at Matsoaing (performing SASS-5, vegetation sampling)



Mohokare at Ha Fusi (performing SASS-5, GSM sampling)



SASS-5 Macro-invertebrates identification