Macroinvertebrate communities and environmental dynamics in relation to land use activities along the Nyl River, South Africa

By

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ABSTRACT

Rivers are dynamic freshwater ecosystems that play a crucial role in sustaining biodiversity, ecological processes, and human well–being. They function as natural pathways, transporting water, nutrients, and sediment across regions, shaping habitats and supporting diverse biological communities. In this study I have (i) assessed the broad use of developed biological tools of assessing river health in Southern African countries, (ii) assessed the spatio– temporal variation of metal pollution in water and sediments of a semi–arid river basin and, (iii) compared the taxonomic and functional characteristics of benthic macroinvertebrate communities in protected and non–protected areas. The findings of the study highlighted that the use of biological tools is increasing greatly across the country, although it shows some limitations in other countries where the indices/tool require modification. However, the method has shown effectiveness and reliability, as the indicator organisms are susceptible to a wide range of disturbances. Metal concentrations in water and sediments showed variations across sites and seasons, where metals in water were mostly high during dry season in the nonprotected areas, while metals in sediments were high during the dry and wet seasons in the protected areas. However, metals levels in the current study fell within the no effect levels and low effect levels, when compared with the Canadian sediment quality standards. Furthermore, all metals in water were below the baseline of the previous research, however, metals in sediments showed great variation with literature, as the results show decreased levels when compared to most studies and a sharp increase when compared to Ramogale (2008). Macroinvertebrate communities showed spatial and temporal variations, where results indicate that the non–protected area had a high diversity when compared to the protected area. However, the area was mostly dominated by the tolerant species which suggest that the area is not supporting a balanced diversity as results only indicate more tolerant species than sensitive species. The SASS and ASPT scores highlighted fair levels with few good levels for both sites and seasons. ANOSIM results also highlighted significant variation among macroinvertebrate community structure across sites and seasons. Overall, the results show a need for a continued monitoring of the system, in order to ensure its sustainability.

Keywords: Rivers, biomonitoring indices, macroinvertebrates, southern Africa, metal concentration and ecosystem health

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DECLARATION

I, Anna Malungani, student number 240650247, hereby declare that the study on "Macroinvertebrate communities and environmental dynamics in relation to land use activities along the Nyl River, South Africa" has not been submitted by anyone else or to any other institution and is my own research work, and that all sources that I used have been acknowledged by means of references at the end of this dissertation.

Signature: AMalungani Date 30/11/2025

CHAPTER ONE: GENERAL INTRODUCTION

General background

Rivers are very important freshwater resources as they provide a wide range of benefits to the community living around them and many of the water treatment infrastructures around the world abstracts water from them (Madilonga et al., 2021). The inadequate access to clean and safe water in rural areas lead to the dependence of river water for domestic and agricultural purposes in developing countries (Edokpayi et al., 2017). Rivers are often used for recreational purposes such as swimming, fishing, boat diving and as a tourist attraction area. However, due to the exploitation of this resource and the large number of dependences on it, its quality is easily degraded by various forms of pollution thereby threatening the potential of its use (Hoang et al., 2020; Edokpayi et al., 2021).

Major threats to river water quality includes point sources of pollution such as the discharge of partially treated and untreated wastewater (Aniyikaiye et al., 2019), the direct deposition of solid waste (i.e., household hazardous waste, construction and demolition debris, industrial/commercial waste) into and within the river courses (Edokpayi et al., 2021). Other threats are from non–point sources such as storm water runoff from agricultural lands into water bodies. Generally, in a river system all living organisms need a certain amount of metal concentration for effective and proper metabolic functioning (Eneji et al., 2011; Khan et al., 2023). Metals such as iron (Fe), copper (Cu) and zinc (Zn) are essential micronutrients for aquatic organisms since they participate in enzymatic reactions, photosynthesis and respiration (Khan et al., 2023).

However, regardless of being essential or not, all metals in a system with concentrations which exceeds naturally occurring quantities, produce toxic effects on the living organisms in the system (Greenfield et al., 2012). Fang et al. (2023) added that metals such as lead (Pb), cadmium (Cd) and mercury (Hg) can disrupt biological processes since they accumulate in organisms and affect their growth, reproduction and overall health. When metals are released into river water bodies, they are distributed between the water and sediments (Knox et al., 2016), where a small amount of the metals is dissolved in water and a larger portion is adsorbed in the sediment. Edokpayi et al. (2016) reported that metals which are known to adsorb to sediment particles under favourable conditions they leach back into the water column thus constituting a secondary source of pollution.

Moreover, metals bind to sediment particles and their presence influences sediment stability and affect erosion rates and nutrient transportation (Fang et al., 2023), and can also lead to species loss, altered community structures and reduced ecosystem resilience (Khan et al., 2023). Sediments are also known for causing destruction on the biological integrity (i.e., a balanced, integrated and adaptive system) of water bodies by causing physical and chemical problems such as filling streams, clogging storm sewers and ditches, altering the flow of water and reducing the water depth (Jones et al., 2012; Wharton et al., 2017; Wilkes et al., 2018; Gupta et al., 2023). However, the removal of sand and gravel from riverbeds for building materials, and the discharge of untreated sewage and wastewater continues to affect river water quality and threatens aquatic biota (Boyd, 2015; Damseth et al., 2024; Ihinmikaiye and Ojo, 2025). Sand and gravel extraction alters river morphology, increases turbidity, disrupts sediment balance, and leads to habitat loss, reduced biodiversity, and changes in aquatic community structure (Rentier and Cammeraat, 2022; Damseth et al., 2024; Ihinmikaiye and Ojo, 2025). Moreover, the discharge of untreated sewage and wastewater introduces excessive nutrients, organic matter, heavy metals, pharmaceuticals, and microplastics into river systems, resulting in eutrophication, oxygen depletion, toxicity, and shifts in microbial and aquatic community composition (Preisner, 2020; Grzywna et al., 2024; Yang et al., 2025).

Macroinvertebrates are invertebrates (i.e., animals without a backbone) that can be seen without the use of microscope or magnifying glasses such as snails, worms, crayfish and clams (Tarkowska-Kukuryk, 2012), and they play key ecological roles in aquatic and soil ecosystems (Nieto et al., 2017; Sri and Atapaththu, 2024) They are highly diverse and widely used as bioindicators of environmental quality and ecosystem health due to their sensitivity to changes in water quality, habitat conditions, and climate factors (Cai et al., 2022; Li et al., 2025). Their diversity plays an important role in maintaining ecosystem functioning, such as resistance to disturbances, biomass production and leaf decomposition in river systems (Manning et al., 2018; Rideout et al., 2022; Cai et al., 2023; Del et al., 2025). Due to them being consumers at intermediate trophic levels they regulate nutrient cycling, primary and secondary productivity, decomposition and translocation of materials (Wallace and Webster, 1996; Covich et al., 2004; McCary and Schmitz, 2021; Chakraborty et al., 2022). Regardless of their vital role in freshwater ecosystems their lives are threatened by pollution, since they are mostly sensitive to anthropogenic pressure and they respond to the change in aquatic ecosystem conditions (Agboola et al., 2020; Ko et al., 2020; Edegbene et al., 2021).

Freshwater ecosystems have become the most vulnerable and threatened in regions where humans have persistently intensified their use for various purposes such as agriculture, industries, urbanisation and resource extractions (Reid et al., 2019; Albert et al., 2020). Generally, rivers contain naturally occurring metals which are in low concentration for ecosystem metabolic functions (Eneji et al., 2011). The deterioration of river water quality is linked to increased human activities such as industrial pollution, agriculture and mining (Ngatia et al., 2023; Gavrilas et al., 2025; Mpopetsi et al., 2025). Metals released from those activities can be toxic or non–toxic to aquatic organisms, depending on their concentration and whether the element is an essential element or not (Khan et al., 2022; Santhosh et al., 2024; Banaee et al., 2024). But however, sediment burrowing and mixing by macroinvertebrates transform organic detritus from sedimentary storage into dissolved nutrients as a major source of primary producers (Covich et al., 2004; Wan Abdul Ghani et al., 2018; Tampo et al., 2020; Dahlin et al., 2021).

The broad study of macroinvertebrates, water and sediment chemistry dynamics is influenced by the fact that they provide different aspect of water quality status (Duque et al., 2021; Pinto et al., 2021). Water analysis indicates contamination status at present and sediment analysis indicate the potential contamination on a temporal scale and provides information on the system's contamination history while macroinvertebrates indicate the cumulative impacts of pollution in rivers (Strbac et al., 2017; Edegbene et al., 2021; Cai et al., 2022).

Problem statement

The river pollution problem related to human disturbances through various stressors such as land use changes, deforestation, agricultural intensification and urbanisation is intensifying around the world (Faridah et al., 2012; Luo et al., 2020; Edegbene et al., 2021; Mpopetsi et al., 2025) has many adverse impacts on river ecosystems that have required managers to increase water assessment efforts in order to restore the ecological status of their rivers (Fayiga et al., 2018; Wantzen et al., 2019). In many studies, biological methods are valuable for determining natural and anthropogenic influences on water resources and habitats because biota responds to stressors from multiple spatial or temporal scales (Resende et al., 2010; He et al., 2020; Kurthen et al., 2020). However, the use of aquatic organisms (i.e., macroinvertebrates) in ecological studies has proven to be more effective than using environmental variables alone, since the aquatic community integrates structural and functional characteristics and reflects the health of the studied river system (He et al., 2020). Macroinvertebrate responses to the change in aquatic ecosystem condition is universally recognised, and their responses are used in indices to monitor freshwater ecosystem for integrity, aiding in decision making in management (Kaboré et al., 2016; Agboola et al., 2019; Tampo et al., 2020; Edegbene et al., 2021). Nonetheless the decrease in intolerant pollution macroinvertebrates is alarming, since it reveals that the ecological significance of the river is being degraded.

Land use activities such as game rangers, settlements, road construction, agricultural practices, mining, and treatment facilities along the Nyl River significantly threaten the ecological habitat, water, and sediment chemistry (Greenfield et al., 2012). Human activities which impact the land can cause changes in nutrient levels and sediment concentrations through runoff and land disturbance, causing degradation in water quality and harm in aquatic ecosystems (Aghsaei et al., 2020; Serrão et al., 2021; Neumann et al., 2025). Changes in land cover, such as increase in agriculture and urban areas, have been shown to increase sediment yield, nutrient loads, and pollutant concentrations, which degrade habitat quality and disrupt water cycles (Aghsaei et al., 2020; Serrão et al., 2021; Ajid et al., 2025). Riparian land use influences stream habitat and water chemistry, with natural riparian zones helping to reduce nutrient export and maintain water quality, while disturbed areas contribute to increased nutrient and sediment inputs (Ramião et al., 2020). Mining and agricultural pesticides introduce toxic substances into soils, sediments, and surface waters, posing a huge ecological risk to aquatic organisms (Pérez et al., 2021). Good land management and restoration projects can lessen these negative effects by decreasing runoff, soil erosion, and nutrient pollution, which improves the benefits we get from water ecosystems and protects freshwater habitats (Anjinho et al., 2024).

Despite previous studies on the Nyl river basin, the most recent assessment was in 2017-2018 creating a significant temporal gap in understanding the current ecological conditions of the system. As over the past six years land use patterns within the system have changed with increasing agricultural expansion, intensified human activities and infrastructure development which may have altered water quality, sediment composition and macroinvertebrate community structure. Moreover, climate variability and prolonged dry periods of semi-arid regions may have influenced the state of the river throughout the past six years. The absence of updated integrated assessments limits the ability of managers to detect emerging pollution trends, evaluate the effectiveness of past management interventions and implement evidence-based conservation strategies. Therefore the current study is both timely and necessary to address these knowledge gaps and reassess ecological integrity across protected and non-protected areas, while also providing an updated baseline data which will inform the sustainable river management and restoration efforts.

Aims

To assess macroinvertebrate communities and environmental dynamics in relation to land use activities along the Nyl River, South Africa.

Objectives

- To evaluate the spatial and temporal patterns of metal pollution in water and sediments of a semi–arid river basin in South Africa: protected and non–protected areas.
- To assess metal contamination in water and sediments using pollution indices and evaluate the associated toxic risks to ecosystem health and functioning.
- To compare the taxonomic and functional characteristics of benthic macroinvertebrate communities in protected *versus* non–protected freshwater sites.

Hypotheses

- Metal contamination in water and sediments will show seasonal variation due to bioturbation and water flow in various section of the river.
- Metal concentrations will increase downstream due to cumulative industrial, mining, and agricultural inputs.
- Protected sites would exhibit high taxonomic richness and diversity, a large proportion of sensitive taxa, distinct community assemblages, greater functional trait diversity, and elevated biological integrity compared with non–protected sites.

Thesis structure

- **Chapter One: General introduction** - This chapter establish the contexts of the research, by presenting the link between macroinvertebrate communities, sediment chemistry, water quality and land use activities along the semi-arid river. It also highlights the importance of evaluating these factors (i.e., macroinvertebrates, sediments and water), as they are the key to ecosystem monitoring. The chapter also outlines the guide of the study (i.e., aims, objectives, hypotheses and problem statement).
- **Chapter Two: Aquatic macroinvertebrates as bioindicators of river health in Southern Africa: a review of past and current use, its effectiveness and challenges -** The chapter provides a comprehensive review of existing literature on biomonitoring tools in the Southern African' rivers, where macroinvertebrates communities are used as bioindicators. It indicates the broad use of the developed yet effective tools which helps in quick assessment of ecosystem health. The chapter also highlight the reasons why macroinvertebrates were chosen as indicators of the systems' health. The review also highlights the effectiveness of the tool and its limitations, especially when adopted by other countries. The chapter also include the recommendations of coupling the biomonitoring tools with multrimetric approaches.

Chapter Three: Spatio-temporal assessment of metal pollution in water and sediments of a semi-arid river basin in South Africa: protected and non-protected areas - This chapter investigates the spatio-temporal variations of metal concentrations in water and sediments of a river basin, where basin protection and non-protection are taken into consideration. The chapter highlights the possible sources of metals in water and sediments, while also exploring the basis that sediments can acts as sink for metals. Moreover, possible impact of extreme metal levels on aquatic ecosystem has been noted to details. The chapter also includes a detailed comparative literature on metals in water and sediments in the Nyl River basin.

- **Chapter Four: Comparative study of benthic macroinvertebrate communities in protected and non-protected freshwater sites** - The chapter focuses on comparing the taxonomic and functional characteristics of macroinvertebrate communities in the protected and non-protected areas, with seasonal impact considered. The chapter explains to detail, the importance of macroinvertebrates in freshwater systems, while also highlighting threats which are posed due to river disturbances. The chapter include results which highlight the role played by basin security and also the effect which arises due to extreme exposure to change/pollution.

- **Chapter Five: General synthesis** - This chapter incorporate all the findings from the preceding chapters, in order to provide substantial information on the role and influence of land use activities on freshwater and aquatic life. It provides a broad understanding of how our inputs can cause severe losses. Furthermore, it highlights the role of natural impact on freshwater systems, which are most likely beyond human control. However, the chapter also includes recommendations, which are likely for educating, monitoring and protecting the ecological systems.

CHAPTER TWO: AQUATIC MACROINVERTEBRATES AS BIOINDICATORS OF RIVER HEALTH IN SOUTHERN AFRICA: A REVIEW OF PAST AND CURRENT USE, ITS EFFECTIVENESS AND CHALLENGES

Abstract

Freshwater ecosystems are among the most threatened ecosystems in the world as they are mostly affected by pollution, habitat degradation, climate change, overexploitation, species invasion and flow modification. However, not only freshwater ecosystems are threatened, but also macroinvertebrate species are at higher risk of extinction due to those factors. Hence, monitoring and management of ecosystem integrity of freshwater bodies (i.e., streams and rivers) has become a growing scientific and public concern. Rapid bioassessment methods (RBMs) have been developed in response to the need in water management for quick and cost–effective methods for assessing water quality given the rising negative impact of anthropogenic activities on water systems. Aquatic macroinvertebrates are well established as dependable indicators of river condition because they are susceptible to various environmental pressures and play an important ecological role in freshwater environments. In Southern Africa, using macroinvertebrates for biomonitoring has become increasingly important in the last thirty years. Methods such as the South African Scoring System (SASS) are commonly used to evaluate and manage river health. This study examines the past and present use of macroinvertebrate bioassessment techniques in Southern African countries, emphasizing their success in identifying ecological damage related to contamination, changes in water flow, alterations in land use, and climate change. Although these techniques have shown to be valuable in providing affordable, quick, and ecologically relevant evaluations, their application faces obstacles. Major weaknesses include variations in procedure consistency between areas, a shortage of specialists in species identification, a lack of connection with new modelling techniques and insufficient attention to local ecological factors. However, there is an increasing opportunity for improvement through combined methods that integrate conventional measurements with hydrological and habitat information. Developing these approaches will be essential for enhancing the trustworthiness and relevance of biomonitoring.

Keywords: Rivers, macroinvertebrates, anthropogenic pressures, biomonitoring, rapid bioassessment methods, ecosystem health

Introduction

Rivers are becoming one of the most endangered precious natural resources on earth (Dudgeon et al., 2006; Dalu and Masese, 2025). Due to the ecological ramifications of land use change, climate change, and human impacts, river health has become a major problem (Hoekstra and Wiedmann, 2014; Zhao et al., 2017; Krajenbrink et al., 2019). According to Stendera et al. (2012), freshwater ecosystems are becoming less able to maintain and support a robust and balanced diversity of organisms that depend on natural habitat. Human activities such as sand mining, agriculture and settlement have a significant impact on the water chemistry and consequently affect the aquatic biota (Mwedzi et al., 2016; Mangadze et al., 2016; Addo–Bediako et al., 2021; Mpopetsi et al., 2025). Henceforth, it is worth noting that economic development and population increase are some of the underlying causes of the ecological integrity loss (Munyika et al., 2015). Furthermore, human intervention has significantly altered the structures of diverse communities, including functional diversity, richness, and individual abundance (Brasil et al., 2019; Addo–Bediako, 2021).

In the past decades, the health of aquatic ecosystems was assessed traditionally (Rocha, 1992), through the physical and chemical measurements, which screened the environmental conditions present at the sampling time and gave a 'snapshot' of water quality parameters at that specific time (Masese et al., 2013; Aschalew and Moog, 2015; Masese et al., 2024). However, presently biological monitoring with the use of macroinvertebrates as bioindicators are used as they provide a 'moving picture' of previous and latest environmental conditions (Masese et al., 2013). Aquatic macroinvertebrates are predominantly used as bioindicators of ecosystems health, as they are able to reflect the change in water quality over time (Li et al., 2010). They are also known to be highly diverse faunal group which inhabit many niches and habitats (Obade and Moore, 2018). Furthermore, they consist of different taxa with a wide tolerance range to environmental pollution (Qu et al., 2013; Rasifundi et al., 2018; Edegbene et al., 2020; Odume et al., 2023), thus providing a strong information for interpreting cumulative effects (Kripa et al., 2013). Kilonzo et al. (2014) further highlighted that macroinvertebrate assemblages respond to the changes in land–use and water quality in terms of community composition and diversity. Moreover, their distribution across river systems are mainly influenced by the availability of microhabitats, interactions among habitat characteristics, physicochemical variables, hydrological characteristics, human activities and food resources (Resh and Rosenberg, 1984; Masese et al., 2025). The ecological branch of biomonitoring is based on the principle that organisms are the ultimate indicators of the health of the environment they live within (USEPA, 2002).

Biomonitoring standards had to be developed to ensure consistency and reliability in assessing freshwater ecosystems. The internationally developed ISO 8689–1:2000 standard offers guidance for interpreting biological quality data derived from benthic macroinvertebrate surveys, establishing a framework for biological classification (ISO, 2000). Moreover, the Water Framework Directive (2000/60/EC) in the European Union requires member of the states to attain "good ecological status" for all water bodies by utilising biological, hydro–morphological, and physicochemical quality aspects (European Commission, 2000). Furthermore, Guidance Document No. 32 under the Water Framework Directive (WFD) delineates procedures for biota monitoring to evaluate adherence to environmental quality criteria (European Commission, 2014). Hence, the internationally recognized protocols, such as the Organisation for Economic Co–operation and Development (OECD) biomonitoring guidelines and the US EPA Rapid Bioassessment Protocols, are widely applied for comparative studies and ecological assessment globally (USEPA, 2006; OECD, 2016). In the region biomonitoring standards are adapted to local ecological and environmental conditions to provide accurate assessments of freshwater ecosystem health. Chutter (1993), developed the South African Scoring System (SASS), a biotic index based on the presence of specific aquatic macroinvertebrate families and how sensitive they are to changes in water quality. The South African Scoring System (SASS) is the predominant tool in South Africa, utilising macroinvertebrate assemblages as bioindicators and integrating habitat weighting to represent local river conditions (Dickens and Graham, 2002; Dallas, 2007). However, this quick bio–assessment technique which was created to quickly evaluate the quality of ambient water has its own drawbacks (Chutter, 1993). Countries such as Namibia, Botswana and Zambia had to modify it so that it can also account to additional tropical invertebrate taxa that occur specifically in their regions. This review aims (1) to broadly discuss the benefits and the limitations which came with the use of biomonitoring as another approach to evaluating ecological systems, (2) to outline the history of biomonitoring as per literature, while also highlighting factors which were said to be considered when using a biomonitoring tool.

Methods

The study applied a structured literature review approach to evaluate the development, application and effectiveness of biomonitoring tools used for assessing river health in Southern Africa. The primary focus was on macroinvertebrate–based indices alongside integrative methods. A broad literature search without year restriction was conducted across databases such as Scopus, Web of Science, Google Scholar, ScienceDirect and ResearchGate. The searching terms included macroinvertebrates, rivers, anthropogenic disturbance, water quality, habitat loss, human impact, biomonitoring and countries (southern Africa). Studies were included if they focused on Southern African freshwater ecosystems, examined biomonitoring tools or indices using macroinvertebrates and were published in English as peer–reviewed articles, thesis or official reports. From each study, data was extracted on author(s), year, study location (river name), and the study' dominant macroinvertebrates group (Table 2.1). The sampling methodology helped in identifying the environmental and anthropogenic factors and reported strengths, limitations and effectiveness of each biomonitoring tool as used in the southern Africa. The data was integrated to compare the performance of traditional biomonitoring ways with the developed macroinvertebrate indices, identifying factors which influence tool selection and application (such as habitat, water quality and anthropogenic pressures). Moreover, the integrated data highlighted research gaps and emerging trends in Southern African river biomonitoring and results were summarized using tables to present, river systems which were studied.

Threats to macroinvertebrates

According to literature the structure and abundance of the macroinvertebrate community are influenced by several factors both inside and outside the river. Palmer et al. (1994) conducted a study which evaluated the role of altitude and slope in structuring the macroinvertebrate community, in that study major changes in macroinvertebrates composition occurred between sites which are separated by the steepest altitudinal change than between any other adjacent sites (Palmer et al., 1994). Several studies have demonstrated that the majority of river systems are simultaneously affected by a multitude of anthropogenic stressors. For example, Mangadze et al. (2015), Nhiwatiwa et al. (2017), Chikodzi et al. (2017), Mwedzi et al. (2017) and Makaka et at. (2018) conducted studies which highlighted how riverine ecosystems are subjected to various environmental pressures including metal and organic pollution, eutrophication and other environmental factors that significantly influence species abundances and community structure. Furthermore, Thiere and Schulz (2004) investigated the impact of agricultural runoff on macroinvertebrate communities, emphasising the risks associated with the use of fertilizers and insecticides. They noted that these agrochemicals often enter aquatic ecosystems through surface runoff, leading to degradation of water quality and adverse effects on aquatic biota, particularly macroinvertebrate diversity and abundance.

Ferreira et al. (2009) and Nakin et al. (2017) added that spatial (natural materials, e.g. soil, rock, water and air) and temporal (the measure of variance in occurrence over time) variations can also influence the distribution of macroinvertebrates communities. Taxa Mollusca are said to be the least tolerant macroscopic invertebrates to heavy metal pollution while midge (Chironomidae) larvae are normally the dominate macroinvertebrate communities in sites which are sleeky polluted by heavy metals (Brkovic–Popovic and Popovic, 1977; Winner et al. 1980). Mass development of oligochaete at a site is usually a sign of large amounts of easily oxidised organic matter and a relatively low concentration of heavy metals salts and poisons (Slepukhina, 1984).

Anthropogenic disturbance and recovery of macroinvertebrates in streams and rivers

According to Mpopetsi et al. (2025), global human population increase leads to rivers and streams' significant exposure to pollutants resulting from various waste discharges such as industrial expansions, constructions and agricultural development. Moreover, Sala et al. (2000), Reid et al. (2019) and Sun et al. (2021), added that human disturbance significantly leads to the global loss of biodiversity at an unprecedented rate leading to pressing environmental challenges. Most aquatic ecosystems are particularly affected by human pressures, including habitat degradation, water abstraction, and pollution from industrial, agricultural, and urban sources (Jackson et al., 2016; Masese and Otieno, 2021; Martins et al., 2021; Hughes and Vadas, 2021). Furthermore, the apparent human pressure affects freshwater ecosystems where habitats are lost and/or degraded while some face extinction which result in macroinvertebrates population decline (Niba and Sakwe, 2018).

Rivers can naturally, although imperfectly, return water quality to pre–pollution levels through biological processes, sedimentation and dilution. However, a significant portion of freshwater systems that have not undergone irreversible change from their initial state due to human activity are probably gone (Elias et al., 2014). Rivers no longer adequately cleanse the pollution that is dumped into them as cities expand and impact is beyond their natural self–purification capacity (Fakayode, 2005; Kassenga and Mbuligwe, 2009). Due to increase in population leading to increased human activity, systems receive higher concentrations of suspended and dissolved solids, nutrients, turbidity, and coliform bacteria which are brought on by an increased pollution load (Adams and Papa, 2000). Thus, the community structure of benthic macroinvertebrates and other aquatic biota within the water column is changed by such alterations (Boyle and Fraleigh, 2003). Additionally, based on their degrees of tolerance and adaptability, freshwater macroinvertebrate species' occurrence, composition, and distribution are influenced by the released wastes from industries, businesses and communities, as rivers are viewed as dumping sites/areas (Suleiman and Abdullahi, 2011; Elias et al., 2014).

Factors that regulate macroinvertebrates in rivers and streams

Temperature

Temperature represents a key environmental control which impact the biology, life cycles, and spatial patterns of lotic macroinvertebrates. It affects their development rates, generation frequency, emergence phenology, and assemblage structure along natural thermal gradients and within anthropogenically modified systems (i.e., deforestation along waterways, dam construction, heated discharges) (Dallas, 2008; Bonacina et al., 2022). Increased water temperatures, frequently associated with decreased streamside vegetation cover, climatic fluctuations, and alterations in land management practices, can diminish the presence of dissolved oxygen (Palmer and Taylor, 2004; Dallas and Day, 2004). This can lead to the elimination of susceptible organisms such as mayflies, stoneflies, and caddisflies (EPT), while promoting the rapid production of more adaptable groups such as midges and aquatic worms (Palmer and Taylor, 2004; Dallas and Day, 2004; Johnson et al., 2024). In contrast, colder, thermally consistent environments typically sustain greater variety and quantity of susceptible organisms, indicating improved ecological integrity (Johnson et al., 2024). Within southern Africa, where numerous rivers undergo substantial seasonal variations, temperature and water flow interact to influence habitat appropriateness and the temporal behaviour of macroinvertebrate communities (Dallas, 2013).

The Intergovernmental Panel on Climate Change (IPCC) predicted that mean land surface temperature in southern Africa is likely to exceed the global mean land surface temperature increase in all seasons (IPCC, 2021). Moreover, this change will alter the life cycle of macroinvertebrates as water temperature regimes are frequently impacted by river/stream flow changes due to natural disasters, particularly in small headwater streams (Dallas and Rivers–Moore, 2014). The pace of the physiological processes in organisms, such as microbial respiration, is influenced by the temperature of the water where higher temperatures encourage faster growth, allowing some macroinvertebrates and fish to establish considerable populations (Water Quality Assessment, 1996). However, the thermal tolerances of individual taxa, and their ability to withstand changes in water temperatures, may ultimately translate into shifts evident at community levels as organisms can grow stressed and die if temperatures are consistently outside their ideal range (Rivers–Moore et al., 2018; Rivers–Moore and Dallas, 2022). Aquatic insects, particularly bottom dwellers (i.e., alderfly larva, caddis flies, mayflies, and others), are temperature sensitive and will migrate to locations in the river/stream where the temperature is ideal for their survival (Hering et al., 2009).

Land use activities

Human activities have significantly increased the speed at which habitats are changing and being lost. Due to the growing demands of a growing population, natural habitats are being transformed into plantations, grazing areas, infrastructure, and agricultural lands (Bodo et al., 2021; Williams et al., 2021). Moreover, in stream ecosystems, grazing has had a significant impact, altering nutrient loads (Murphy et al., 2015), changing hydrology (i.e., flow regime, predictability, and water quantity) (Fulgoni et al., 2020), and causing erosion that leads to soil loss and sediment input (Cavaillé et al., 2018). Due to livestock trampling riverbeds, habitats for benthic organisms are destroyed leading to organism population declines (González–Trujillo et al., 2019).

Global population increase serves as a major destruction tool in both rivers and streams, as an increase in population leads to an increase in service demand (IERE, 2005). Increasing global demand for minerals and metals creates opportunities for the economy and employment, yet exploitation of these resources may result in unexpected environmental impacts and consequences on biodiversity while also freshwater ecosystem is also tempered with (Byrne et al., 2017). Additionally, riverbed morphology is mostly altered during placer mining as fertile topsoil is removed, resulting in rivers being subjected to increased turbidity, sediment deposition and introduction of nutrients, especially phosphorus and heavy metals which pollutes the water and endanger aquatic insects (Stubblefield et al., 2005; Inam et al., 2011).

As rivers and streams are expected to meet the livelihood requirements they are mostly exploited as surrounding communities depend on natural resources for various purposes, such as food provision, leisure activities and water for domestic use (Reid et al., 2004). However, these activities have in turn, altered some rivers from perennial to being seasonal while converting some habitat from lotic to lentic (García et al., 2010; Wu et al., 2019). Moreover, most forest have been converted to agricultural development, industries, households and other constructions leading to loss of diversity, abundance and biomass of macroinvertebrate shredders and other sensitive taxa that are adapted to cooler forested steams (Masese et al., 2014; Fugère et al., 2018; Yegon et al., 2021).

As a result of land–use activities such as agriculture (Figure 2.1), infrastructure development, and water abstraction river habitats have been lost, posing a threat to freshwater biodiversity (Mpopetsi et al., 2025). Although water abstractions and dam construction provide reliable water supplies and reduce drought, they can adversely affect sediment quality and flow patterns, which can negatively impact biodiversity (Reid et al., 2004; Bredenhand et al., 2009; Darwall et al., 2009; Mpopetsi et al., 2025). Moreover, dam constructions are mostly known for decreasing macroinvertebrates taxa richness in downstream reaches due to altered flow regimes, changes in water quality (sedimentation, temperature, nutrients) and habitat degradation which favour certain species and make it harder for others to survive (Wu et al., 2019). It is commonly known that a variety of aquatic life in rivers are susceptible to variations in flow and temperature brought on by dams, as well as decreased river connectivity and changed hydrochemistry (Reid et al., 2019; Mellado–Díaz et al., 2019). In addition, the decline in species diversity has been widely observed in all types of dammed rivers, including large (Castello and Macedo, 2016), to small rivers (Lessard and Hayes, 2003; Couto and Olden, 2018).

Figure 2.1. An illustration of most common land use activities.

River vegetation

In aquatic ecosystems both riverbank (riparian) and submerged vegetation (macrophytes and filamentous algae) (Figure 2.2) significantly influence the communities of macroinvertebrates. They create varied habitats, providing sustenance, affecting the local environment and influencing water flow and channel form (Dallas and Rivers–Moore, 2014; Dalu et al., 2022). Vegetation increases the diversity of the riverbed through its roots, stalks and decaying matter while offering sites for attachment and protection that benefit organisms which consume decaying matter, scrape surfaces and prey on others (Dallas and Day, 2004; Birkett et al., 2017; O'Hare et al., 2018; Bunn et al., 2022). Furthermore, it regulates water speed and sediment accumulation, which impacts the distribution of organisms that collect particles or dig them (Obade and Moore, 2018; Chakona et al., 2020).

Organic matter originating from external sources and attached algae from vegetation also modify food supply and the composition of functional feeding groups, often leading to increased macroinvertebrate numbers and local species richness when water quality is high (Wallace and Webster, 1996; Dickens and Graham, 2021). On the contrary, the removal of vegetation along the riverbank and within the water bodies due to land use changes, controlled river flow/excessive nutrients can significantly alter available habitat and favours adaptable species while reducing functional diversity (Buss et al., 2002; Allan, 2004; Joubert et al., 2020a; Dalu et al., 2022). However, this weakens the capacity of macroinvertebrate indicators to accurately assess river health (Buss et al., 2002; Dalu et al., 2022). Vegetation significantly influences hydrological characteristics, current regime and sediment transport (Dallas and Rivers–Moore, 2014; Chakona et al., 2020). Therefore, evaluating riparian zone attributes and aquatic plant distribution is crucial for understanding macroinvertebrate community patterns (Dalu et al., 2022). Integrating these habitat characteristics into biomonitoring protocols enhances the reliability of river condition evaluations within southern African ecosystems (Joubert et al., 2020b; Dickens and Graham, 2021).

Figure 2.2. Different macrophyte vegetation types along the Nyl River system.

Macroinvertebrates represent one of the most diverse groups of aquatic organisms and serve as a crucial intermediate link in freshwater food webs, consuming a variety of food sources including plankton, benthic algae, leaf litter, and fine particulate organic matter, while also providing prey for fish and other large aquatic organisms (Figure 2.3; Wallace and Webster, 1996; Covich et al., 1999). Their high diversity allows them to occupy numerous niches and habitats within freshwater systems (Obade and Moore, 2018). The distribution of macroinvertebrates is primarily determined by microhabitat availability and food resources, but is also influenced by interactions among habitat structure, physicochemical variables, hydrology, and anthropogenic activities (Resh and Rosenberg, 1984). Consequently, alterations in water body characteristics, habitat quality and environmental resources strongly shape benthic community patterns (Buss et al., 2002). However, habitat loss and degradation remain major threats to freshwater ecosystems, leading to declines in species populations and increased risk of local extinctions (Brooks et al., 2002).

Figure 2.3. An illustration of unprocessed macroinvertebrate samples from the Nyl River.

Water quality

In rivers and streams, macroinvertebrate ecosystems are mostly regulated by water quality factors such as dissolved oxygen, temperature, pH, conductivity, turbidity and nutrient concentrations. Dissolved oxygen is very crucial as different organisms require a certain concentration for their survival. Tolerant species such as Chironomidae flourish in low–oxygen environments, while many sensitive taxa such as Ephemeroptera, Plecoptera, and Trichoptera need high oxygen levels (Dallas and Day, 2004; Chakona et al., 2020). However, the metabolic processes, development rates and life cycles of macroinvertebrates are significantly influenced by temperature where high temperatures resulting from urban runoff or the loss of riparian vegetation frequently reduce variety and favour species that can withstand high temperatures (Dallas and Rivers–Moore, 2014; Dalu et al., 2017). Moreover, extreme values of pH and conductivity reduce species richness and change the composition of communities towards tolerant taxa, reflecting underlying geology and human pollution (Dallas, 2008; Gerber et al.,2015). Land use change causes increased turbidity and sedimentation, which cover benthic substrates and restrict light penetration while lowering habitat quality and affecting the availability of food from periphyton and detritus (Matlou et al., 2017; Dalu et al., 2022). In addition, the eutrophication and algal blooms caused by elevated nutrient loads from urban effluents and agriculture frequently reorganise communities by giving preference to filter–feeders and collector–gatherers over sensitive functional groups (Joubert et al., 2020a; Dickens and Graham, 2021). Through altering macroinvertebrate assemblages and their dependability as bioindicators of river health in the area, these water quality factors work together to create powerful environmental filters.

Degraded stream/river water quality is globally becoming a common predicament where the emerging impacts from climate change, river regulation and flow diversion create several challenges for the management of freshwater ecosystem water quality (Cha et al., 2017). For example, the Olifants River Basin is under threat from a growing human population and water demands associated with economic development which in turn have an impact on biodiversity (Matlou et al., 2017). Furthermore, the declining water quality and quantity present a pressing challenge, largely driven by intensified human activities. Industrialization and urban expansion have increased pollution loads in rivers and streams, while mining contributes heavy metal contamination and acid mine drainage that severely impact aquatic ecosystems (Gerber et al., 2015; Matlou et al., 2017). Agricultural intensification, including the use of fertilizers and pesticides, has led to nutrient enrichment and sedimentation, further degrading water quality and altering aquatic habitats (Chetty and Pillay, 2019). Moreover, large–scale afforestation and power generation activities have modified hydrological regimes, reduced water availability and disrupted ecological processes critical for sustaining aquatic biodiversity (Addo–Bediako, 2020). The Mohlapitsi River which has been known for supplying water of good quality to the Olifants River has been affected by the increasing human activities which takes place along the river midstream and downstream resulting in adverse impact on water quality (Matlou et al., 2017).

Climate change

Growing evidence points to climate change as a key determinant of macroinvertebrate assemblages in southern African rivers and streams, largely due to its effects on water quality, flow patterns, and temperature. By promoting warm–adapted taxa and decreasing the prevalence of cold stenothermic groups, rising air and water temperatures change species ranges, resulting in changes to community composition and possible biodiversity reductions (Dallas, 2008; Dalu et al., 2017). Baseflows and habitat availability are decreased by altered rainfall patterns and more frequent droughts (Figure 2.4), which frequently lead to the local extinction of sensitive taxa and the dominance of tolerant, generalist species (Le Roux et al., 2019; Chakona et al., 2020). According to Arimoro et al. (2015), extreme flood events linked to climatic variability also disrupt benthic ecosystems, uprooting invertebrates and reorganising populations. Additionally, aquatic invertebrates experience physiological stress due to climate–driven decreases in dissolved oxygen and elevated salinity, which further limits their ability to survive and operate ecologically (Dallas and Rivers–Moore, 2014; Odume, 2021). When taken as a whole, these effects show how climate change is a major factor influencing the dynamics of macroinvertebrates in river systems in southern Africa, which has consequences for ecosystem health and biomonitoring initiatives.

Variations in river flow, especially during flood or drought events, can also cause riparian vegetation to change and this include a drop in cover and a loss or shift of species, with non–riparian species becoming more prevalent as flow declines (Mpopetsi et al., 2025). Moreover, climate change is likely to have an impact on aquatic assemblages operating at multiple levels (individual, population, and community), with species–specific susceptibility to climate change differing according to key biological characteristics such as thermal tolerance ranges (Dallas, 2008; Woodward et al., 2010), life history traits (Desta et al., 2012), dispersal ability (Domisch et al., 2011), reproductive strategies and habitat specialisation (Chakona et al., 2020). For example, species with narrow thermal tolerance, limited dispersal capacity, long generation times, or strong dependence on specific habitats and resources are often more vulnerable to shifts in temperature and flow regimes, while generalist taxa with broader physiological plasticity and higher reproductive rates may exhibit greater resilience (Desta et al., 2012).

Figure 2.4: The effect of climate change acceleration: A vicious cycle (image credits: wikimedia).

Biotic indices

Due to the rapid destruction of river systems and the narrow National water quality monitoring programs, different indices and sampling protocols were developed as an aid to evaluate water quality of rivers and streams. Biotic indices have been developed, starting with the saprobic system which is a system used to evaluate the degree of water pollution by means of taxonomic and quantitative analysis (Zahradkova and Soldan, 2008). Other biomonitoring indices includes the Biological Monitoring Working Party Score System – BMWP (Armitage et al., 1983) which is a score that equals the sum of the tolerance scores of all macroinvertebrate families in the sample, where a higher score is considered to reflect a better water quality, Family Biotic Index– FBI (Hilsenhoff, 1987) which is mostly based on the tolerance of species to reduced oxygen associated with organic pollution (Chutter, 1972), South African Scoring System – SASS (Dickens and Graham, 2002; Chutter, 1994, 1998) which is a qualitative, multi–habitat, rapid field–based method which requires the identification of macroinvertebrates to family level (Dallas, 2004a, 2004b), Average Score Per Taxa – ASPT (Dickens and Graham, 2002) which is a water quality index that rates benthic invertebrates families according to their sensitivity to

dissolved oxygen depletion as it was developed for detecting the water pollution caused by organic substances (Armitage et al., 1983; Sidagyte et al., 2013) and Stream Invertebrate Grade Number Average Level Version 2 – SIGNAL2 (Chessman, 2003) which is a simple scoring system for macroinvertebrates (water bugs) samples from Australian Rivers and it suits both the family and order–class phylum identification. Moreover, some of the Southern Africa (e.g. Namibia, Botswana and Zambia) countries modified and standardised SASS into the Namibian Scoring System – NASS (Palmer and Taylor, 2004), Okavango Assessment System – OKAS (Dallas, 2009) and Zambian Invertebrate Scoring System – ZMSS (Lowe et al., 2013; Dallas et al., 2018) in order to account for additional tropical invertebrate taxa that occur specifically in these regions as SASS is not universally applicable as the tolerance of organism varies under a different set of environmental conditions (Cairns and Dickson, 1973). All the developed indices are tools which assess water quality based on the different responses of organisms in relation to their environmental changes (Borisko et al., 2007). Moreover, several biotic indices, especially those which depend on rapid, field–based protocols, use family–level taxonomic resolution as it is simple, quick and less expensive
(Bonada et al., 2006).

Biomonitoring in Southern Africa

South Africa

Physical and chemical analysis was initially used to evaluate the health or state of rivers, but this method was costly, time–consuming, and limited to that sampling period (Rocha, 1992; Masese et al., 2013; Aschalew and Moog, 2015). Thus, the shortcomings of physical and chemical water assessments have therefore necessitated the adoption of biological monitoring as it is quick, easy and cost effective when assessing the effects of environmental changes on aquatic ecosystems (Bere and Tundisi, 2010; Dalu and Froneman, 2016; Mangadze et al., 2017). However, United Kingdom was the first nation to officiate the use of Rapid Bio– monitoring methods for assessing river pollution in 1970 (Elias et al., 2014). To follow the suit in 1972 there was a developed biotic index for South African rivers, but it was labour–intensive which in turn led to it not being a preferred biomonitoring tool (Chutter, 1972; Chutter, 1998). In response to criticisms about the inadequacy of the method, the Biological Monitoring Working Party (BMWP) index was developed in 1976 and recommended for use in river pollution surveys (Hawks, 1997). A variety of Rapid Bioassessment Methods (RBMs) based on macroinvertebrates were created and put into use globally in response to the demand for rapid and affordable techniques for evaluating the quality of water in water management

(Dallas, 1997).

For example, SASS protocol was derived from the BMWP (Hawkes, 1997) and modified for water quality and condition assessments of South African rivers (Dallas,1997; Chutter, 1998). However, SASS being the first rapid bioassessment protocol in Africa, it was used less since people did not have adequate information on it until the year 2000 when it gained momentum and was widely used (Phiri, 2000). According to Munyika et al. (2015) SASS protocol could not establish whether land–use activities alone had an influence in the water quality of the Orange River or not. In addition, Chikodzi et al. (2017) highlighted that changes in species composition, abundance and distribution within a family in relation to environmental water quality changes cannot be detected by SASS, since its rapid assessment nature, taxonomic resolution is at the family level. Palmer et al, (2004) further indicated that although biomonitoring seems to gain momentum it has disadvantages such as (1) it cannot be used to identify a specific pollutant instead it merely provides an indication that there is something wrong with the water quality and (2) ecosystem monitoring is partially successful during high flow periods which leads to the information detected/gathered not being precise. The SASS protocol, which has undergone extensive testing in South Africa and been shown to be capable and reliable for assessing water quality and overall river condition, has been modified into

NASS, OKAS, and ZISS (Dallas, 1997; Dallas, 2004a; Dallas, 2004b; Dallas et al., 2010).

Table 2.1: Detailed published literature on rivers/streams for macroinvertebrates, biomonitoring, management of ecosystems and anthropogenic disturbances in southern Africa.

Country	**System**	**Dominant taxa**	**References**
South Africa	Mutshundudi River	**Cool–dry**	Munyai et al. (2024)
		Upstream – Libellulidae and Dytiscidae	
		Midstream – Chironomidae and Libellulidae	
		Downstream – Chironomidae and Hydrophilidae	
		Hot–wet	
		Upstream – Chironomidae and Notonectidae	

		Midstream – Chironomidae and Dytiscidae	
		Downstream – Chironomidae and Libellulidae	
	Luvuvhu River	**Week 1**	Mutshekwa et al. (2024)
		Macadamia orchard – *Chironominae* and *Pseudagrion* sp.	
		*Communal area – Other taxa and *Chironominae*	
		Week 2	
		Macadamia orchard – *Chironominae* and *Radix natalensis*	
		Communal area –*Chironominae* and other taxa	
		Week 3	
		Macadamia orchard – *Chironominae* and *Trithemis* sp.	
		Communal area –*Chironominae* and other taxa	
		Week 4	
		Macadamia orchard – *Chironominae* and *Ostracoda/pantala flavescens*	
		Communal area – *Chironominae* and other taxa	
		Week 5	
		Macadamia orchard – *Chironominae* and *Planaria* sp.	
		Communal area – *Chironominae* and other taxa	
		Week 6	
		*Macadamia orchard – *Chironominae* and *Radix natalensis*	
		*Communal area –*Chironominae* and *Ostracoda*	
	Lovu, Matikulu, Mdloti, iMfolozi, Mhlathuze, Mkomazi, Mkuze, Mtamvuna, Mzimkhulu, Phongolo, Thukela, Tongati, uMlazi, uMngeni and uMvoti Rivers	Odonata, Gastropoda and Chironomidae	Agboola et al. (2019)
	Eyre, Elands and Lushington Rivers	**Agriculture disturbed –** *Baetis* and *Dicentroptelum*	Matomela et al. (2021)
		Land use invaded – *Castanophlebia* and *Tricorythus*	

The Kat River	**Nature** – *Tricorythus* and *Baetis*	
Mohlapitsi River	Thiaridae, Baetidae, Planorbidae, Hydropsychidae, and Gomphidae	Raphahlelo et al. (2022)
Zio River	Chironomidae, Leste and Gomphidae	Tambo et al. (2021)
Mthatha River	**Headwaters**	Niba and Sikwe (2018)
	HW1 – *Tricorythus* sp.	
	HW2 – *Tricorythus* sp.	
	HW3 – *Castranophlebia* sp.	
	Langeni	
	LN1 – *Afroceania* sp.	
	LN2 – *Neoperia* sp.	
	LN3 – *Neoperia* sp.	
	Kambi	
	KM1 – *Ceraptogompus pictus*	
	KM2 – *Neoperia* sp.	
	KM3 – *Neoperia* sp.	
	Ntsaka	
	NT1 – *Castranophlebia* sp.	
	NT2 – *Castranophlebia* sp.	
	NT3 – *Oosthuizobdella Stuhlmanni*	
Swartkops River	Chironomidae and Oligochaeta	Odume et al. (2012)
Palmiet River	Odonata, Hemiptera, Crustacea and Trichoptera	Lebepe et al. (2022)
Tsitsa River	Oligonuridae, Baetidae, Coenogranidae and Gomphidae	Ntloko et al. (2021)
Selati River	**Site 1** – Hydropsychidae and Caenidae	Rasifudi et al. (2018)
	Site 2 – Hydropsychidae and Heptageniidae	
	Site 3 – Leptophlebiidae and Caenidae	
	Site 4 – Caenidae and Hydropsychidae	
	Site 5 – Caenidae and Baetidae	
Kleinplaas dam on the Eerste River	**Upstream** – Ephemeroptera and Diptera	Bredenhand and Samways (2009)
	Downstream – Diptera and Ephemeroptera	
Mvoti Rivers	Chironomid and Oligochaete, Ephemeroptera, Plecoptera, Trichoptera, Gastropoda and Midges (Diptera)	Malherbe et al. (2010)
Olifants River	Thiaridae, Tubificidae, Baetidae and Corixidae	Kemp et al. (2014)

	Wilge River	Baetidae, Caenidae and Leptophlebidae.	Farrell et al. (2015)
	Drankensberg Rivers	Baetid mayfly Demoreptus monticola, baetid Baetis harrisoni and heptageniid mayfly *Afronurus sp*	Rivers–Moore et al. (2013)
	Spekboom river and Dwars river	**Dwars River** – Hydropsychidae, Caenidae and Baetidae	Addo–Bediako (2021)
		Spekboom River – Baetidae, Hydropsychidae and Simuliidae	
	Buffalo River	*Leptophlebiids Adenophlebia auriculata Eaton* and *Choroterpes elegans* (Barnard) (brushers), the heptageniid Afronurus harrisoni Barnard and *limpet Burnupia* sp. (scrapers), a *caenid* sp. A (gatherer), the simuliids Simulium nigritarse Coquillet Simulium damnosum s.l. Theobald, *Simulium adersi Pomeroy* and the *tricorythid Neurocaenis* reticulata Barnard (passive filterers)	Palmer et al. (1996)
	Groot Letaba River	Diptera and Trichoptera	Kekana et al. (2023)
	Lourens River	**Lourens River Upstream** – Corydalidae and Glossomatidae	Thiere and Schulz (2004)
		Lourens River Downstream – Corydalidae and Veliidae	
	Eerste River	Leptophlebiidae and Baetidae, with Castanophlebia calida and Baetis	King (2010)
	Marico River	Baetidae and Chironomidae	Wolmarans et al. (2016)
	Mthatha River	*Simulium adersi, Baetis harrisoni, Pseudocloeon* sp. and *Tricorythus* sp.	Niba and Mafereka, (2015)
	Blyde River of the Olifants River	Hydropsychidae, Caenidae, Baetidae, Simuliidae, Chironomidae and Elmidae	Malakane et al. (2020)
	Buffalo River	Site 0 – Baetis capensis and Castanophlebia calida Site 1 – Blephariceridae and *Ecnomus* sp.	Palmer et al. (1994)
		Site 5 – *Hydra* sp. and *Chimarra* sp.	
		Site 6 – Baetis glaucus and Simulium adersi	
	Kat River	Ephemeroptera, Diptera and Trichoptera	Akamagwuna et al. (2023)
	Keurbooms River and Kowie/Bloukrans River	Simulium nigritarse, Afroptilum sudafricanum, Baetis harrisoni, Ancylidae (Mollusca) and Rhabdocoela (Platyhelminthes)	Eady et al. (2013)

	Steelpoort River	Baetidae, Hydropsychidae and Simuliidae	Makgoale et al. (2022)
	Bloukrans River	Trichoptera and Chironomidae	Odume and Mgaba, (2016)
	Vuvu River	This study showed that upstream sites were mostly dominated by benthic macroinvertebrates that have high sensitivity scores and there was also higher benthic macroinvertebrate diversity observed upstream than at the downstream	Nakin et al. (2017)
		Baetidae was the most abundant Turbellaria, Oligoneuridae, Polymitarcidae, Lestidae, Psychomyiidae, Blephariceridae, and Physidae had the lowest number of individuals	Munzhelele et al. (2024)
	Steelpoort River and Olifants River	Diptera, Odonata, Coleoptera, Ephemeroptera, Trichoptera, Mollusca, Hemiptera and Crustacea	Matlou et al. (2017)
	Blyde and Mohlapitsi rivers	**Blyde River –** Hydropsychidae, Caenidae, Baetidae and Simuliidae	Addo–Bediako (2023)
		Mohlapitsi River – Thiaridae, Baetidae and Planorbidae	
	Nyl River	Diptera and Coleoptera	Baker and Greenfield (2019)
	Olifants River	Thiaridae, Tubificidae, Baetidae	Wolmarans et al. (2014)
		and Corixidae.	
	Boesmanspruit River	Heptageniidae, Leptophlebiidae and Caenidae	Tate and Husted (2015)
	Komati River	Thiaridae, Muscidae, Veliidae, and Aeshnidae	Dlamini et al. (2010)
	Tsitsa River	**Collector–gatherers** – *Baetis* sp., *Oligoneuropsis lawrencei* and *Aphenicerca* sp.	Akamagwuna et al. (2022)
		Collector–filterers – *Pseudocloeon vinosum*, *glaucum*, *P. piscis* and *Euthraulus* sp.	
		Shredders – *Cheumatopsychethomasetti*, *C. afra* and *Hydropsyche* sp.	
	Elands River	Corbiculidae, Philopotamidae and Baetidae	Ferreira et al. (2009)
	Dwars River	**Site 1** – Hydropsychidae and Elmidae	Mmako et al. (2021)
		Site 2 – Hydropsychidae and Baetidae	
		Site 3 – Caenidae and Hydropsychidae	

	Site 4 – Caenidae and Baetidae	
Spekboom	**Site 1** – Hydropsychidae and Baetidae	Nukeri et al. (2021)
	Site 2 – Leptophlebiidae and Caenidae	
	Site 3 – Chironomidae and Simuliidae	
	Site 4 – Baetidae and Hydropschidae	
Bloukrans River	In this study, the abundance of shredders was very low even in less impacted stream order 1 sites; however, abundances decreased further along the land–use gradient.	Mangadze et al. (2019)
	The abundance of collector–gatherers in Bloukrans River was possibly linked to the increased levels of organic matter,	
Bloukrans River	**Mainsteam river** – Ancylidae, Chironomidae, Physidae, Hirudinae and Tetragnathidae	Dalu et al. (2017)
	Stream site – Aeshnidae and Gyrinidae	
Bloukrans River	**Spring and winter** – *Paratrichocladius* sp. and *Cladotanytarsus* sp.	Odume and Muller (2011)
	Spring– *Nonacladius* sp., *Parakiefferilla* sp., *Eukiefferiella* sp., *Cryptochironomus* sp., *Coelotanypus* sp., *Procladius* sp, *Trissopelopia* sp. and *Clinotanypus* sp.	
	All season– *Cricotopus* sp., *Cricotopus trifasciata gr*, *Orthocladius* sp., *Tanytarsus* sp.	
Tsitsa River	The box plot showed that FFGs structures of EPT across the site groups examined were affected by a gradient of sediment stress. Except for predators, the relative abundance of EPT FFGs significantly decreased in the influenced site categories 1 and 2 sites when compared with the less influenced site groups 3 and 4 sites.	Akamagwuna and Odume (2020)
Tsitsa River	Ephemeroptera and Trichoptera	Akamagwuna et al. (2019)
Western cape– Eerste, Plamiet and Berg Rivers	**Stones** – Plecoptera, Ephemeroptera, Coleoptera and Trichoptera	Dallas (2007)

	Mpumalanga– Sabie and Crocodile Rivers	**Vegetation** – Hemiptera and Odonata	
	Western cape– Eerste, Plamiet and Berg Rivers	**Spring assemblages** – Heptageniidae, Helodidae, Tipulidae and Oligochaeta	Dallas (2010)
	Mpumalanga– Olifants, Sabie and Crocodile Rivers	**Autumn assemblages** – Hydropsychidae, Philopotamidae, Veliidae, Aeschnidae, Chlorolestidae, Brachyura and Planariidae	
	Kaaimans, Silwer, Touw, Diep, Hoogekraal, Karatara, Plaat, Homtin, Millwood, Rooiels and Gouna Rivers	*Simulium medusaeforme, S. dentulosum, S. nigritarse s.l., S. rutherfoordi, S. merops and S. bequaerti*	Rivers–Moore et al. (2018)
	Wit River	The abundances of all large bodied and/or conspicuous macroinvertebrates (Gomphidae, Aeshnid, Notonectidae, Belostomatidae, Nepidae, Dytiscidae and Gyrinidae) were significantly lower in, or they were even absent from, the sections with *M. salmoides*.	Weyl et al. (2010)
		The abundance of the medium sized aquatic macroinvertebrates was not significantly higher in the presence of M.salmoides, with the exception of the Libellulidae which there were significantly more abundant.	
		Small aquatic macroinvertebrates were unaffected by the presence of M. salmoides, with the exception of the Hydropsychidae which were significantly more abundant in the presence of M. salmoides	
	New years River and Bloukrans River	41 macroinvertebrate families were collected in association with duckweed mats from the New Years and Bloukrans rivers. Community composition in both systems was similar, with predators accounting for 42.9% and 37.9%, detritivores for 25.7% and 27.6%, herbivores for 5.7% and 6.9% and families with species that fitted into more than one functional feeding group accounting for 25.7% and 27.6% of macroinvertebrates in the New Years and Bloukrans rivers, respectively	Muskett et al. (2016)

	Cunene River	Ephemeroptera, Trichoptera, Chironomidae and Simuliidae	de Moor et al. (2000)
	Orange River	Trichopterans (e.g. Hydropsychidae)	Munyika et al. (2014)
	Zambezi River	Sand bars – Gomphidae, Backwater areas – mayflies (Ephemeroptera) and Veliidae	Palmer and Taylor (2010)
Zimbabwe	Shagashe and Mucheke rivers	**Mucheke upstream** – Anodonta sp., Caenidae sp. and Psephenidae sp.	Chikodzi et al. (2017)
		Mucheke downstream – Glossiphonidae sp. and Erpobdellidae sp.	
		Shagashe upstream – Antocha sp., Leptophlebiidae and Perlidae	
		Shagashe downstream – Chironomidae sp., Ceratopogonidae sp. and Lymnaeidae sp.	
	Gwebi and Mukuvisi Rivers	**Gwebi**	Phiri (2000)
		GS1 – Caenidae and Dytiscidae	
		GS2 – Dytiscidae and Chironomidae	
		GS3 – Caenidae and Libelludidae	
		GS4 – Coenagrridae and Chironomidae	
		GS5 –Hydrobiidae and Lymnaeidae	
		Mukuvisi	
		MS1 – Hydrophilidae and Libelludidae	
		MS2 – Libelludidae and Chironomidae	
		MS3 – Chironomidae and Glossiphoniidae	
		MS4 – Chironomidae and Anthomycidae	
		MS5 – Chironomidae and Simulidae	
	Manyame River	Psephenidae, Hydropsychidae, Baetidae, and Leptoceridae.	Mwedzi et al. (2017)
	Manyame River	Decapoda, Plecoptera, Ephemeroptera, Odonata, Hemiptera, Coleoptera, Diptera, Gastropoda and Pelecypoda	Mangadze et al. (2016)
	Manyame River	**Urban sites** – Chironomidae	Bere et al. (2016)
		Great Dyke sites – Gyrinidae	
		Communal sites – Simuliidae and Thiaridae	

	Manyame River	**Communal and park edge area stations (except Station 9)** – Chironomidae, Physidae, Corixidae, Ancylidae, Hydrophilidae, Pleidae, Elmidae and Thiaridae	Bere et al. (2016)
		Stations 9–15 (park area, except for Station 9) – Baetidae, Hydraenidae, Corduliidae and Gerridae	
	Ngamo River	Chironomidae, Culicidae and Hydroptilidae	Nhiwatiwa et al. (2017)
	Tokwe River	Filterers, Predators, Collector–gatherers, Scrappers and Shredders	Makaka et al. (2018)
		Coleoptera, Ephemeroptera, Odonata, Hemiptera, Diptera and Mollusca	
	Kwando and Linyanti River	Chironomidae, Bulininae, Caenidae, Lymnaeidae and Libellulidae	Lyczkowski (2006)
Botswana	Okavango Delta	**Marginal vegetation** – Mayflies (Heptageniidae, Leptophlebiidae, Polymitarcyidae), Bugs (Nepidae) and Flies (Simuliidae).	Dallas and Mosepele (2020)
		Seasonally inundated floodplains – Bugs (Naucoridae), Beetles (Scirtidae), Flies (Stratiomyidae, Syrphidae, Tabanidae) and Snails (Bithyniidae, Hydrobiidae, Thiaridae, Viviparidae and Sphaeridae).	
	Okavango Delta	Ephemeroptera (Baetidae, Caenidae, Heptageniidae, Polymitarcyidae and Tricorythidae), Odonates (Aeschnidae, Gomphidae and Libellulidae).	Dallas and Mosepele (2007)
Zambia	Upper Zambezi Floodplain (UZF), Bangweulu–Mweru (BM), Zambezian Headwaters (ZH), Kafue Flats (KF), Middle Zambezi–Luangwa (MZL) and Lower Zambezi (LZ)	Ephemeroptera (*Dicercomyzidae, Ephemerythidae, Machadorythidae*), Hemiptera (*Aphelocheiridae*), Gastropoda (*Ampulariidae*) and Pelecypoda (*Mutelidae*).	Dallas et al. (2018)
	Chimvu River	Damselflies, Dragon flies, Water scavenger beetles, Predacious diving beetles, Marsh beetles, Minute–moss beetles and Caddisflies	Sabiti (2021)
	Chamba, Chunga, Namando.	**Chamba River**	Kochelani (2016)

	Ngwerere, Chongwe and Musangashi Rivers	Site 1 and 2 – *Coenagrionidae* (Order: Odonata, Suborder: Zygoptera) and *Chironomidae* (Order: Diptera; Suborder: Nematocera)	
		Chunga River	
		Site 1 – *Chironomidae* (Diptera)	
		Site 2 – Culicids (mosquitoes) and Psychodidae (sewage flies/moths)	
		Namando River – Chironomidae and Hydrosychidae	
		Ngwerere River	
		Site 1 – Oligocheates (Annelida) and Gastopod (Thiaridae)	
		Site 2 – Chironomidae and Class Hirudinae	
		Chongwe River	
		Site 1 – Chironomidae, Atyidae (Order Decapoda; Common name: Shrimps), Calamoceratidae (Trichoptera) and Leptophlebidae (Ephemeroptera)	
		Site 2 – Chironomidae, Anthericidae, Elmidae (Order Coleoptera) and the Hydrosychidae (Trichoptera)	
		Musangashi River	
		Chironomidae, Planobidae and Bulininae	
	Chibefwe River	Ecnomidae, Hydropsychidae, Libellulidae, Chlorolestidae (Synlestidae), Elmidae and Aeshinidae	Namafe (2020)
Tanzania	Karanga, Rau, Lumbanga, Sere and Umbwe Rivers	Ephemeroptera, Odonata, Diptera and Trichoptera	Elias et al. (2014)
	Msimbazi River	Gastropoda and Diptera	Shimba et al. (2018)
	Mpanga River	Dicercomyzidae, Ephemerythidae and Neritidae	Tumusiime et al. (2019)
	Pinyinyi River	Mosquito larva, Diptera, Aquatic caterpillar and Lepidoptera	Omary et al. (2023)
	Mkondoa River	Diptera, Ephemeroptera, Coleoptera, Oligochaeta, Trichoptera, Hemiptera, Gastropoda and Odonata	Shimba and Jonah (2016)
	Nzovwe stream	Coleoptera, Odonata and Diptera	Ojija et al. (2017)
	Morogoro River	Hemeroptera, Coleoptera, Plecoptera, Odonata, Trichoptera, Decapoda, Diptera, Melagoptera, Isopoda, Hemiptera and Aranaea	Shirima (2019)

Angola	Cubango, Cuito and Cuanavale Rivers	Baetidae, Caenidae, Heptageniidae and Leptophlebiidae	James and Ferreira (2019)

Zimbabwe

In Zimbabwe, utilizing aquatic macroinvertebrates for biomonitoring is proving to be a valuable approach for evaluating the condition of freshwater environments, acting as a supplement to conventional physicochemical water quality assessments. Research conducted in the Mucheke and Shagashe rivers of Masvingo revealed that macroinvertebrate derived metrics, such as the South African Scoring System version 5 (SASS5), accurately mirror pollution gradients. Locations characterized by taxa susceptible to pollution (i.e., mayfly and stonefly larvae) exhibited elevated SASS5 scores and improved water quality, while downstream areas displayed a widespread presence of pollution–tolerant species such as Chironomidae and Lymnaeidae, indicating compromised environmental status (Bere and Nyamupingidza, 2014). Within Lake Kariba's Sanyati Basin, the structure of benthic macroinvertebrate communities, encompassing EPT (Ephemeroptera, Plecoptera, Trichoptera) diversity, demonstrated significant associations with nutrient and organic enrichment markers, with SASS and ASPT scores effectively indicating anthropogenic influences (Dalu et al., 2022; Mangadze et al., 2022). Further investigations, including analyses of community composition in the Gwebi and Mukuvisi rivers near Harare, studies of the Umfurudzi river under differing land–use scenarios, and examinations following Cyclone Idai in Chimanimani, consistently emphasize the vulnerability of macroinvertebrate communities to habitat deterioration, land–use alterations, and hydrological disruptions. This reinforces their significance as expedient, comprehensive biomonitoring instruments across a variety of freshwater systems in Zimbabwe (Mwedzi et al., 2016; Mangadze et al., 2022). Studies by Phiri, (2000); Ndebele–Murisa, (2012); Bere and Nyamupingidza, (2014); Mwedzi et al. (2016) highlighted that the effective application of SASS protocol in Zimbabwe does not require modification as it was able to reflect water quality and ecological health of lotic systems in Bere and Nyamupingidza, (2014) study.

Zambia

In Zambia SASS protocol had to be modified where apparent changes from SASS to ZISS had to occur, additionally south African invertebrate families (SASS taxa) which are not found in Zambian rivers had to be removed while Zambian families which are not found in South Africa had to be added, so that the Indices can account for other taxa' (Dallas et al., 2018). However, despite the development of ZISS protocol in 2012, the only information and data which is currently available for characterizing river health is unpublished limiting the progress of evaluating the effectiveness of the indices (Namafe, 2020). In 2018, when SASS protocol was applied in wadable in Zambia, sampling of vegetation biotope had to be adapted in line with that developed for OKAS (Dallas, 2009). It is important to always note the variation in biotope availability on taxa recorded and biotic indices generated, as the use of biotic indices depend on biotope availability (Dallas, 2007).

Namibia

de Moor et al. (2000) applied SASS in Namibia during the Cunene River assessment, it was not fully effective as it was difficult for them to recommend the exact figures for instream flow requirements needed to ensure continued survival of key species and species assemblages. Additionally, SASS protocol was tested in the perennial rivers (Chutter, 1997) were, it was concluded that SASS protocol could be used in those areas, but it should be modified to account for additional taxa which exist in those regions. SASS was modified to NASS where organisms such as Snails *Pila occidentalis* (Mousson), *Lanistes ovum* (Peters), *Gabbiella kisalensis* (Pilsbry and Bequaert), *Etheria elliptica* (Lamarck) and *Cleopatra elata* (Dautzenberg and Germain) were added in the modified protocol as SASS demonstrated distribution limit in northern Namibia (Brown, 1994). Taylor (1999) further explained that aquatic species such as snails *Pila occidentalis*, *Lanistes ovum* and *Gabbiella kisalensis*, are the most common species in northern Namibia hence there was a need for protocol modification.

Tanzania

Bioassessment indices, especially macroinvertebrate–based indices, produced in one region can be utilised in other regions with calibration and modification (Dallas et al., 2010; Kaaya et al., 2015). For example, indices produced from non–tropical countries typically need to be adjusted before they are applied in the tropical countries. Kaaya et al. (2015) proposed the Tanzanian Rivers Scoring System (TARISS), the only tropical system derived from SASS, for East African nations, and it was recently implemented in a few Rwandan rivers (Dusabe et al., 2019). Additionally, the system's dependability as a biomonitoring system for Ugandan rivers, such as the Mpanga River in mid–western Uganda, which rises from the Rwenzori Mountains in the Nile River basin, has not been evaluated. As adaption of bioassessment in tropical countries such as Uganda is challenging, due to the poor knowledge of its taxonomical resolution and the sensitivity of macroinvertebrate taxa to pollution (Jacobsen et al., 2008). However, it is noted that changes in climate, geology, longitude and latitude, and other geo graphic qualities may contribute to differences in the physical and chemical characteristics of rivers between two nations such as between Tanzania and Uganda (Ojija and Laizer, 2016).

Rivers in tropical nations such as Kenya and Uganda have not yet benefited from continuous efforts in southern tropical Africa to create, test, refine, and validate bio–monitoring indices (Jacobsen et al., 2008). Elias et al. (2014b) highlighted the ideal need for the development of rapid bioassessment protocols in tropical African regions as they have been depending on indices which are developed from other geographical areas, especially the non–tropical regions. Oigara and Masese (2017) added that more literature in tropical regions concluded that before SASS protocol is extended beyond south Africa testing and validation of the protocol should take place.

The challenges and limitations of using biotic indices

With all this international practice and development of indices, most developing countries have not yet made significant progress on methodological advances, indicator refinement, and integration with management and policies (Suriano et al., 2011). As a results developing countries adapt from developed countries. However, adaptation of indices has shown great limitations as its application requires testing and modification to accommodate different environments (Wong and Fikri, 2021). Monaghan (2016) explained that this is due to organisms responding differently to various types of degradation in the environment, while Gaufin (1973) cautioned on the weaknesses of basing the conclusion of water quality on a specific indicator organism since, an organism or taxa which an individual may consider as being a characteristic/Indicator of polluted water may be used by others as an organism or taxa which is only found in clean water conditions. Chikodzi et al. (2017) further explained that SASS is a single biotic index hence, it masks ecological information from all levels (individual, population and community) of the ecosystem. Lücke and Johnson (2009) recommended the use of multiple metrics from different ecosystem levels and functions as they provide more robust ecological information for the management of water resources than dependence on a single biotic index. According to literature [Rosenberg et al. (1993); Karr (1999); Dickens and Graham (2002); Bonada et al. (2006); Stoddard et al. (2006); Dallas (2007); Baird and Hajibabaei (2012)], the following are to be considered when adopting/using a biomonitoring tool:

- Selection of indicator organisms – Selection is mostly influenced by ecosystem type, stressors of interest and ecological relevance.
- Reference Conditions and Baseline Data – Comparing the collected information with a baseline or benchmark from undisturbed locations or past measurements is essential. Knowing how much natural change happens in the baseline is important to prevent drawing incorrect conclusions.
- Environmental and Habitat Variability – Naturally occurring changes (e.g., seasonal shifts, water flow patterns, elevation, rock types, and plant life) influence living organisms in an area. Hence, during planning there is a need to consider how these factors differ across locations and time.
- Taxonomic Resolution and Expertise – The precision of species identification varies depending on the taxonomic rank (e.g., species, genus, family). While molecular methods such as DNA barcoding and eDNA can assist, significant knowledge of taxonomy is crucial.
- Sensitivity and Specificity of the Tool – The method used should be capable of identifying ecological shifts at the appropriate level of detail (e.g., increased nutrient levels or harmful contaminants). Different methods are sensitive to different environmental pressures (e.g., certain algae react rapidly to nutrient increases, while fish populations indicate the overall health of their environment).
- Practical Considerations (Cost, Time, and Logistics) – Methods should be economical, fast, and consistent. Data collection techniques in the field (e.g., kick sampling, artificial substrates, or eDNA sampling) require uniform procedures to ensure data can be compared across different studies.
- Integration with Management and Policy – The tool needs to generate clear and easy–to–understand data to support decision–making. Compatibility with established systems such as the EU Water Framework Directive or South Africa's River Health Programme guarantees its real–world usefulness.

Discussion

Due to the limitations of biotic indices, ecological research moved towards the use of multimetric indices to incorporate information from multiple biological organisations to capture a wide variety of responses to various environmental stressors (Collier, 2008; Elliott et al., 2018; Singh and Saxena, 2018). However, studies by Hawkins et al. (2010), Gonçalves and Menezes

(2011), Suriano et al. (2011), Odume et al. (2012) and Feld et al. (2014), highlighted that it is always crucial for the metrics to undergo evaluation/tests and be validated before they are included as multimetric indexes. As the inclusion of a metric depends on certain factors such as its sensitivity to the investigated stressor/factor, its respond to seasonal changes and understandable response to the stressors (Mwedzi et al., 2020). Li et al. (2009) further explained that several biomonitoring techniques are currently employed in river ecosystems, and the selection of a suitable technique depends on factors which are assessed and the availability of resources. Suriano et al. (2011) and Feld et al. (2014) explained that the metrics differ in sensitivity, complexity and regional implementations. Potential biomonitoring methods include diversity indices, biotic indices, multimetric approaches, multivariate approaches, functional feeding groups (FFGs) and multiple biological traits (Mwedzi et al., 2016). Palmer et al. (1995) used functional feeding groups as indicators of water quality where he discovered that sampling sites which were most polluted according to study by Palmer and O'Keeffe, (1990) were not distinguished by either change in taxonomic or functional feeding group composition. Therefore, it is crucial to note that either the organisms that have managed to live in the system are robust, or the scale and resolution at which the data was gathered or examined is not suitable. A sector that focusses exclusively on specific groups of macroinvertebrates, such as Ephemeroptera, Plecoptera, and Trichoptera (EPT), has been formed by the advancements of multivariate methodologies for system assessments through functional feeding groups.

Moreover, literature highlights that coupling biotic indices with the multimetric approaches yield clearer results. Phiri, (2000) revealed that SASS and ASPT could not explain the variation between the water quality of Gwebi and Mukuvisi rivers, hence he adopted the diversity indices (i.e., Margalef, Shannon and Simpson). In addition, the decline in the diversity of the macroinvertebrate community in Gwebi River and Mukuvisi River were identified using diversity indices, which also measured correlation with the majority of the physical and chemical variables (Phiri, 2000). Therefore, using the Simpson's index instead of the Margalef and Shannon indices would provide a better representation of changes in the macroinvertebrate community in relation to water quality, as Phiri (2000) research revealed a stronger relationship between the physical and chemical variables and the variability of the macroinvertebrate community as measured by the Simpson's index (Phiri, 2000). According to Nhiwatiwa et al. (2017) using a multivariate combined approach of nutrients, diatom and macroinvertebrate community metrics and biological indices is imperative as it allows further ecological diagnostics of the evaluated system and it also helps in decision making in relation to river conservation and restoration measures.

The SASS, ASPT, Simpson, Shannon, Margalef, Canonical correspondence analysis (CCA), principal component analysis (PCA), principal coordinate analysis (PCO) and redundancy analysis (RDA) are the most widely used methods in assessing ecological systems in the Southern Africa. However, they are not entirely feasible, thus indicating the urgency to develop a biological (and other ecological) data analysis which are straightforward and can facilitate a translation for management application. Moreover, there is a need to develop a new bioassessment tool which will assess aquatic habitat irrespective of the area of assessment or the sources/type of pollution, so that there could be a reduction of tests and evaluations when a tool is being adapted from another area. Hering et al. (2006a) explained that the knowledge of how organisms respond to different types of stress can and should be used to design more robust and cost–effective monitoring programs. Therefore, developing nations must carry out and record more research because Africa is reportedly lacking in documented ecological data for organism groups (Bere et al., 2014). This restricts the usage and development of biotic indices because ecological data is necessary for the creation of a biotic index (Bere et al., 2014).

Conclusions and recommendations

This review demonstrates that while traditional biotic indices remain valuable for rapid ecological assessments, they are limited in their ability to capture the full complexity of ecosystem responses to environmental stressors. Multimetric and multivariate approaches drawing from multiple biological, functional and environmental indicators offer a broad understanding of river condition, particularly because individual metrics differ widely in sensitivity, seasonal responsiveness and regional applicability. Evidence from southern African studies shows that combining biotic indices with complementary metrics, such as diversity indices and community–based analyses, provides clear results of ecological change and improves the detection of pollution–related impacts. The integration of these methods therefore strengthens ecological interpretation, supports more robust decision–making and enhances the reliability of river health assessments.

To improve biomonitoring accuracy and management outcomes, it is recommended that future assessments couple biotic indices with multimetric approaches. This combined strategy maximises the strengths of each method: biotic indices provide rapid biological signals, while multimetric and multivariate tools capture slight shifts in community structure, functional traits and environmental gradients. Incorporating techniques such as Simpson's diversity index, nutrient profiling, diatom metrics and macroinvertebrate community analyses will improve stressor–specific detection and increase confidence in ecological interpretations. Additionally, all proposed metrics should undergo performance testing and validation before incorporation into multimetric systems. There is also an urgent need for simplified, regionally adaptable bioassessment tools that reduce the need for repeated recalibration across different catchments.

CHAPTER THREE: SPATIO–TEMPORAL ASSESSMENT OF METAL POLLUTION IN WATER AND SEDIMENTS OF A SEMI–ARID RIVER SYSTEM IN SOUTH AFRICA

Abstract

Rivers in semi–arid catchments are essential for maintaining biodiversity, agricultural productivity and local livelihoods. However, increasing anthropogenic stressors particularly from effluent discharge, agricultural runoff, and land–use changes pose a growing risk to water and sediment quality. This study assessed the spatiotemporal variation of metal concentrations in river water and sediments across protected and non–protected areas of a semi–arid river system. To evaluate potential ecological threats, the study applied ecological risk assessment using pollution indices, such as contamination factor (CF), geo–accumulation index (Igeo) and enrichment factor (EF), to determine the extent and sources of metal contamination in the Nyl River system. Ecological risk assessment showed clear spatial and seasonal differences, with higher contamination at the protected area during the dry season. Aluminium (Al) posed great risk (CF ≈ 7.1; Igeo ≈ 2.2; EF ≈ 5.0), followed by moderate enrichment of chromium (Cr), copper (Cu), and zinc (Zn). Arsenic showed minor enrichment in the wet season, while manganese, lead, and cadmium remained at unpolluted levels. Overall contamination followed the order Al > Cr > Cu > Zn > arsenic (As) > manganese (Mn) > lead (Pb) > cadmium (Cd), indicating low to moderate ecological risk. When comparing sediment metal levels with the Canadian Sediment Quality Guidelines, the study' findings were within the no effect level and low effect level. The results emphasise the importance of continuous water and sediment quality monitoring, severe regulation of effluent discharges, and the implementation of catchment–based management strategies that integrate agricultural practices, erosion control, and wastewater treatment improvements. Additionally, such measures are essential to maintain sediment integrity, prevent pollutant remobilisation, and ensure the long–term ecological resilience and sustainability of river systems.

Keywords: Nyl River basin; metals; sediments; water quality; anthropogenic pressures; ecosystem health; ecological risks assessment.

Introduction

Rivers are dynamic ecosystems that provide essential services including freshwater supply, habitat for aquatic biota, nutrient cycling, and roles in supporting human livelihoods (Cañedo–

Argüelles et al. 2019; O'Brien et al. 2021; Dalu and Masese, 2025). However, the condition of river water and sediments continues to decline as a result of increasing metal and nutrient inputs from natural and human–related sources (Bhat and Qayoom, 2021; Mpopetsi et al., 2025). As metals are known to be non–biodegradable, once released into aquatic environments they tend to persist for long periods, compared to organic pollutants that degrade over time (Tchounwou et al., 2012; Varol, 2020; Dalu and Tavengwa, 2022). Instead of breaking down they mostly accumulate in water, biota and sediments where their persistence and ability to bind contaminants lead to harmful environmental and ecological effects (Ali et al., 2019; Samuel et al., 2023). The accumulation of metals always leads to long-term ecological and health risks, as metals are released back into the water column under changing physicochemical conditions which increases their bioavailability (Förstner and Wittmann, 2012; Tchounwou et al., 2012; Varol, 2020).

Various studies (i.e., Dahms et al., 2017; Hossain et al., 2024; Sharma et al., 2025; Saifullah et al., 2025) have indicated that the chemical composition of water and sediments can be utilised to evaluate the condition of aquatic environments. The health of aquatic environments depends not only on sediment quality but also on a range of interconnected environmental factors, including water flow dynamics, land use practices, water chemistry, and habitat structure (Pejman et al., 2015). Therefore, the assessment of water quality integrity should focus on various ecotoxicological parameters that reflect the bioavailability, toxicity, and cumulative impacts of contaminants on ecosystem functioning. Other studies (i.e., Beltaos and Burrell, 2021; Aouiche et al., 2023) documented that changes in water flow regimes affect the transport and deposition of sediments, whereas modifications in land management practices such as agriculture and urban development can influence surface runoff patterns and potentially increase the influx of contaminants into river systems. Metals present in sediments, regardless of their location are susceptible to displacement during flooding events, which may result in the discharge of substantial quantities of metals into the water column (Wang et al., 2007; Greenfield et al., 2007; Hauser–Davis and Wosnick, 2022; Alfee and Bloor, 2024; Gavrilaș et al., 2025). Therefore, examining metal concentration in sediments can provide us with a long–term view of pollution levels (Dalu et al., 2022; Mukwevho et al., 2025).

Metals are introduced into aquatic systems through both geogenic (i.e., breakdown of organic material and leaching of rocks and minerals) and anthropogenic mechanisms (i.e., industrial discharge, agricultural practices, and extractive industries) (Palaniappan and Karthikeyan 2009, Dahms et al., 2017; Dalu and Tavengwa, 2022). They are distributed across four primary compartments within an aquatic system: particulate matter, pelagic water, interstitial water, and sediments (Sanders, 1997). Furthermore, they interact with sediment through physical processes, such as the trapping of metal–containing particles, particularly in systems where reduced water flow facilitates sedimentation (Sanders, 1997; Buykx et al., 2002; Omo–Okoro et al., 2025; Cihan and Sezgin, 2025), and chemical processes, such as the binding of contaminants to sediment particles.

While most metals are essential micronutrients, they can become toxic at elevated levels or under altered environmental conditions such as pH changes (Newman, 2009; Olaniran et al., 2014; Mbanje, 2025; Francis, 2025). A small concentration of trace metals is important for biotic metabolic processes, but their presence in aquatic environments poses a threat when their levels exceed naturally occurring background concentrations in both water and sediments (Galvin, 1996; Ali et al., 2025; Galasso et al., 2025). Metals can be transferred across trophic levels through bioaccumulation and biomagnification processes, where aquatic organisms readily absorb and accumulate metals leading to increased metal concentrations in organisms occupying higher trophic positions (Maceda–Veiga et al., 2012; Tchounwou et al., 2012; Huang et al., 2024; Oros, 2025). This accumulation poses significant ecological and human health risks by altering species diversity and ecosystem function, while toxic metals concentrate in the tissues of aquatic organisms particularly benthic species in constant contact with sediments leading to potential effects throughout the food web (Duruibe et al., 2007; Wang et al., 2012; Jaishankar et al., 2014).

The Nyl River basin, in the Limpopo Province, South Africa, is of great ecological significance, especially its floodplain wetlands which are Ramsar–declared wetlands of international importance (BirdLife South Africa, 2023). They play important roles in filtering pollutants, trapping sediments, and maintaining ecological balance in this part of the Limpopo Province (Dalu and Wasserman, 2022; Ndlovu, 2023; Rapholo, 2024). However, the river basin is subject to various land use activities, including agriculture, urban settlements, and wastewater treatment discharges which contribute to metal and nutrient entering the river system (Molepo et al., 2021). Dahms et al. (2017) has shown that metal concentrations generally increase downstream and that floodplain carry high risk indices during both high- and low-flow periods.

Baseline monitoring and research in the Nyl River system indicated high concentrations of several heavy metals in both water and sediments, and studies applying sediment quality indices and fractionation methods report spatial variation in contaminant levels and ecological risk across the basin (McQuade, 2002; Molepo et al., 2021). The latter studies showed that while some metal concentrations may reflect geogenic background, anthropogenic sources (e.g., wastewater treatment works and diffuse agricultural inputs) contribute to observed patterns and potential ecological risks. Given that the floodplain lies in a semi–arid savannah region, conserving water is critical (Jacobs et al., 2011; Chomba et al., 2021; Christie, 2024). This is especially important because Limpopo is identified as the country's poorest and most arid province (Aliber, 2003; Mmbadi, 2019; Mashamba, 2023). However, floodplain also help in groundwater recharge and thus makes water more available to farmers in the area throughout the year (McQuade, 2002; Chomba et al., 2021; Nkosi et al., 2021; Dlamini et al., 2021)

The Nyl River, a vital tributary of the Limpopo River system in South Africa, supports diverse ecological habitats and provides essential water resources for agriculture, industry, and local communities (Higgins et al., 1996; Greenfield, 2004; McCarthy et al., 2011; Dahms, 2015; Baker, 2018). However, increasing anthropogenic pressures, including mining, agricultural runoff, and urbanisation, have raised concerns regarding metal contamination and its ecological implications. This study aims to (i) evaluate the spatial and temporal patterns of metal pollution along the Nyl River by integrating newly collected field data with historical records obtained from published literature within protected and non–protected areas, and (ii) to assess metal contamination in water and sediments using pollution indices and evaluate the associated toxic risks to ecosystem health and functioning. This study hypothesized that (i) metal contamination in water and sediments will show seasonal variation due to bioturbation and water flow in various section of the river and (ii) metal concentrations will increase downstream due to cumulative industrial, mining, and agricultural inputs. Ultimately, this research will contribute to sustainable water resource management and environmental policy formulation in the Limpopo River basin by bridging the gap between scientific data and environmental governance.

Material and methods

Study area

The study area falls within the Waterberg catchment of the Limpopo Province, South Africa, and it follows the course of both the Klein and Groot Nyl Rivers, from their sources to their confluence, and the downstream course of the Nyl River to Moorddrift Dam near Mokopane (Huggins and Roger, 1993; Greenfield et al., 2007). The Nyl River flows through or is impacted on by the towns of Modimolle (formerly Nylstroom) and Moogkopong (formerly Naboomspruit), flowing in a north–easterly direction from Modimolle to Mokopane. At Mokopane, the river shifts northward and changes its name to the Makgalakwena River, which ultimately joins the Limpopo River (Huggins and Roger, 1993; Greenfield et al., 2007). The region's geology is made up of sandstone formations from the Waterberg Group, mixed with granites and gneisses from the Swazian and Bushveld complexes (UP, 2021; DFFE, 2023). This geological makeup contributes to the area's mineral richness and influence sediment composition (UP, 2021; DFFE, 2023). The landscape features rolling hills, wide floodplains, and isolated mountain ridges, creating a varied range of ecological niches and shaping water flow patterns. The Waterberg catchment is a significant water source area, receiving mean annual rainfall ranging from 450 mm to 750 mm, with most precipitation falling in the summer months (October to March). This seasonal rainfall drives river flow and floodplain flooding, which are important for maintaining wetland health and sediment movement (Junk and Wantzen, 2006; Baker et al., 2009).

The system is increasingly affected by anthropogenic pressures, including agriculture and urban developments (Smith et al., 2015). The rich floodplain soils support intensive cultivation of crops such as maize, groundnuts, tobacco, citrus, cotton, millet, wheat, rice, and sunflowers (Greenfield, 2004). Cattle farming is common in the floodplain, while game farming dominates the upper drainage basin, near the river sources, with many private reserves (Greenfield, 2004). These land–use practices, combined with runoff from formal and informal settlements, contribute to metal enrichment, sediment loading, and hydrological alteration, posing risks to the ecological integrity of the Nyl River floodplain and downstream systems. The sites of the study were selected following Greenfield (2004), which serves as a base literature for studies in the Nyl River basin. The study sampled the Nyl River basin and some permanent tributaries (i.e., Nylsvley Nature Reserve (Nyl), Klein Nyl Oog (KNO), Donkerpoort Dam (DPD), Golf Course (GC), Sewege Treatment Works (STW), Jasper (JAS) and Moorddrift Dam (MDD)). In the above selected sites, different land use practices are taking place.

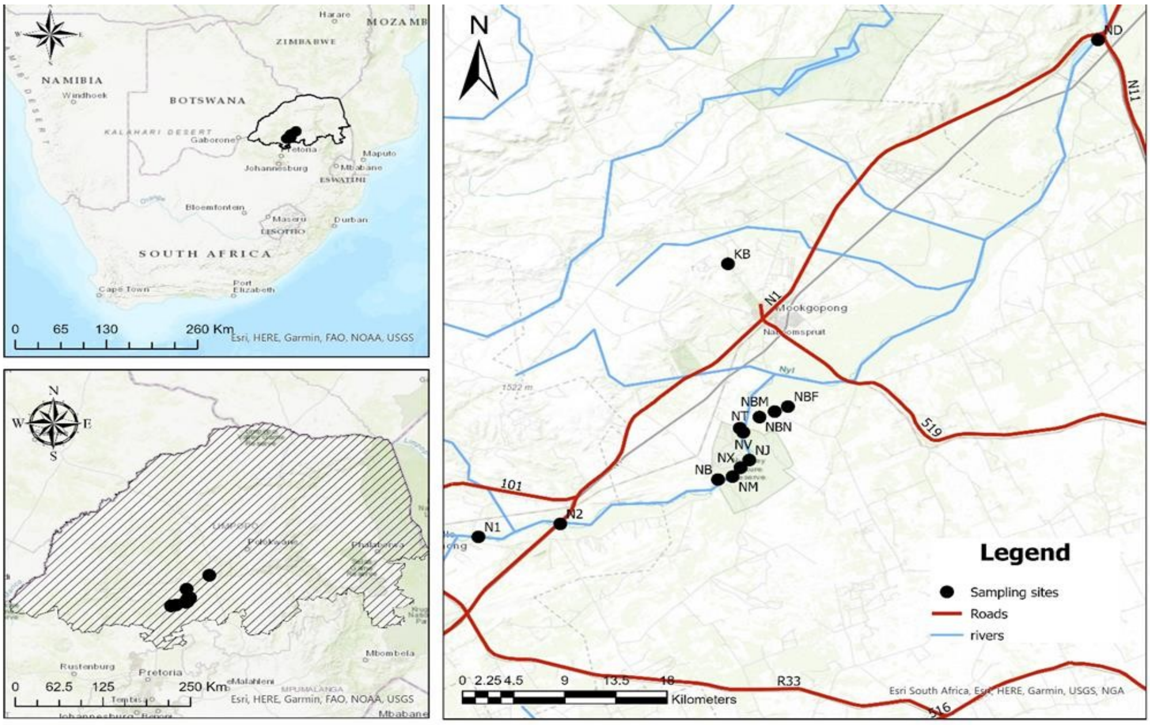

Figure 3.1. A study area map of the Nyl River system in Limpopo Province, South Africa.

Water variables

Water samples were collected seasonally [i.e., dry (May 2024) and wet (March 2025)] from thirteen (13) different sites (Figure 3.1). The 13 sites were from both the protected (5 sites within the Nylsvley Nature Reserve) and non–protected (8 sites outside the reserve) areas along the Nyl River system. A portable hand–held multi–parameter probe PCTestr 35 (Eutech/Oakton Instruments) was used to measure conductivity (µS/cm), salinity (PSU), dissolved oxygen (mg/L), total dissolved solids (mg/L), pH and temperature (°C) onsite.

Two polyethylene bottles (250 mL) were filled with water from each site for metal analysis. Metal analyses were performed at WaterLab (Pretoria), a laboratory accredited by the South African National Accreditation System (SANAS). The concentrations of cations (i.e., B, Ca, K, Mg and Na) and heavy metals (i.e., Al, Fe, Mn, and Zn) were determined using an Inductively Coupled Plasma–Atomic Emission Spectrometer (ICP–AES, ACTIVA–M; Horiba Advanced Techno, Kisshoin, Japan). The detection limits for the metals were 0.0001 mg/L, 0.0001 mg/L, 0.0001 mg/L, 0.0002 mg/L, respectively. Standard solutions (De Bruyn Spectroscopic Solutions 500 MUL20–50STD2) with recoveries within 10 % of known concentrations were used to ensure analytical accuracy. The metal recoveries were between

91.8 % and 106.3 %. A certificated reference material (River Water Reference Material for Trace Metals, NRC Canada, SLRS–4) was analysed after every five samples to verify the accuracy of the instrumental methods. This quality control measure was consistently applied throughout the process.

Sediment variables

Two integrated sediment samples per site for each season were collected from the river using a plastic hand shovel. The sediment samples were then placed in a polyethylene Ziplock bags and placed on ice in a cooler box before being taken to the laboratory for further processing. In the laboratory, sediment samples were oven dried at 60 °C for 48 to 72 hours before disaggregation in a porcelain mortar and sieving for the removal of plant roots and debris. The sediment sampled were then properly mixed using a riffle splinter and a subsample (10 g) was weighted out for further analysis. The sediment samples were sent for analysis at Central Analytical Facilities (CAF), Stellenbosch University. The metals studied were chosen based on prior research conducted on the river system (e.g., Greenfield et al., 2012; Dahms et al., 2017). Metals such as sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), boron (B), chromium (Cr), aluminium (Al), manganese (Mn), iron (Fe), cobalt (Co), molybdenum (Mo), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) were analysed using an Agilent 7900 quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) equipped with a High Matrix Introduction system and Agilent Mass Hunter software (version 4.4) for instrument control and data processing (McCurdy, 2009). Phosphorus was analysed using a SEAL AutoAnalyzer 3 high resolution and Bray–2 extract as per Bray and Kurtz (1945). To evaluate the reliability of this approach, two standard certified reference soils, specifically SARM–51 (MINTEK) and SL–1 (IAEA), were processed and measured twice, and these results were used to determine recovery rates. The percentage recoveries of the certified values ranged between 88 % and 118 % for all metals.

Data analysis

The data was assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's test and it was found to conform to parametric assumptions. All water and sediment data were subjected to descriptive statistical analysis to determine the mean and standard deviation for each physicochemical parameter and metal concentrations, thereby summarising the overall trends and variability in the dataset. Comparative analyses between sampling sites (i.e., protected and non–protected) and seasons (i.e., dry and wet) were conducted using two–way ANOVA, to determine statistically significant differences across sites and seasons. Statistical analyses were performed using SPSS (version 23), with significance thresholds set at $p < 0.05$.

To evaluate the degree of metal contamination in both protected and non–protected sites, the measured concentrations were compared against international sediment quality guidelines (SQGs), which serve as threshold values indicating concentrations that may cause harmful biological effects in aquatic ecosystems. This research employed the Canadian Sediment Quality Guidelines (CSQGs) (Persaud et al., 1993), comprising the Low Effect Level (LEL) and Severe Effect Level (SEL). These guidelines were chosen due to their broad international use, especially in areas such as South Africa that currently lack their own sediment quality benchmarks.

Ecological risk assessment

To evaluate the level of sediment contamination and linked ecological risks, pollution indices such as the Contamination Factor (CF), Geo–accumulation Index (Igeo), and Enrichment Factor (EF) were calculated. The application of these indices is critical, as they offer quantitative measures for identifying pollution sources, measuring contamination intensity, and assessing the potential impacts of metals on aquatic ecosystems. Iron (Fe) was used as the reference metal, following Dahms et al. (2015) in the Nyl River study, because of its relatively stable distribution and lack of correlation with other metals in aquatic environments.

Contamination factor

It is a widely used pollution index that quantifies the degree of contamination of a given metal in sediments by comparing its measured concentration to a selected reference value (Swain et al., 2024). Contamination factor allows quantification of the degree of contamination for each individual element, helping in differentiating anthropogenic input from natural geological sources (Esmaeilzadeh et al., 2021; Rahman et al., 2022). The CF is calculated using the following equation:

$$CF = \frac{\mathrm{C\ (metals)}}{\mathrm{C\ (background)}}$$

In this equation C (metal) is the concentration of the metal in question measured at a particular site. C (background) is the level of the metal in question measured at the reference site in this study (Thomilson et al., 1980).

Geo–accumulation index

Assess the degree of heavy metal pollution in aquatic sediments by comparing current concentrations with pre-industrial background levels (Muller, 1969). This index is important because it provides a standardised, quantitative metric to classify sediments into seven contamination categories ranging from "uncontaminated" (Igeo ≤ 0) to "extremely contaminated" (Igeo > 5), thereby allowing comparisons of pollution intensity across sites (Addo–Bediako et al., 2021; Chen et al., 2022). By applying Igeo in sediment analyses, researchers can identify anthropogenic enrichment of metals, distinguish spatial patterns of contamination, and better inform ecological risk assessments and remediation strategies (Dahms et al., 2017; Addo–Bediako et al., 2021). The index levels are calculated using the following equation.

$$Igeo = \log_2 \frac{\mathrm{Cn}}{1.5 \times \mathrm{Bn}}$$

In this equation Cn is the concentration of the metal in question measured at a certain site and Bn is the geochemical background metal levels (Muller, 1979).

Enrichment factor

It is the ratio of a metal's concentration to that of a reference element (such as Fe or Al) in the sediment, compared to the same ratio in background material, which shows how much the metal levels exceed natural background concentrations (Abrahim and Parker, 2008). Due to the use of a consistent baseline element, the EF method assists in regulating inherent variations in sediment composition. This allows for a clear distinction between metal accumulation caused by human activities and background levels originating from natural geological formations (Abrahim and Parker, 2008). EF is calculated using the following equation.

$$EF = \frac{(C_i / C_{ref})sample}{}$$

$$(C_i / C_{ref})\ background\ value$$

In this equation C_i is the metal element concentration with index i, measured in milligrams per kilogram (mg/kg). C_{ref} represents the concentration of the reference element, also measured in mg/kg (Loska et al., 2004; Li et al., 2013; Kara et al., 2014).

Table 3.1. Description of pollution indices, Contamination Factor (CF) (Thomilson et al., 1980), Geo–Accumulation Index (Igeo) (Muller, 1979) and Enrichment Factor (EF) (Loska et al., 2004; Li et al., 2013; Kara et al., 2014).

Index	**Value**	**Description**
CF	CF < 1	Low contamination
	1 < CF < 3	Moderate contamination
	3 < CF < 6	Considerable contamination
	CF > 6	Highly contaminated
EF	EF < 1	No enrichment
	1 < EF < 3	Minor enrichment
	3 < EF < 5	Moderate enrichment
	5 < EF < 10	Moderately severe enrichment
	10 < EF < 25	Severe enrichment
	25 < EF < 50	Very severe enrichment
	EF > 50	Extremely severe enrichment
Igeo	≤ 0	Unpolluted
	0 – 1	Unpolluted to moderately polluted
	1 – 2	Moderately polluted
	2 – 3	Moderately to strongly polluted
	3 – 4	Strongly polluted
	4 – 5	Strongly to extremely polluted
	>6	Extremely polluted

Results

Water quality results

The pH values were slightly acidic across all sites and seasons, ranging between mean 6.3 and mean 6.5. Water temperature showed clear seasonal variation, with low temperatures during the dry season, with a mean of 15.0 °C and 16.1 °C for the protected area and non–protected area,

respectively. High temperatures during the wet season, with means of 24.0 °C and 24.3 °C were observed for protected and non-protected areas, respectively (Table 3.2). Total dissolved solids (TDS) and conductivity were high in the dry season than in the wet season at both sites. Within the protected area, TDS averaged (mean 50.4 mg/L) in the wet season and (mean 102.9 mg/L) in the dry season, while at the non–protected area, it increased from mean
68.6 mg/L (wet season) to 138.4 mg/L (dry season). Conductivity followed a similar trend, with mean values of 98.9 µS/cm (wet season) and 205.6 µS/cm (dry season) in the protected area, while in the non–protected area, the mean was 137.5 µS/cm (wet season) and 276.6 µS/cm (dry season). Salinity remained constant across all sites and seasons at mean 0.1 PSU (Table 3.2). The ANOVA results (Table 3.3) indicated that total dissolved solids, salinity, and conductivity were significantly ($p < 0.05$) different across sites and seasons. Temperature varied significantly across seasons (F= 134.21, $p < 0.001$) but not across sites (p= 0.598). The pH exhibited no significant differences between sites or seasons ($p > 0.05$).

Metals in river water

Sodium (Na) concentrations were high at the non–protected area, ranging between mean 11.6 mg/L (wet season) and 20.8 mg/L (dry season), compared to mean 7.1 mg/L (wet season) and 16.0 mg/L (dry season) in the protected area. Potassium (K) and calcium (Ca) showed similar patterns, with high levels during the dry season for both sites (Table 3.2). Magnesium (Mg) levels were generally low, ranging between mean 0.0 mg/L and 0.4 mg/L across sites and seasons. Al concentrations reached mean 0.1 mg/L and 0.2 mg/L in the wet and dry seasons, respectively at the protected area, and mean 0.1 mg/L (wet season) and 0.5 mg/L (dry season) at the non–protected area. Manganese (Mn) concentrations were low, ranging from mean 0.1 mg/L to 0.4 mg/L, while Zn remained low across all sites, varying from mean 0 to 0.2 mg/L. Among the metals, Fe levels were recorded in high concentrations compared to other metals, with concentrations ranging from mean 1.6 mg/L (wet season) to 4.2 mg/L (dry season) in the protected area and mean 2.1 mg/L (wet season) to 4.3 mg/L (dry season) in the non–protected area (Table 3.2). Based on the ANOVA analysis, Na (F = 8.17, $p = 0.006$), Ca (F = 9.68, $p = 0.003$), and Mg (F = 4.40, $p = 0.041$) differed significantly across sites, while K (F = 9.40, $p = 0.004$), copper (F = 27.89, $p < 0.001$), Mn (F = 7.24, $p = 0.010$), and Zn (F = 4.97, $p = 0.031$) showed significant seasonal variation. Other metals, such as Al, Fe, Pb and P showed no significant variation across sites and seasons ($p > 0.05$) (Table 3.3).

Metals in River sediment

Sediment analysis showed variation in metal concentrations between sites and seasons, with metals such as Na, Mg, K, Ca, and P varying across sites and seasons. At the protected area, Na ranged from mean 181.3 mg/kg in the wet season to 332.7 mg/kg in the dry season, while in the non–protected area, it ranged from mean 134.5 mg/kg in the wet season to 266.9 mg/kg in the dry season. Magnesium showed similar pattern, with mean 1 770.6 mg/kg in the wet season and 2 047.5 mg/kg in the dry season of the protected area and mean 1306.7 mg/kg (wet season) and 2 190.2 mg/kg (dry season) in the non–protected area (Table 3.2). Phosphorus had mean values of 320.0 mg/kg (wet season) and 349.5 mg/kg (dry season) in the protected area, whereas mean values of 239.5 mg/kg (dry season) and 255.4 mg/kg (wet season) in the non– protected area were recorded. Potassium and Ca showed relatively high values compared to other metals (Table 3.2). In the protected area, K averaged 7 841.4 mg/kg (wet season) and 8 778.4 mg/kg (dry season), while in the non–protected area, K was slightly low with a mean of 3 845.7 mg/kg (dry season) and 4 115.6 mg/kg in the wet season. Calcium ranged from mean 1 554.5 to 1 577.3 mg/kg in the protected area and 2 180.1 to 2 334.4 mg/kg in the non– protected area.

Aluminium and Fe recorded high concentrations in all sites and seasons . Within the protected area, Al ranged between mean 49 219.0 mg/kg (wet season) and 55 953.1 mg/kg (dry season), while Fe ranged from mean 25 313.3 mg/kg (wet season) to 25 989.7 mg/kg (dry season). The non–protected area showed slightly low average concentrations for both elements, with Al (mean 25 401.6–30 731.6 mg/kg) and Fe (mean 18 292.7- 21 864.9 mg/kg). Manganese and Zn concentrations were moderate across both sites (Table 3.2), ranging from mean 240.8 to 421.2 mg/kg for Mn and 45.7 to 89.4 mg/kg for Zn. Copper concentrations varied between the protected (mean 29.2 to 43.5 mg/kg) and non–protected (mean 24.4 to 26.4 mg/kg) areas, while nickel (Ni) ranged from mean 25.0 to 47.8 mg/kg. Lead (Pb) concentrations had mean range values of 42.6–52.5 mg/kg in the protected area and 20.6–28.4 mg/kg in the non– protected area. Arsenic (As) concentrations ranged from mean 3.9 to 12.7 mg/kg in the protected area and mean 2.8 to 3.3 mg/kg in the non–protected area (Table 3.2). Other elements such as selenium (Se), molybdenum (Mo), Cd, tin (Sn), and antimony (Sb) occurred in low concentrations (< 2 mg/kg) (Table 3.2). Barium (Ba) was high at the protected area (mean range 331.3–377.1 mg/kg) compared to the non–protected area (mean range 169.6–223.9 mg/kg). Significant site variation were observed for K ($p < 0.001$), Al ($p < 0.001$), silicon (Si) ($p <$ 0.001), Zn ($p < 0.001$), Ba ($p < 0.001$), and Pb ($p < 0.001$), V ($p = 0.014$), B ($p = 0.026$), As ($p = 0.039$), Se ($p = 0.007$), and Sn ($p = 0.002$). Seasonally, Si ($p < 0.001$), Na ($p < 0.001$) and Cd

(p = 0.032) showed significant differences (Table 3.3).

Table 3.2. Water and sediment chemistry variables (mean ± SD) measured along the Nyl River floodplain from the protected and non–protected areas during the dry and the wet seasons.

Variables	**Units**	**Protected**		**Non–protected**	
		Dry	**Wet**	**Dry**	**Wet**
Water					
pH		6.5 ± 0.6	6.5 ± 0.2	6.4 ± 0.5	6.3 ± 0.2
Temperature	°C	15.0 ± 0.5	24.3 ± 1.4	16.1 ± 3.7	24.0 ± 2.4
TDS	mg/L	102.9 ± 10.4	50.4 ± 5.8	138.4 ± 41.6	68.6 ± 33.3
Conductivity	µS/cm	205.6 ± 20.7	98.9 ± 11.8	276.6 ± 83.7	137.5 ± 66.4
Salinity	PSU	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Na	mg/L	16.0 ± 4.8	7.1 ± 2.4	20.8 ± 7.9	11.6 ± 4.8
K	mg/L	3.5 ± 2.3	1.2 ± 0.5	3.8 ± 2.7	2.6 ± 1.1
Ca	mg/L	11.2 ± 2.3	6.0 ±2.3	13.8 ± 4.4	8.9 ± 2.1
Mg	mg/L	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.3	0.0 ± 3.6
Al	mg/L	0.2 ± 0.1	0.1 ± 0	0.5 ± 1.2	0.1 ± 0.1
Fe	mg/L	4.2 ± 4.8	1.6 ± 1.1	4.3 ± 8.3	2.1 ± 1.2
Mn	mg/L	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.2
Zn	mg/L	0.1 ± 0.1	0 ± 0	0.2 ± 0.2	0.1 ± 0.2
Sediment					
Na	mg/kg	332.7 ± 63.7	181.3 ± 69.2	266.9 ± 168.2	134.5 ± 120.0
Mg	mg/kg	2047.5 ± 594.7	1770.6 ± 609.8	2190.2 ± 2515.3	1306.7 ± 1263. 9
P	mg/kg	349.5 ± 100.8	320.0 ± 80.5	239.5 ± 177.6	255.4 ± 218.6
K	mg/kg	8778.4 ± 2075.4	7841.4 ± 2198.9	3845.7 ± 2600.4	4115.6 ± 3573.0
Ca	mg/kg	1577.3 ± 359.0	1554.5 ± 511.2	2180.1 ± 2402.2	2334.4 ± 4179.8
B	mg/kg	6.7 ± 1.2	6.2 ± 1.8	5.9 ± 3.5	3.8 ± 2.5
Al	mg/kg	55953.1 ± 14210.6	49219.0 ± 15211.0	30731.6 ± 20218.4	25401.6 ± 19506.4
Si	mg/kg	3525.1 ± 274.0	1983.8 ± 390.6	2832.2 ± 475.1	1413.3 ± 390.1

V	mg/kg	64.3 ± 13.8	61.8 ± 11.4	50.1 ± 24.0	43.8 ± 30.4
Cr	mg/kg	105.6 ± 19.2	119.4 ± 58.7	94.3 ± 45.3	69.0 ± 37.6
Mn	mg/kg	246.4 ± 127.5	240.8 ± 140.8	421.2 ± 393.0	201.3 ± 180.4
Fe	mg/kg	25989.7 ± 9156.9	25313.3 ± 8724.1	21864.9 ± 13044.4	18292.7 ± 13337.2
Co	mg/kg	11.2 ± 3.3	10.3 ± 3.5	13.8 ± 13.0	7.1 ± 7.2
Ni	mg/kg	47.8 ± 10.4	40.5 ± 13.4	39.2 ± 26.9	25.0 ± 24.2
Cu	mg/kg	29.2 ± 4.4	43.5 ± 40.5	26.4 ± 19.4	24.4 ± 24.1
Zn	mg/kg	88.2 ± 28.8	89.4 ± 35.2	49.7 ± 36.5	45.7 ± 33.2
As	mg/kg	3.9 ± 0.8	12.7 ± 20.3	2.8 ± 1.1	3.3 ± 3.1
Se	mg/kg	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.2
Sr	mg/kg	15.8 ± 3.3	14.7 ± 3.7	12.4 ± 8.2	13.6 ± 16.3
Mo	mg/kg	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.3 ± 0.2
Cd	mg/kg	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0
Sn	mg/kg	1.9 ± 0.4	1.9 ± 0.5	1.0 ± 0.9	1.4 ± 0.9
Sb	mg/kg	0.2 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	0.5 ± 1.1
Ba	mg/kg	377.1 ± 102.2	331.3 ± 107.0	223.9 ± 143.4	169.6 ± 128.8
Pb	mg/kg	42.6 ± 10.0	52.5 ± 19.0	20.6 ± 13.0	28.4 ± 21.5

Sediment quality guidelines

When compared against the Canadian standards three metals (i.e., Mn, Zn and Cd) were within the no effect level across sites and seasons (Table 3.4). Copper levels were within the lowest effect levels (LEL) across sites and seasons. Iron concentrations in the protected area during the dry and wet seasons, and in the non–protected area during the dry season, fell within the LEL threshold. The Fe concentrations within the non–protected area during the wet season was within the no effect level. The Ni concentrations in protected area for both seasons were within the LEL threshold, while in the non–protected area for both seasons, the Ni concentrations were within the no effect level. For As concentrations only protected area (i.e., wet season) was within the LEL, while others were within the no effect level (Table 3.4).

Table 3.3. The two-way analysis of variance results for water and sediment concentrations along the Nyl River basin during two seasons (i.e., hot–wet and cool–dry). Bold highlights significance level at $p < 0.05$.

Variable	Sites		Season		Sites * Season	
	F	***P***	**F**	***p***	**F**	***p***
Water						
TDS (mg/L)	**9.020**	**0.004**	**62.854**	**<0.001**	1.973	0.167
pH	1.529	0.222	0.308	0.581	0.077	0.783
Salinity (PSU)	**7.911**	**0.007**	**48.867**	**<0.001**	1.590	0.214
Conductivity (µs/cm)	**9.365**	**0.004**	**62.905**	**<0.001**	1.769	0.190
Temperature (° C)	0.282	0.598	**134.208**	**<0.001**	0.790	0.379
Na (mg/L)	**8.174**	**0.006**	**30.259**	**<0.001**	0.006	0.941
K (mg/L)	2.671	0.109	**9.402**	**0.004**	0.992	0.324
Ca (mg/L)	**9.679**	**0.003**	**32.300**	**<0.001**	0.047	0.829
Mg (mg/L)	**4.396**	**0.041**	0.345	0.560	0.113	0.739
Al (mg/L)	1.000	0.322	1.584	0.214	0.761	0.387
Cu (mg/L)	0.158	0.693	**27.888**	**<0.001**	0.158	0.693
Fe (mg/L)	0.060	0.807	2.575	0.115	0.013	0.910
Pb (mg/L)	0.655	0.422	2.676	0.109	0.478	0.493
Mn (mg/L)	0.362	0.550	**7.242**	**0.010**	2.477	0.122
P (mg/L)	0.530	0.470	0.141	0.709	3.322	0.075
Zn (mg/L)	2.610	0.113	**4.974**	**0.031**	3.187	0.081
Sediments						
Na (mg/kg)	2.482	0.122	**15.892**	**<0.001**	0.078	0.782
Mg (mg/kg)	0.119	0.731	1.540	0.221	0.422	0.519
P (mg/kg)	3.419	0.071	0.034	0.856	0.182	0.672
K (mg/kg)	**36.983**	**<0.001**	0.575	0.452	0.240	0.627
Ca (mg/kg)	0.904	0.346	0.023	0.880	0.033	0.858
B (mg/kg)	**5.252**	**0.026**	3.878	0.055	1.456	0.234
Al (mg/kg)	**23.753**	**<0.001**	1.689	0.200	0.000	0.998
Si (mg/kg)	**28.939**	**<0.001**	**162.408**	**<0.001**	0.404	0.528
V (mg/kg)	**6.454**	**0.014**	0.587	0.447	0.146	0.704
Cr (mg/kg)	**6.856**	**0.012**	0.314	0.578	2.842	0.098
Mn (mg/kg)	0.804	0.375	2.373	0.130	2.146	0.150
Fe (mg/kg)	3.593	0.064	0.723	0.400	0.416	0.522
Co (mg/kg)	0.012	0.913	2.357	0.131	1.332	0.254
Ni (mg/kg)	3.707	0.060	2.936	0.093	0.305	0.583
Cu (mg/kg)	2.340	0.133	0.730	0.397	1.301	0.260

Zn (mg/kg)	**19.015**	**<0.001**	0.070	0.793	0.153	0.697
As (mg/kg)	**4.497**	**0.039**	2.958	0.092	2.940	0.093
Se (mg/kg)	**7.817**	**0.007**	3.249	0.078	0.042	0.838
Sr (mg/kg)	0.543	0.465	0.002	0.963	0.152	0.698
Mo (mg/kg)	0.995	0.324	1.233	0.273	1.094	0.301
Cd (mg/kg)	1.194	0.280	**4.865**	**0.032**	0.430	0.515
Sn (mg/kg)	**11.188**	**0.002**	0.580	0.450	0.445	0.508
Sb (mg/kg)	0.156	0.695	1.852	0.180	0.004	0.951
Ba (mg/kg)	**20.066**	**<0.001**	2.244	0.141	0.051	0.823
Pb (mg/kg)	**26.257**	**<0.001**	2.777	0.102	0.188	0.666

Table 3.4. Sediment quality guidelines for Mn, Fe, Cu, Zn, Pb, Ni, Cd and As concentrations (mg/kg) for different sites (i.e., protected and non–protected) and seasons (i.e., dry and wet), along the Nyl River system. Canadian Sediment Quality Guidelines (Persuad et al., 1993), no effect level (blue shade), LEL – lowest effect level (green shade) and SEL – severe effect level (yellow shade): Cu (LEL – 16, SEL – 110 mg/kg), Fe (LEL – 20000; SEL, 40000 mg/kg), Mn (LEL – 460, SEL – 1100 mg/kg), Zn (LEL – 120, SEL – 820 mg/kg), Pb (LEL – 31, SEL – 250 mg/kg), Ni (LEL – 16, SEL – 75 mg/kg), Cd (LEL – 0.6, SEL – 10 mg/kg), As (LEL – 6, SEL – 33 mg/kg).

Sites	**Seasons**	**Mn**	**Fe**	**Cu**	**Zn**	**Pb**	**Ni**	**Cd**	**As**
Protected	Dry	246.4	25989.7	29.2	88.2	42.6	47.8	0.1	3.9
	Wet	240.8	25313.3	43.5	89.4	52.5	40.5	0	12.7
Non–protected	Dry	421.2	21864.9	26.4	49.7	20.6	39.2	0	2.8
	Wet	201.3	18292.7	24.4	45.7	28.4	25.0	0	3.3

The ecological risk assessment based on CF, Igeo, and EF indices showed clear differences across sites a season (Tables 3.1 and S1). The overall, contamination levels followed the order Al > Cr > Cu > Zn > As > Mn > Pb > Cd, with high metal loads observed at the protected sites, particularly during the dry season (Figure 3.2, Table S1).

In the dry season, Al recorded high contamination for sites, with CF values of mean 3.9 (non–protected area) and 7.1 (protected area), indicating high to considerable contamination levels (Figure 3.2a). For Pb, contamination remained low to moderate in both seasons. Zinc showed moderate to considerable contamination across both sites and seasons. Arsenic concentrations were low to moderate, in the protected site during the wet season with CF (mean 2.2). Copper showed moderate to considerable contamination, especially in the protected site during the dry season (CF= 4.1). Manganese showed low contamination across all sites and seasons.

Chromium showed moderate to considerable contamination (CF= 2.7–3.5) for sites. Cadmium remained consistently low in all samples (CF < 0.1)

The Al Igeo values (non-protected – mean 1.4, protected – mean 2.2) placed both sites in the moderately to strongly polluted category (Figure 3.2b). The Pb Igeo values consistently below zero, indicating unpolluted conditions (Table S1). Copper has Igeo values of 1.4 corresponding to moderately polluted levels (Table S1). The Mn with negative Igeo values showed unpolluted sediments. Cadmium remained consistently low in all samples (Igeo < 0)

The Al EF values (non-protected – 3.2, protected – 5.0) reflected moderate enrichment (Table S1, Figure 3.2c). Zinc had slightly high enrichment during the dry period (EF ≈ 1.7–2.7). Zinc EF values (1.6) indicated minor enrichment. Chromium EF values (~2.0–2.5) suggested minor enrichment (Figure 3.2c). Cadmium showed no enrichment across sites and seasons.

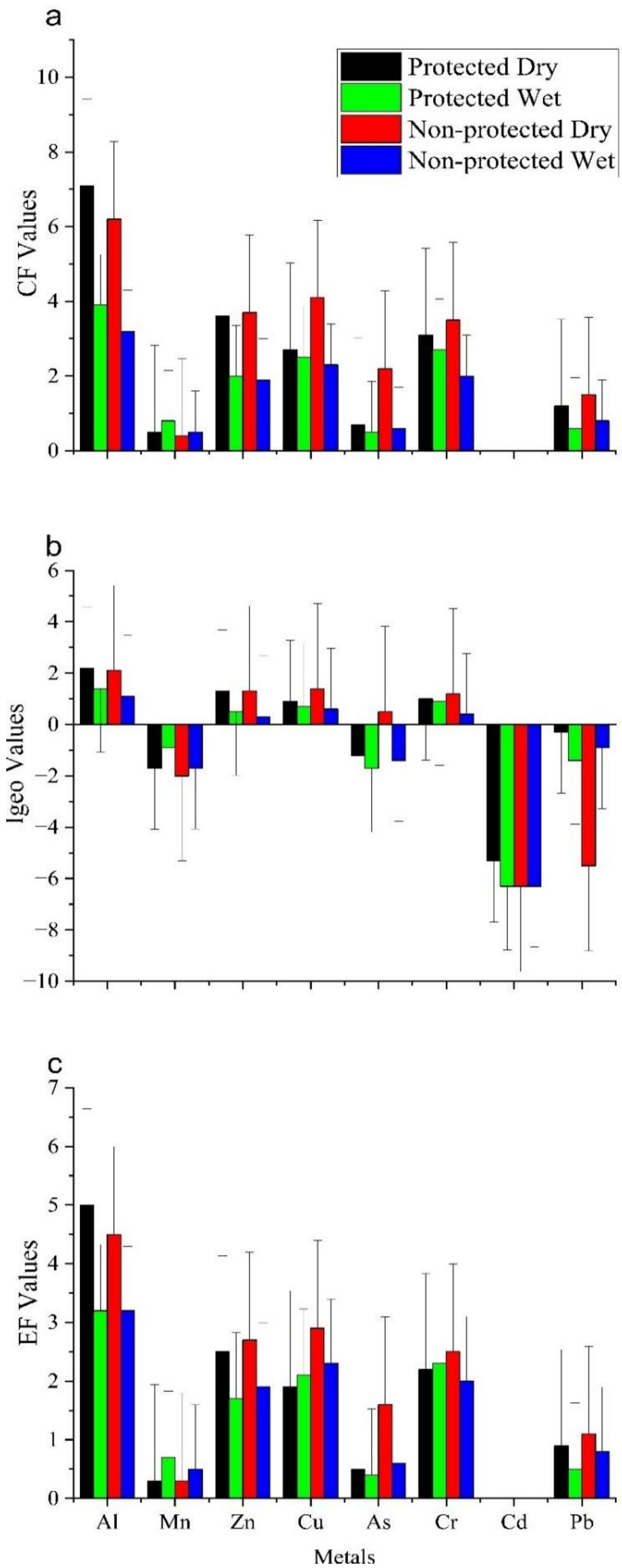

Figure 3.2. Risk assessment indices: (a) contamination factor (CF; mean ± standard deviation), (b) geo–accumulation indices (Igeo; mean ± standard deviation), and (c) enrichment factor (EF; mean ± standard deviation) of metals in sediments collected along the Nyl River basin from the protected and non–protected areas, during the dry and wet seasons.

Historical assessment of metal along the Nyl River in comparison to the current study

Water

Among the various studies conducted along the Nyl River system, metal concentrations in water showed considerable temporal and spatial variability (Table 3.5). The current Al concentrations were low compared with historical values. During the dry season, mean Al levels were 0.4 ± 0.9 mg/L (range 0–3.8 mg/L), and wet–season concentrations decreased to 0.1 ± 0.05 mg/L (range: 0–0.2). These values are substantially lower than the high wet–season peaks previously reported by Greenfield et al. (2012) and Gabbi (2018), which reached 913.6 mg/L. Similarly, Fe concentrations were below historic baselines, with dry–season means of 4.3 ± 7.1 mg/L (range 0–25.8 mg/L) and wet–season means of 1.9 ± 1.2 mg/L (range 0–4.0 mg/L). These values were particularly low in comparison to those observed by Ramogale (2008) and Gabbi (2018), where concentrations exceeded 2.0 mg/L (Table 3.5). Manganese concentrations were also minimal compared with earlier studies, with dry–season Mn concentrations averaging 0.3 ± 0.3 mg/L (range 0–1.0 mg/L), while wet–season means were 0.1 ± 0.2 mg/L (range 0–0.8 mg/L). These values show a sharp decline from the high historical concentrations of up to 1 910 mg/L reported by Jooste and Polling (2000). Zinc levels remained consistently low, with dry–season means of 0.2 ± 0.3 mg/L (range 0–1.1 mg/L) and wet–season concentrations of 0.03± 0.02 mg/L (range 0–0.1 mg/L). These results fall far below the peaks documented in earlier research, particularly the elevated values (>400 mg/L) observed by

Greenfield et al. (2012) (Table 3.5).

Sediments

Sediment metal concentrations in the current study were generally within lower to moderate ranges compared with earlier findings from the Nyl River basin (Table 3.5). Lead concentrations during the dry season averaged 29 ± 16 mg/kg (range 0–53 mg/kg), with wet–season means of 37.6 ± 23.4 mg/kg (range 0–86.2 mg/kg). These values remained substantially below the high wet–season levels reported by Greenfield (2004) of up to 1 188 mg/kg. Manganese concentrations also remained moderate, ranging from 354 ± 325.6 mg/kg (range 0–1 114.5 mg/kg) during the dry season to 216.5 ± 164.4 mg/kg (range 0–724.1 mg/kg) in the wet season. These were within the lower range compared to previous studies where concentrations exceeded 5 000 mg/kg (Table 3.5). Zinc concentrations were within the low range of historical data, with dry–season means of 64.5 ± 38.3 mg/kg (range 0–124.5 mg/kg) and wet–season means of 62.5 ± 39.7 mg/kg (range 0–154.5 mg/kg). Copper levels remained moderate, with dry–season means of 27.5 ± 15.3 mg/kg (range 0–58 mg/kg) and wet–season means of 31.7 ± 32.1 mg/kg (range 0–152.4 mg/kg), consistent with the mid–range values reported in Gabbi (2018).

Arsenic concentrations were low during the dry season (3.2 ± 1.2 mg/kg, range 0–4.3 mg/kg) but increased slightly during the wet season (7.0 ± 13.3 mg/kg, range 0–68.6 mg/kg), though still lower than historic highs documented by Greenfield et al. (2007). Aluminium and Fe were the most abundant metals in sediments. Aluminium levels were higher than previous records (i.e., Greenfield, 2004; Greenfield et al., 2007) during the dry–season and lower than previous records during the wet-season (Table 3.5). Iron levels were abundant but low when compared to the records of Dahms et al. (2016) and Gabbi (2018). Cadmium concentrations were consistently at or below 0.1 mg/kg while Dahms et al. (2016) recorded 11.5 ± 10.5 mg/kg, which was the highest level according to literature (Table 3.5). Chromium ranged between 98.6 ± 37.3 mg/kg (range 0–128.7 mg/kg) during dry season and was higher than previous studies, wet – season ranged 88.4 ± 52.1 mg/kg (range 0–255.1 mg/kg) and was lower than literature, except for Ramogale (2008). Nickel concentrations were moderate, varying from 39.3 ± 25.5 mg/kg (range 0–85.6 mg/kg) in the dry season to 25.1 ± 19.1 mg/kg (range 0–62.7 mg/kg) in the wet season, However, the levels were higher than Dahms et al. (2016) and lower than Gabbi (2018) (Table 3.5).

Table 5. Metal concentrations comparison between previous studies and current study along the Nyl River in water and sediment among seasons.

Metals	Season	Jooste and Polling (2000)			Ramogale (2008)		Greenfield et al. (2012)		Gabbi (2018)		Current study	
		1998			2002		2001–2002		2017–2018		2024–2025	
Water (mg/L)												
		Mean ± SD	Range		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Al	Dry						122.3±50.1	0–212.3	107.4±149.8	0–261.0	0.4±0.9	0–3.8
	Wet						913.6 ±189.2	0–267.5	37.9±19.0	0–38.0	0.1±0.05	0–0.2
Fe	Dry						1595±1724	0–2439	219.3±288.6	0–532.0	4.3±7.1	0–25.8
	Wet						2316±1042	0–1474.6	6.5±0.7	0–1.4	1.9±1.2	0–4.0
Mn	Dry	703.6±663.6	0–1910				235.4±242.8	0–343.4	11.9±8.6	0–16.8	0.3±0.3	0–1.0
	Wet	165.7±101.3	0–300				177.5±93.8	0–132.7	8.7±3.4	0–6.7	0.1±0.2	0–0.8
Zn	Dry				0.1 ±0.01	0–0.1	56.6±78.2	0–110.7	3.8±1.5	0–2.7	0.2±0.3	0–1.1
	Wet				0.3 ±0.4	0–0.0	479.4±58.1	0–82.2			0.03±0.02	0–0.1
Pb	Dry	220±137.7	0–470		0.3 ±0	0–0	4.6±3.6	0–5.2				
	Wet	257.1±186.9	0–700		0.1 ±0.1	0–0.2	55.1±24.0	0–34.0				
Cu	Dry	153.6±273.5	0–860		0.1 ±0	0–0	2.17±1.4	0–2				
	Wet	35±25.9	0–70		0.0 ±0	0–0	37.4±23.1	0–32.6				
Sediments (mg/kg)												
Sampling time	**Season**	**Greenfield (2004)**		**Greenfield et al. (2007)**	**Ramogale (2008)**		**Dahms et al. (2016)**		**Gabbi (2018)**		**Current study**	

		2001–2003		**2001–2002**		**2002**		**2014**		**2002**		**2024–2025**	
Pb	Dry	33.0±9.8	0–20.5			12.8±99.9	0–17.1			25.8±3.2	0–5.8	29±16	0–53
	Wet	595.7±667.6	0–1189			9.1±2.4	0–6.47			5.7±4.2	0–8.3	37.6±23.4	0–86.2
Mn	Dry	950.4±1026.4	0–1213	2400±374.2	0–900			34.0±14.5	0–20.5	532.2±245.9	0–431.2	354±325.6	0–1115
	Wet	1387.6±524.2	0–1113	5350±6467.1	0–13600					87.2±53.1	0–105.1	216.5±164.4	0–724.1
Zn	Dry	1895±1840	0–4054	2050±1646.2	0–3500	13.4±11.8	0–24.9	2169±3048	0–4310.3	61.9±40.3	0–78.4	64.5±38.3	0–124.5
	Wet	2271±2670	0–5059	7950±14703	0–29700	8.1±4.7	0–11–2			11.4±10.0	0–19.8	62.5±39.7	0–154.5
Cu	Dry	15.0±31.1	0–61.1	227±382.4	0–782	13.7±11.6	0–22.3	78.8±100.5	0–142.1	43.8±22.4	0–39.7	27.5±15.3	0–58
	Wet	31.7±26.2	0–36.8	56.3±43.9	0–95	8.3±3.5	0–9.1			2.1±1.9	0–3.4	31.7±32.1	0–152.4
As	Dry	10.4±13.7	0–16.0	5.3±3.3	0–7			4.5±1.5	0–2.2			3.2±1.2	0–4.3
	Wet	31.1±30.9	0–58.1	34.8±36.0	0–73.5							7.0±13.3	0–68.6
Al	Dry	12541±8438	0–2663	26750±5377	0–12000			30755±43491	0–61505			40432±21784	0–79759
	Wet	59880±78915	0–14186	33250±5377	0–12000							34562±21242	0–66654
Cd	Dry	1.3±1.4	0–2.51	0.7±1.2	0–2.5	0.6±0.5	0–9	11.5±10.5	0–14.9			0.1±0	0–0.1
	Wet	0.4±0.5	0–1	0.7±0.3	0–0.8	0.3±0.1	0–0.3					0±0	0–0.1
Cr	Dry	42.8±33.7	0–43.9	89.5±8.2	0–20	13.0±12.0	0–23.3	62.5±76.5	0–108.2			98.6±37.3	0–128.7

	Wet	82.6±110	0–209.7	146.3±103.6	0–225	20.5±15.6	0–36.9					88.4±52.1	0–255.1
Ni	Dry							29.3±30.9	0–57.8	59.1±39.3	0–69.1	39.3±25.5	0–85.6
	Wet									4.7±3.4	0–6.8	25.1±19.1	0–62.7
Fe	Dry							29708±36922	0–52215	71133±25759	0–44776	21490±13407	0–44185
	Wet									9748±7446	0–14888	17969±12289	0–39539

Discussion

The concentrations of metals in water and sediments showed spatial and seasonal variation across the study period, which is consistent with our first hypothesis, where we hypothesised that metal contamination in water and sediments will show seasonal variation due to bioturbation and water flow in various sections of the river. However, metals in water decreased and highlighted a contrast with our second hypothesis, where we hypothesised that metal concentrations will increase downstream due to cumulative industrial, mining and agricultural inputs. Contrary to metals in water, some metals (i.e., Fe, Cr, Al) in sediments were consistent with the second hypothesis. The pH remained slightly acidic across sites and seasons, indicating strong buffering capacity and minimal influence from runoff or organic inputs. This stability is similar to findings in the Crocodile and Olifants rivers (Renshaw, 2012; Tshivhase, 2019). Temperature followed expected climatic patterns, increasing in the wet season and decreasing in the dry season (Nembilwi et al., 2021; Mupepi et al., 2025), with wet season warming influenced by shallow water and solar radiation (Jacobs et al., 1998; Yue et al., 2015; Boyd, 2020).

High TDS and conductivity during the dry season reflect evaporation and reduced dilution (Adjovu et al., 2023), a trend widely reported in semi–arid systems, where low flow conditions lead to soluble ion buildup (Gossweiler et al., 2019). The elevated values in the non–protected sites indicate increased runoff and anthropogenic inputs (Slingenberg et al., 2009), similar to the Palala River (Sibiya, 2023). Stable salinity further confirm that the Nyl River remains a freshwater system with less salt intrusion (Greenfield, 2004), aligning with other South African inland rivers such as the Vaal and Molopo rivers (Akpotu, 2021). These findings suggest that the rivers water chemistry is mainly influenced by natural hydrological cycles flow, evaporation, anthropogenic stressor and dilution.

Dissolved ions such as Na, K, and Ca increased during the dry season at the non–protected site due to concentration under low flow, high evaporation rates and allochthonous inputs (Anderson and Dietrich, 2001), a pattern observed in the upper Crocodile River (Zondi, 2017). Iron concentrations were generally high compared to other metals, and this was consistent with findings from South African rivers such as the Vaal and Olifants rivers, where Fe dominates the dissolved metal fraction due to natural geological sources and anthropogenic sources (Netshitungulwane et al., 2015; Addo–Bediako, 2022). Low Zn and Mn contradict findings from industrial catchments such as the Steelpoort and Olifants rivers, where mining increase metal loads (Addo–Bediako et al., 2018; Addo–Bediako, 2022, 2025). These patterns indicate that while the Nyl River is exposed to some natural metal inputs and anthropogenic inputs, it remains relatively unpolluted in terms of dissolved metals (Greenfield et al., 2012).

Findings by Greenfield et al. (2012) highlighted that while some metal concentrations exceeded the desired water quality ranges/limits (i.e., Target Water Quality Recommendations) for aquatic ecosystems, the levels remained relatively constant throughout the system and generally considered to pose little or no immediate threat. However, the small but detectable concentrations of Fe, Mn, and Zn may represent baseline geogenic contributions rather than anthropogenic enrichment. Seasonal variation further indicates that hydrological processes particularly dilution during high flow strongly influence the concentration of metals in water (Papafilippaki et al., 2008). Metals such as Na, Ca and Mg showed significant spatial differences which are consistent with studies that attribute site level contrast to surrounding geology or land use. However, the lack of significant variation in metals such as Al, Fe, Pb, and P is in contrast with reports by Duruibe et al. (2007) and Jaishankar et al. (2014), which observed greater metal fluctuations driven by anthropogenic activities.

Sediment results showed dominance of lithogenic metals such as Al and Fe, indicating strong geological control where metals are primarily derived from natural weathering of mineral rich soils and rocks (Shozi, 2015). Dry–season increases reflect fine–particle deposition under low flow, similar to the Msunduzi River (Shozi, 2015). Metals (i.e., Zn, Cu, Cr, Ni, Pb, As) occurred in small amounts typical of partial impact, and the Cd and Sb concentrations further indicate minimal anthropogenic influence. High sediment metal loads in the protected site may reflect greater sediment retention and organic accumulation (Shozi, 2015). These results resemble patterns in relatively undisturbed rivers such as the Msunduzi River (Shozi, 2015) but differ from mining impacted systems such as the Spekboom and Olifants, where Cr, Ni, and Zn exceed thresholds (Ayisi et al., 2023). The results are also contrast with Dahms et al. (2016), who observed that metals exceeded the Canadian guidelines downstream during both flow periods. The combination of lithogenic dominance and moderate trace–metal presence in this study therefore suggests that sediment composition is primarily natural with minor anthropogenic impact, though ongoing monitoring is needed to detect any increasing inputs.

Sediment metal concentrations along the Nyl River were low and mostly within the no effect level and LEL based on Canadian guidelines, indicating minimal ecological risk. Metals such as Mn, Zn, and Cd consistently fell within safe limits across sites and seasons, reflecting limited anthropogenic inputs. However, Cu, Ni, and Fe were mostly within the LEL, with slightly high values in the protected sites which are linked to natural geological enrichment.

The ecological risk assessment based on CF, Igeo and EF showed clear spatial and seasonal variation, with the dry season generally exhibiting high metal accumulation. Aluminium showed high contamination, and the Igeo indicated moderate to strong pollution, with EF confirming moderate enrichment. This pattern aligns with findings from Shozi (2015) and Ayisi et al. (2023), who also linked elevated Al to natural weathering of rich soils. The high values compared to Dahms et al. (2016) suggest strong geogenic loading during low–flow periods (i.e., dry season). Chromium, Cu and Zn displayed moderate to considerable contamination, with EF suggesting minor to moderate enrichment. These results are similar to Greenfield et al. (2007), though much lower than levels reported in mining–impacted rivers such as the Olifants and Steelpoort (Addo–Bediako et al., 2018; Ayisi et al., 2023). Arsenic showed low to moderate contamination with minor enrichment, primarily in the wet season in the protected sites, indicating natural and runoff–driven inputs. Lead, Mn and Cd exhibited low contamination (negative Igeo, CF < 1), similar to previous observations in relatively undisturbed parts of the Nyl system (Greenfield et al., 2007).

Metal concentrations in both water and sediments of the Nyl River showed clear temporal and spatial variation, consistent with previous studies (Greenfield et al., 2012; Dahms et al., 2016; Dalu et al., 2020). In general, dissolved metals in water, particularly Al and Fe, were considerably low in comparison to historical records, indicating a reduction in metal loads over time (Ramogale, 2008; Greenfield et al., 2012; Gabbi, 2018). This decline may suggest effective dilution or metal adsorption as compared with earlier periods. Similarly, Mn and Zn levels were minimal across sites and seasons, consistent with more clean conditions, contrasting with past reports that recorded elevated concentrations, especially in areas influenced by industrial and mining activities (Jooste and Polling, 2000). Sediment metals such as Pb, Mn, Zn, As and Cd were generally within lower to moderate ranges as compared to historical records, while metals such as Al, Fe and Cr were higher than historical records. Cadmium remained consistently low suggesting minimal contamination. Seasonal differences, with slightly high concentrations during the dry season, are driven by reduced flows, increased sediment deposition, and concentration of metals on fine particles, as observed in other semi–arid river systems (Greenfield et al., 2007; Dahms et al., 2016). The trends observed from historical and current records show no consistency as records indicate great fluctuations throughout the years, which likely leads to unstable baselines (Greenfield, 2004; Greenfield et al., 2007; Ramogale, 2008; Dahms et al., 2016; Gabbi, 2018).

Conclusion and recommendations

The current study assessed metal concentrations in water and sediment along the Nyl River system focusing on the protected and non–protected areas. The mean concentrations showed a low impact trend across the system as alluded by the Contamination factor, geo–accumulation index and the enrichment factor. The indices indicated that the system is still in its natural state except for minor contaminations which can be linked to excessive weathering of mineral rich soils and rocks and anthropogenic sources. Moreover, the metal levels were within the no effect level and low effect levels when compared against the Canadian sediment quality guidelines, which highlights that the metal present have less to no effect on aquatic ecology. The Nyl River currently maintains a stable physicochemical balance and relatively low contamination status. The system reflects a resilient freshwater environment where natural geochemical processes exceed anthropogenic influences. The dominance of lithogenic elements and low enrichment of trace metals indicate that sediment and water quality remain within acceptable ecological limits. The observed patterns point to a system that is hydrologically dynamic yet environmentally stable, supporting the rivers role as an important ecological protection within a semi–arid landscape.

To preserve this ecological integrity, regular monitoring should continue to track potential shifts in metal dynamics, especially under changing climatic and land–use pressures. Future studies should include biological indicators and sediment water interaction analyses to improve understanding of ecological risks. Catchment conservation measures such as vegetation maintenance, erosion control, and responsible land–use practices are essential to sustain water quality and prevent future contamination. Collaborative management among conservation authorities, local communities, and researchers will further increase long–term river health and ecosystem resilience.

CHAPTER FOUR: COMPARATIVE STUDY OF BENTHIC MACROINVERTEBRATE COMMUNITIES IN PROTECTED AND NON–PROTECTED FRESHWATER SITES

Abstract

Freshwater ecosystems are among the most biodiverse yet most threatened habitats globally, with benthic macroinvertebrates serving as reliable indicators of ecological conditions. This comparative study assessed benthic macroinvertebrate communities in protected and non–protected areas, to understand how conservation status influences ecological integrity. A total of 10 304 macroinvertebrate individuals from 28 families were identified, with community structure showing significant differences between sites but not seasons. Protected areas were dominated by *Pseudagrion* sp., *Ceriagrion glabrum*, and *Hydrocanthus* sp., while non–protected areas exhibited higher abundances of pollution–tolerant taxa such as *Tanypus* sp. and greater overall community dissimilarity. Boosted regression tree models revealed that sediment (i.e., Mn, Mo, As) and water (i.e., conductivity, K, Fe) chemistry were key predictors of macroinvertebrate richness, with both positive and negative relationships observed. Diversity metrics, including the Shannon–Wiener, Margalef, Pielou evenness, and Simpson indices, indicated that both diversity and richness peaked during the low flow period, while evenness remained relatively stable across seasons. Macroinvertebrate communities were more dominant during the dry–seasons than the wet–seasons. ANOSIM analysis highlighted significant differences in community composition among sites, but not across seasons. SIMPER analysis identified key taxa contributing most to dissimilarity between sites (i.e., *Ceriagrion glabrum*, *Pseudagrion* sp., *Hydrocanthus* sp., *Tanypus* sp.), highlighting the influence of environmental gradients on community structure. Non–metric multidimensional scaling ordination further illustrated clear separation of macroinvertebrate communities by site and seasons. These findings highlight the importance of local environmental conditions and habitat heterogeneity in shaping macroinvertebrate diversity and composition and provide a foundation for using these communities as indicators of ecosystem health.

Keywords: Benthic macroinvertebrates, Nyl River, protected and non–protected areas, environmental variable, anthropogenic stressors, sediment metals, community composition, ecological integrity.

Introduction

Freshwater ecosystems are globally recognised as some of the most biodiverse yet most threatened habitats on Earth (Dudgeon et al., 2007; Reid et al., 2019; Feio et al., 2023). Rivers and streams in particular support high levels of species richness and provide essential ecosystem services such as water purification, nutrient cycling, and habitat provision for a wide range of aquatic and terrestrial species (Mdluli et al., 2022; Munyai et al., 2024; Leshaba et al., 2025). These systems also play a vital role in maintaining hydrological connectivity and landscape productivity, making them critical to both ecological integrity and human well–being (Haase et al., 2023; Guan et al., 2023). However, despite their ecological and socio–economic significance, freshwater environments are experiencing unprecedented levels of degradation worldwide (Ottoni et al., 2023; Dudgeon and Strayer, 2025). A growing body of research indicates that land–use change, agricultural intensification, urbanisation, industrial effluents, and hydrological alterations collectively threaten the diversity and functioning of freshwater systems (Dudgeon et al., 2007; Feio et al., 2022). In southern Africa, these threats are particularly severe due to expanding agricultural activities, catchment deforestation, mining, and poor wastewater management, which mainly leads to excessive nutrient loading, sedimentation, and contaminant accumulation in rivers and streams (Odume et al., 2015; Dube et al., 2020; Lebepe et al., 2022). Odume et al. (2015), highlight that many of these pressures originate from diffuse sources such as agricultural runoff and urban stormwater, which makes them difficult to monitor and regulate. Furthermore, developing regions often face limited institutional and financial capacity to implement effective management and long–term monitoring, resulting in intensified declination of freshwater biodiversity (Odume et al., 2015). Moreover, these combined pressures lead to habitat degradation, reduced water quality, and biodiversity loss while threatening the stability, resilience, and service provision of river ecosystems (Dube et al., 2020; Feio et al., 2022).

Among the biological components of freshwater ecosystems, benthic macroinvertebrates play an important ecological role (Dallas, 2007; Odume et al., 2015; Bonacina et al., 2022; Leshaba et al., 2025). Benthic macroinvertebrates represent a broad and diverse assemblage that includes insect larvae such as mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies

(Trichoptera), and true flies (Diptera), as well as non–insect groups such as crustaceans (amphipods, isopods, copepods), molluscs (gastropods and bivalves), oligochaetes, and aquatic worms (Dickens and Graham, 2002; Dallas, 2007; Odume et al., 2015). Furthermore, these organisms occupy multiple ecological niches within aquatic environments, from fast–flowing riffles to depositional zones, and perform important functions in decomposition, nutrient cycling, energy transfer, and sediment mixing (Santos and Ferreira, 2020; Bonacina et al., 2022; Leshaba et al., 2025). Their relative immobility varied sensitivity to pollutants, and well–known life histories make them ideal indicators of localized environmental conditions and long–term ecological changes (Dallas, 2007; Eriksen et al., 2021). Macroinvertebrate assemblages respond rapidly and predictably to disturbances such as organic enrichment, sedimentation, flow modification, and chemical pollution, thereby integrating multiple stressors over time (Eriksen et al., 2021; Mureithi et al., 2025). This makes them a key biological tool for assessing water quality and ecological integrity beyond the direct snapshot offered by physico–chemical parameters (Dallas, 2007; Odume et al., 2015; Bonacina et al., 2022). As a result of their crucial assessment several bioassessment approaches have been developed globally, including multi–metric indices and rapid bioassessment protocols that use macroinvertebrate community composition to deduce ecosystem conditions (Dallas et al., 2021).

In southern Africa, the South African Scoring System (SASS) is the most widely applied method for river health assessment (Dickens and Graham, 2002; Dallas, 2007). Where SASS assigns sensitivity scores to macroinvertebrate families, with higher scores indicating better water quality (Dickens and Graham, 2002; Dallas, 2007). However, the system has been locally calibrated for various ecoregions and has proven to be effective for rapid, cost–efficient biomonitoring across a wide range of stream types (Dickens and Graham, 2002; Dallas, 2007; Palmer et al., 2021). Furthermore, these bioassessment frameworks continue to evolve with advances in functional trait analysis and molecular taxonomy, improving accuracy and comparison across regions (Eriksen et al., 2021; Mureithi et al., 2025).

In addition, the use of benthic macroinvertebrates as bioindicators has several strengths that make them particularly suitable for ecological monitoring in data–limited contexts (Rosenberg and Resh, 1993; Boulton and Lake, 1990; Bonada et al., 2006; Hering et al., 2006; Dallas, 2007; Odume et al., 2015; Bonacina et al., 2022). As they are sensitive to both long–term and short–

term pollution events, integrating multiple stressors, and providing direct evidence of the biological state of aquatic habitats (Dallas, 2007; Odume et al., 2015). Since macroinvertebrates are ever–present, relatively easy to sample, and well–studied in terms of tolerance and functional traits, they allow for standardization across catchments and long–term monitoring programs (Odume et al., 2015). However, bioassessment using macroinvertebrates also has inherent challenges (Lenat, 1993; Dallas, 2007), where taxonomic resolution can be limited by regional expertise, natural variability can conceal anthropogenic signals, and calibration of sensitivity scores must consider local conditions such as altitude, flow regime, and substrate type (Dallas et al., 2021). Despite these limitations, macroinvertebrate–based assessments remain the foundation of river biomonitoring in Africa and globally, complementing physico–chemical and hydro–morphological analyses (Odume et al., 2015; Bonacina et al., 2022).

In addition to assessing ecological condition, macroinvertebrates are also valuable for evaluating the effectiveness of conservation interventions, particularly the role of protected areas in maintaining aquatic biodiversity (Nieto et al., 2017; Orozco–Gonzlez and Ocasio–Torres, 2023). A central assumption in freshwater conservation is that formally protected riparian or catchment areas experience fewer anthropogenic disturbances and therefore sustain higher biodiversity and better ecological condition than unprotected landscapes which are exposed to agricultural runoff, urbanisation, or industrial pollution (Dallas and Day, 2004; Odume et al., 2015; Bere et al., 2016; Mwedzi et al., 2016; Dube et al., 2020). Protected areas, including nature reserves and biosphere zones, are designed to conserve biodiversity, maintain natural processes, and safeguard ecosystem services through the restriction of intensive land uses (Bere et al., 2016; Mwedzi et al., 2016; Dube et al., 2020). Studies across southern Africa, such as those in Zimbabwe and South Africa, have consistently shown that protected catchments display greater macroinvertebrate diversity, higher SASS scores, and better overall water quality compared to non–protected or disturbed sites (Mwedzi et al., 2016; Bere et al., 2016; Mabidi et al., 2017; Ntloko et al., 2021). These findings emphasize the ecological value of riparian and catchment protection as a mechanism for sustaining aquatic biodiversity and ecosystem functioning.

Nevertheless, the assumption that protection automatically bestow ecological integrity is not universally upheld (Rodrigues et al., 2004; Le Saout et al., 2013). In some cases, the benefits of protection may be diminished by external catchment influences, inadequate enforcement, or legacy impacts from historical land use (Gordon, 2013; Larned et al., 2019; Blanckenberg et al., 2020). Edge effects, agricultural intrusion, and pollution inputs from upstream or adjacent unprotected lands can still affect water quality and biotic communities within protected boundaries (Rodrigues et al., 2004; Le Saout et al., 2013). Furthermore, the time required for ecological recovery following protection can be long, meaning that recent designations may not yet reflect significant biological improvements (Bailey et al., 2015; Jewitt, 2015; Dube et al., 2019; Musetsho et al., 2021). Limited connectivity between protected river sections and surrounding landscapes can also hinder recolonization by sensitive taxa, reducing the resilience of aquatic communities (Bailey et al., 2015; Jewitt, 2015; Musetsho et al., 2021).

Additionally, lateral hydrological connectivity has been found to differentially affect the community characteristics of multiple groups of aquatic invertebrates in tropical wetland pans in South Africa (Dube et al., 2019). These factors highlight the importance of empirical evaluations that directly measure the biological effectiveness of protection under real environmental conditions rather than assuming its benefits (Bailey et al., 2015). Comparative studies examining macroinvertebrate assemblages across protected and non–protected freshwater sites are therefore essential for testing the conservation effectiveness of riparian protection (Dickens and Graham, 2002; Weyl et al., 2010; Dalu et al., 2016). Such comparisons can reveal whether protected areas truly act as refuges for sensitive taxa, whether they support more balanced functional trait distributions, and whether ecological processes such as decomposition and nutrient cycling are better maintained (Weyl et al., 2010). They also help identify the thresholds at which anthropogenic stressors begin to overwhelm the buffering capacity of protected systems (Chutter, 1998; Dalu et al., 2016). In many subtropical and semi–arid regions, particularly in developing countries, these relationships remain underexplored despite the growing need to manage freshwater resources sustainably (Sundar et al., 2020; Akamagwuna et al., 2023; Leshaba et al., 2025).

Addressing this knowledge gap is fundamental for evaluating the success of conservation policy and guiding adaptive management of river ecosystems in southern Africa and similar regions worldwide (Weyl et al., 2010; Cowx et al., 2016). Empirical studies that link macroinvertebrate community structure, functional diversity, and environmental variables across protection gradients provide not only ecological insight but also practical guidance for land and water managers (Weyl et al., 2010). Such studies contribute to the refinement of biomonitoring frameworks such as SASS, inform riparian buffer design, and help optimize the allocation of limited conservation resources. In doing so, they enhance the evidence base for sustaining freshwater biodiversity and ecosystem services in the face of ongoing environmental change.

The overall study aim was to compare the taxonomic and functional characteristics of benthic macroinvertebrate communities in protected *versus* non–protected freshwater sites. Specifically, the research (i) quantified differences in macroinvertebrate taxa richness, abundance and diversity indices between locations, (ii) assess whether protected sites support a greater proportion of pollution–sensitive taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT), (iii) test for significant differences in community composition while accounting for environmental variables, and (iv) compare functional trait distributions including feeding guilds and life–history traits. We hypothesised that protected sites would exhibit high taxonomic richness and diversity, a large proportion of sensitive taxa, distinct community assemblages, greater functional trait diversity, and elevated biological integrity compared with non–protected sites. By testing these hypotheses, the study will generate robust evidence of whether protection status confers measurable ecological benefits for freshwater biodiversity, while also highlighting the role of macroinvertebrates as effective indicators of conservation success.

Materials and methods

Study area

The study area falls within the Waterberg catchment of the Limpopo Province, South Africa, and it follows the course of both the Klein and Groot Nyl rivers, from their sources to their confluence, and the downstream course of the Nyl River to Moorddrift Dam near Mokopane (formerly Potgietersrus) (Huggins and Roger, 1993; Greenfield et al., 2007). The Nyl River flows through or is impacted on by the towns of Modimolle (formerly Nylstroom) and Moogkopong (formerly Naboomspruit), flowing in a north–easterly direction from Modimolle to Mokopane. At Mokopane, the river shifts northward and changes its name to the Makgalakwena River, which ultimately joins the Limpopo River (Huggins and Roger, 1993; Greenfield et al., 2007). The region's geology is made up of sandstone formations from the Waterberg Group, mixed with granites and gneisses from the Swazian and Bushveld complexes

(UP, 2021; DFFE, 2023). This geological makeup contributes to the area's mineral richness and influence sediment composition (UP, 2021; DFFE, 2023). The landscape features rolling hills, wide floodplains, and isolated mountain ridges, creating a varied range of ecological niches and shaping water flow patterns. The Waterberg catchment is a significant water source area, receiving mean annual rainfall ranging from 450 mm to 750 mm, with most precipitation falling in the summer months (October to March). This seasonal rainfall drives river flow and floodplain flooding, which are important for maintaining wetland health and sediment movement (Junk and

Wantzen, 2006; Baker et al., 2009).

The system is increasingly affected by anthropogenic pressures, including agriculture and urban developments (Smith et al., 2015). The rich floodplain soils support intensive cultivation of crops such as maize, groundnuts, tobacco, citrus, cotton, millet, wheat, rice, and sunflowers (Greenfield, 2004). Cattle farming is common in the floodplain, while game farming dominates the upper drainage basin, near the river sources, with many private reserves (Greenfield, 2004). These land–use practices, combined with runoff from formal and informal settlements, contribute to metal enrichment, sediment loading, and hydrological alteration, posing risks to the ecological integrity of the Nyl River floodplain and downstream systems.

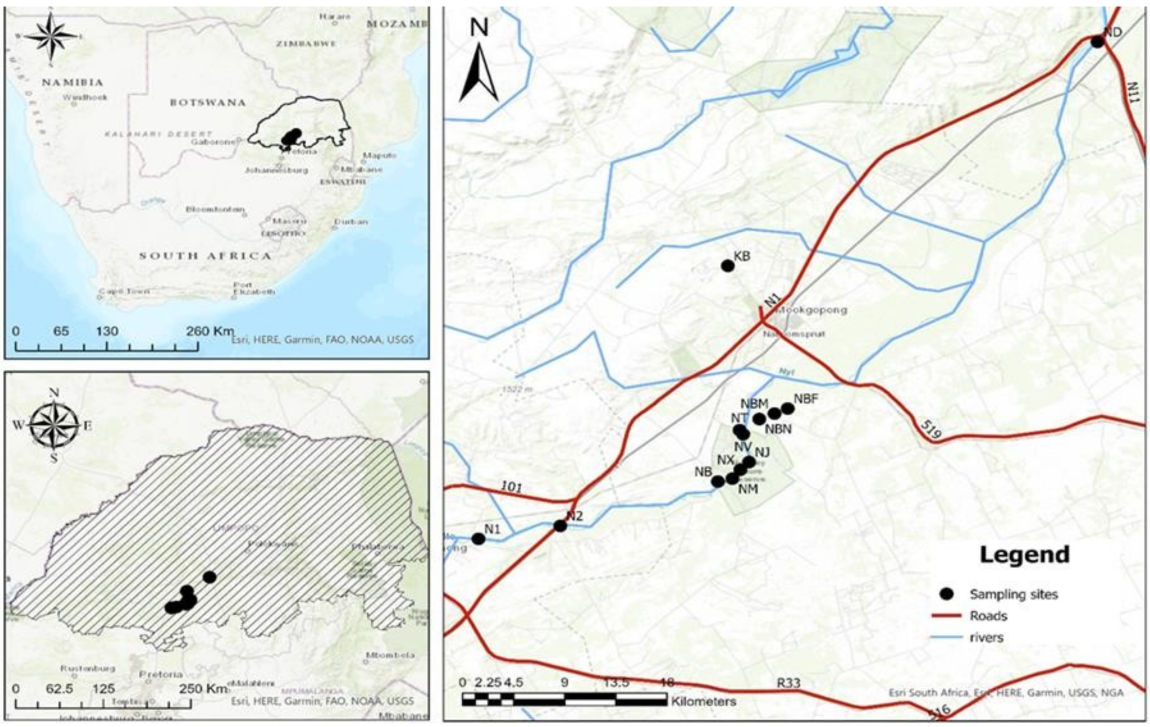

Figure 4.1. A study area map of the Nyl River system in Limpopo Province, South Africa.

Macroinvertebrate sampling

At each site (Figure 4.1), macroinvertebrates were sampled using the kick sampling method according to Dickens and Graham (2002). In summary, a hand–held kick net (dimensions 30×30 cm, mesh size 500 µm, 1.5 m handle) was used to sample all the available habitat types (i.e., gravel, sand and mud (GSM), macrophytes, pools, bedrock). The handheld kick net was submerged in the water and swept within a certain demarcated area and this involved walking with the net throughout the water, dragging and kicking macrophyte vegetation, rocks and sediments in order to dislodge macroinvertebrates attached. Active animals were prevented from escaping in the net by quickly lifting it out of the water and emptying its contents into a tray, where all detrital organic matter was removed. The macroinvertebrates were then stored in a labelled 500–mL polyethylene bottles and preserved in 70 % ethanol (Dalu and Chauke, 2020). Identification and counting of macroinvertebrates were done under a disserting Olympus microscope using a field guide to Freshwater macroinvertebrates of southern Africa (Fry, 2021).

Data analysis

Environmental variable data were evaluated for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test), and the assumptions for parametric analyses were satisfied. Two–way ANOVA was then applied to assess seasonal (i.e., dry, wet) and location (i.e., protected, non–protected) differences in water chemistry variables and macroinvertebrate diversity metrices (i.e., Shannon–Wiener, Simpsons, evenness, richness, abundances) using SPSS v25. Macroinvertebrate assemblage patterns were analysed using distance–based PERMANOVA (Bray–Curtis and Euclidean dissimilarities; 9999 permutations with Monte Carlo tests) in PRIMER v6 with the PERMANOVA+ add–on. Community composition differences were further tested with two–way ANOSIM (9999 permutations), and SIMPER analysis was applied to identify taxa contributing to dissimilarities. Furthermore, relationships between macroinvertebrate taxa richness and environmental variables were modelled using boosted regression trees (BRTs). The BRT analyses were implemented in R (R Core Team, 2025) with the gbm package (Ridgeway, 2006) following the approach of Elith et al. (2008), using gbm.step for model fitting and gbm.simplify for variable reduction.

Several macroinvertebrate indicator taxa metrics were also calculated: % EPT – proportion of total taxa or individuals that belong to EPT, with a high % EPT indicating good water quality, % Chironomidae often increases in disturbed or enriched habitats (pollution–tolerant), % Oligochaeta with high values indicating organic pollution and sediment enrichment, and EPT/Chironomidae ratio (i.e., ratio of sensitive taxa (EPT) to tolerant taxa (Chironomidae)) useful for contrasting clean *vs* impacted sites. The South African Scoring System (SASS) is a biomonitoring tool widely applied in river health assessments, based on the presence and diversity of macroinvertebrate families with differing sensitivities to pollution (Thirion et al., 1995; Dallas, 1995; Dallas, 2021). Each family is assigned a sensitivity score, and the cumulative total represents the SASS score for a site. To account for sample size and habitat differences, the Average Score Per Taxon (ASPT) was calculated by dividing the total SASS score by the number of taxa present. The ASPT provides a measure of the average sensitivity of the community and is less influenced by absolute richness. Together, SASS and ASPT provide complementary indices of river condition. The SASS5 and ASPT scores were used as a measure of each site condition: excellent (SASS5 score > 100 and ASPT score > 7), good (80–100 and 5–7), fair (60–80 and 3–5), poor (40–60 and 2–3) and very poor (< 40 and < 2) (Thirion et al. 1995).

Results

Environmental variables

The level of TDS was high during the cool–dry season (mean range 50.40–68.63 mg/L) as compared to the hot–wet season (mean 102.90–138.38 mg/L). Salinity concentrations were low during the hot wet season with a mean value of less than 0.10 PSU however, the cool–dry season had higher concentrations. Electrical conductivity levels were high during the cool–dry season with mean range values of 205.60 µS/cm to 276.56 µS/cm. Furthermore, temperature levels were high during the hot–wet season (mean 24.26 – 24.02 °C) as compared to the cool–dry (mean 15.02 –16.07 ° C). Additionally, the above–mentioned variables showed significance ($p < 0.05$) across both seasons and sites. Most of the environmental (i.e., water and chemistry) variables are presented in Chapter 3.

Macroinvertebrate communities

A total of 10 304 macroinvertebrate taxa from 28 families were identified along the Nyl River during the dry and wet seasons. *Pseudagrion* sp. and *hydrocanthus* sp. were dominant during both seasons and sites, *Ceriagrion glabrium* was dominant during dry (non–protected) and wet (protected and non–protected) seasons. *Tanypus* sp. was dominant during dry season for the non–protected area, while *Enitharus* sp. was dominant during wet season in the non–protected area. Families such as Gomphidae, Chaoboridae, Ceratopogonidae and Hydrochidae were found only during the dry season while, Corixidae were found only during the wet season (Table 4.1). Furthermore, the PERMANOVA analysis highlighted significant differences across locations (Pseudo–F = 3.168, df = 1, p = 0.0003) and seasons (Pseudo–F = 10.391, df = 1, p =

0.0001) for macroinvertebrate taxa.

Table 4.1: The mean dominant macroinvertebrate taxa relative abundances (%) identified within the protected and non–protected areas within the Nylsvley Wetlands region, South Africa.

Taxa	**Dry**				**Wet**			
	Protected		**Non–protected**		**Protected**		**Non–protected**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Oligochaeta								
Tubifex sp.			0 – 27.02	3.34 ± 8.57	0 – 0.73	0.07 ± 0.23	0 – 6.52	0.6 ± 1.65
Trombidiformes								
Hydrachna sp.			0 – 13.33	1.55 ± 4.13	0 – 0.69	0.06 ± 0.21	0 – 0.81	0.05 ± 0.2
Hydrophilidae								
Anacaenini	0 – 5.78	2.32 ± 1.8	0 – 11.11	2.98 ± 3.83	0 – 6.13	3.26 ± 2.17	0 – 9.75	1.52 ± 3.12
Regimbartia sp.	0 – 2.92	0.53 ± 1.02	0 – 0.48	0.03 ± 0.12	0 – 1.53	0.23 ± 0.52	0 – 1.35	0.09 ± 0.33
Dytiscidae								
Copelatus sp.	0 – 1.67	0.18 ± 0.52			0 – 12.57	2.32 ± 4	0 – 18.69	3.46 ± 6.5
Rhantus sp.					0 – 27.3	4.26 ± 8.77	0 – 11.42	1.55 ± 3.32
Noteridae								
Hydrocanthus sp.	0 – 78.18	35.55 ± 26.14	0 – 61.05	10.59 ± 16.05	0 – 5.77	0.83 ± 1.91	0 – 11.11	1.03 ± 3.01
Hydrochidae								
Hydrochus sp.			0 – 1.88	0.11 ± 0.47				
Hydroptilidae								
Hydroporinae	0 – 17.47	3.34 ± 5.22	0 – 9.43	1.33 ± 2.49	0 – 3.47	0.52 ± 1.17	0 – 5.47	0.49 ± 1.45
Atyidae								

Caridina sp.			0 – 56.92	5.84 ± 15.26			0 – 12.96	1.3 ± 3.43
Culicidae								
Anopheline	0 – 7.51	0.75 ± 2.37	0 – 3.14	0.52 ± 1.12	0 – 12.23	2.25 ± 4.03	0 – 8.64	1.8 ± 3.1
Ephydridae								
Brachydeutera sp.	0 – 6.35	1.16 ± 2	0 – 6.91	0.91 ± 1.74	0 – 0.73	0.14 ± 0.3		
Ceratopogoninae			0 – 8.1	0.74 ± 2.13				
Chaoboridae								
Chaoborus sp.	0 – 3.7	0.37 ± 1.17						
Chironomidae								
Chironominae			0 – 6.8	0.42 ± 1.7	0 – 3.59	1.21 ± 1.29	0 – 18.79	2.54 ± 5.31
Tanypus sp.	0 – 5.78	1.25 ± 1.82	0 – 90.32	15.66 ± 27.73	0 – 9.55	1.34 ± 3	0 – 51.85	5.87 ± 12.79
Psychodidae								
Clogmia sp.			0 – 84.37	5.53 ± 21.04	0 – 9.48	2.16 ± 3.27	0 – 13.53	1.56 ± 3.48
Athericidae								
Suragina bivittate	0 – 4.58	0.51 ± 1.44						
Caenidae					0 – 30.14	4.78 ± 10.5	0 – 44.98	5.32 ± 13.6
Notonectidae								
Anisops sp.			0 – 0.42	0.02 ± 0.1	0 – 21.51	2.76 ± 6.65	0 – 8.57	0.89 ± 2.27
Enithares sp.	0 – 2.88	0.92 ± 1.17	0 – 29.72	3.24 ± 7.95	0 – 0.69	0.06 ± 0.21	0 – 88.3	8.43 ± 24.26
Aplelocheiridae								
Aphelocheirus sp.	0 – 1.83	0.18 ± 0.58			0 – 7.18	4.2 ± 2.43	0 – 20.3	5.32 ± 6.92
Belostomatidae								

Appasus sp.	0 – 8.33	2.97 ± 3.09	0 – 22.22	4.74 ± 6.25	0 – 3.67	0.64 ± 1.14	0 – 6.01	1.11 ± 1.86
Gerridae								
Aquarius sp.	0 – 1.46	0.14 ± 0.46	0 – 7.14	0.44 ± 1.78	0 – 9.55	1.28 ± 3.02	0 – 12.5	2.87 ± 3.6
Gerris Swakopensis					0 – 2.53	0.47 ± 1.00	0 – 3.7	0.32 ± 0.97
Neogerris sp.			0 – 0.21	0.01 ± 0.05	0 – 0.59	0.13 ± 0.22	0 – 5	0.35 ± 1.25
Hydrometra sp.			0 – 2.55	0.4 ± 0.89	0 – 3.47	0.98 ± 1.38	0 – 2.85	0.34 ± 0.79
Nepidae								
Ranatra sp.	0 – 0.57	0.07 ± 0.18	0 – 1.52	0.23 ± 0.46	0 – 5.06	0.83 ± 1.68	0 – 2.73	0.44 ± 0.91
Corixidae								
Sigara sp.					0 – 5.06	0.54 ± 1.59	0 – 7.5	1.04 ± 2.28
Planorbidae								
Helisoma duryi	0 – 9.69	3.68 ± 4.08	0 – 4.92	0.42 ± 1.29	0 – 15.52	6.34 ± 5.27	0 – 9.75	1.41 ± 2.72
Ampillariidae								
Pila occidentalis	0 – 0.97	0.16 ± 0.35	0 – 2.73	0.5 ± 0.94	0 – 0.73	0.11 ± 0.25	0 – 17.5	1.34 ± 4.34
Coenogrionidae								
Ceriagrion glabrum	0 – 3.47	0.86 ± 1.42	0 – 42.22	11.72 ± 13.72	15.64 – 56.11	33.69 ± 11.71	0 – 74.41	27.31 ± 24.23
Pseudagrion sp.	1.46 – 87.15	33.53 ± 31.94	0 – 67.64	19.05 ± 20.29	0 – 29.86	8.47 ± 11.32	0 – 41.09	9.29 ± 11.5
Libellulidae								
Chalcostephia flavifrons	0 – 14.45	1.99 ± 4.7	0 – 16.66	2.4 ± 4.35	0.84 – 22.15	8.51 ± 7.71	0 – 25.71	7.17 ± 8.28
Sympetrum sp.	0 – 13.19	3.88 ± 5.35	0 – 10	2.25 ± 3.11	0 – 6.14	0.61 ± 1.94	0 – 10	0.88 ± 2.62
Gomphidae								
Paragomphus sp.			0 – 9.53	1.08 ± 2.97				
Aeshnidae								

Pinheyschna sp.	0 – 6.99	1.01 ± 2.25	0 – 3.81	0.3 ± 0.97	0 – 2.63	0.47 ± 1	0 – 1.57	0.09 ± 0.39
Diversity metrices								
Taxa richness	7 – 16	11.5 ± 2.63	11 – 21	16.6 ± 2.87	7 – 18	11.37 ± 3.44	8 – 18	12.37 ± 3
Individuals	103 – 553	266.8 ± 170.42	79 – 326	194.9 ± 78.64	27 – 576	188.31 ± 161.23	35 – 488	167.06 ± 159.91
Dominance	0.1 – 0.76	0.41 ± 0.21	0.14 – 0.35	0.2 ± 0.06	0.12 – 0.81	0.34 ± 0.21	0.1 – 0.78	0.29 ± 0.17
Simpson	0.23 – 0.89	0.58 ± 0.21	0.64 – 0.85	0.79 ± 0.06	0.18 – 0.87	0.65 ± 0.21	0.21 – 0.89	0.7 ± 0.17
Shannon–Wiener	0.56 – 2.46	1.41 ± 0.56	1.56 – 2.23	2.03 ± 0.18	0.48 – 2.34	1.57 ± 0.53	0.54 – 2.43	1.71 ± 0.49
Evenness	0.25 – 0.73	0.39 ± 0.15	0.34 – 0.74	0.47 ± 0.11	0.16 – 0.8	0.49 ± 0.22	0.21 – 0.76	0.49 ± 0.18
%EPT	0 – 1.83	0.25 ± 0.59	0 – 30.14	6.16 ± 10.04	0 – 4.29	0.34 ± 1.07	0 – 44.98	5.32 ± 13.6
%Chironomidae	0 – 1.83	0.18 ± 0.58	0 – 30.14	4.78 ± 10.5	0 – 0.85	0.05 ± 0.21	0 – 44.98	5.32 ± 13.6
EPT/Chironomidae ration	0 – 1	0.2 ± 0.44	0 – 3.5	1.54 ± 1.46	0 – 0.68	0.06 ± 0.2	0 – 16.7	1.83 ± 4.85
% Oligochaeta	0 – 0	0 ± 0	0 – 0.73	0.07 ± 0.23	0 – 27.02	3.34 ± 8.57	0 – 6.52	0.6 ± 1.65
SASS	32 – 54	41 ± 6.89	33 – 83	53.6 ± 15.75	26 – 66	40 ± 12.78	19 – 64	38 ± 13.08
ASPT	3.58 – 5.33	4.32 ± 0.57	3.66 – 5.18	4.33 ± 0.54	3.66 – 4.83	4.15 ± 0.32	3.11 – 5.12	4.08 ± 0.63

Taxa richness ranged from 7 to 21, with high mean richness (16.6 ± 2.9) recorded within the non–protected sites during the dry season compared to lower values at protected sites in both dry (11.5 ± 2.6) and wet (11.4 ± 3.4). Dominance values were high in the dry season in the protected sites (0.41 ± 0.21), while in the wet season for the non–protected sites had low dominance (0.29 ± 0.17) levels. The Simpson (0.79 ± 0.06) and Shannon–Wiener (2.03 ± 0.18) indices were high in the non–protected sites during the dry–season compared to wet–season, with the protected sites (Simpson 0.65 ± 0.21, Shannon–Wiener 1.57 ± 0.53). Evenness remained moderate across sites (range 0.39–0.49) (Table 4.1). Significant differences were observed for macroinvertebrate taxa richness (location: F = 6.205, df = 1, $p = 0.016$; seasons: F = 12.202, df = 1, $p = 0.001$), dominance richness (season: F = 5.865, df = 1, $p = 0.019$), Simpson (season: F = 5.865, df = 1, $p = 0.019$) and Shannon–Wiener (season: F = 7.543, df = 1, $p = 0.008$) based on ANOVA analysis. The %EPT were high in the non–protected area during both the dry and wet seasons, with ranges of mean 5.32 ± 13.6 (wet) and 6.16 ± 10.4 (dry), while protected had mean 0.25 ± 0.59 (dry) and 0.34 ± 1.07 (wet).

The % Chironomidae were high in non–protected with mean ranges of 4.78 ± 10.5 (dry) and 5.32 ± 13.6 (wet). EPT/Chironomidae ration were also high in non–protected areas for both seasons. %Oligochaeta were only high in the protected area during the wet season (mean 3.34 ± 8.57). SASS mean scores were consistent in the protected areas for both seasons, while in the non–protected area there was a sharp drop during the wet season. ASPT mean scores were consistent across sites and seasons. Furthermore, no significant differences ($p > 0.05$) were observed for %EPT, %Chironomidae, EPT/Chironomidae ration, % Oligochaeta, SASS scores and ASPT scores across sites and seasons. The distribution across sites and seasons showed to fall under fair scores on the SASS and ASPT figure, with few good scores for both sites and seasons (Figure 4.2).

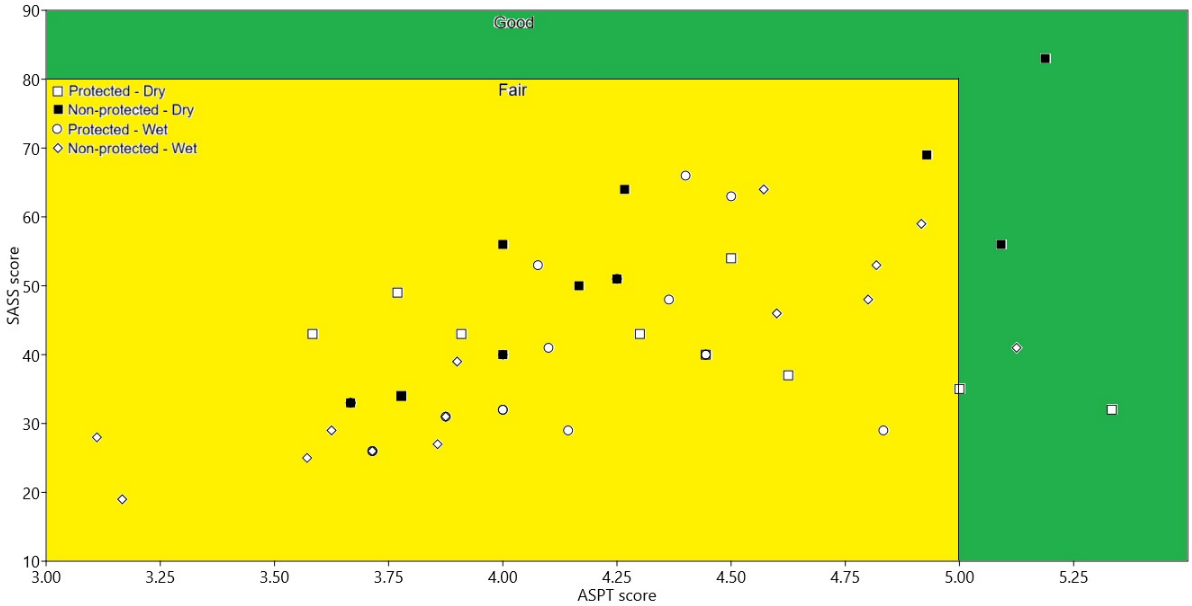

Figure 4.2. Mean average score per taxon (ASPT) and South African scoring system (SASS) scores calculated for protected and non–protected sites over two seasons.

The ANOSIM results highlighted that macroinvertebrate community structure differed significantly across locations (Global R= 0.412, p= 0.0001), with similarities being observed across seasons (Global R= 0.046, p= 0.179) (Table 4.2). Locations showed a high Global R–value signifying greater dissimilarity between protected and non–protected areas, whereas seasons indicated that the differences within and between seasons were relatively equal. The average community dissimilarity values for the areas were 55.9 % protected and 69.4 % non–protected sites. The assemblages in protected site were dominated by *Pseudagrion* sp. (19.1 %), *Ceriagrion glabrum* (16.6 %) and *Hydrocanthus* sp. (14.0 %), *Helisoma duryi* (7.7 %), *Anacaenini* sp. (7.3 %) and *Chalcostephia flavifrons* (6.2 %). The non–protected sites were strongly influenced by *Ceriagrion glabrum* (23.0 %) and *Pseudagrion* sp. (17.5 %), with high representation of pollution–tolerant taxa such as *Tanypus* sp. (9.1 %), *Chalcostephia flavifrons* (8.4 %), *Hydrocanthus* sp. (5.3 %) and *Appasus* sp. (5.1 %). However, the overall dissimilarity between protected and non–protected sites was 67.0 %, with *Pseudagrion* sp., *Hydrocanthus* sp., *Ceriagrion glabrum* and *Tanypus* sp. being the main taxa, driving separation between the two groups. During the dry season, communities were shaped primarily by *Pseudagrion* sp. (24.1 %), *Hydrocanthus* sp. (16.5 %) and *Ceriagrion glabrum* (11.7 %), whereas during the wet season communities were shaped by *Ceriagrion glabrum* (29.8 %) and *Chalcostephia flavifrons* (12.3 %), and *Aphelocheirus* sp. (8.0 %). The average dissimilarity between dry and wet seasons was 76.3 %, driven mainly by the shifts in the abundance of *Ceriagrion glabrum, Pseudagrion* sp., *Hydrocanthus* sp. and *Tanypus* sp. (Table 4.2).

Table 4.2. Analysis of similarities (ANOSIM) and SIMPER of macroinvertebrate taxa across locations and seasons.

Variable	Global R (*p*–value)	Dissimilarity (%)	Contributing dominant taxa
Locations	0.412 (0.0001)		
Protected		55.91	*Pseudagrion* sp. (19.1%), *Ceriagrion glabrum* (16.6%), *Hydrocanthus* sp. (14.0%), *Helisoma duryi* (7.7%), Anacaenini (7.3%), *Chalcostephia flavifrons* (6.2%)
Non–protected		69.43	*Ceriagrion glabrum* (23.0%), *Pseudagrion* sp. (17.5%), *Tanypus* sp. (9.1%), *C. flavifrons* (8.4%), *Hydrocanthus* sp. (5.3%), *Appasus* sp. (5.1%)
Protected *vs* non–protected		67.04	*Pseudagrion* sp. (8.9%), *Hydrocanthus* sp. (8.4%), *C. glabrum* (7.4%), *Tanypus* sp. (5.1%)
Seasons	0.046 (0.179)		
Dry		65.76	*Pseudagrion* sp. (24.1%), *Hydrocanthus* sp. (16.5%), *C. glabrum* (11.7%), *Tanypus* sp. (8.8%), *Appasus* sp. (8.0%), Acanaenini (6.9%)
Wet		65.73	*C. glabrum* (29.8%), *C. flavifrons* (12.3%), *Pseudagrion* sp. (12.0%), *Aphelocheirus* sp. (8.0%)
Dry *vs* Wet		76.34	*C. glabrum* (8.9%), *Pseudagrion* sp. (8.5%), *Hydrocanthus* sp. (7.7%), *Tanypus* sp. (5.6%)

The *n*–MDS showed clear patterns in macroinvertebrate community composition across locations and seasons (Figure 4.3). The ordination highlighted a distinct separation between protected and non–protected sites, indicating high dissimilarity (67.0 %) between the two areas. Protected sites clustered together, dominated by *Pseudagrion* sp., *Ceriagrion glabrum*, and *Hydrocanthus* sp., whereas non–protected sites were more dispersed, influenced by taxa such as *Tanypus* sp., *Chalcostephia flavifrons*, and *Appasus* sp. Seasonal variation appeared less distinct, as samples from dry and wet seasons overlapped considerably, supporting the ANOSIM results (Global R = 0.046, p = 0.179) (Table 4.2), which suggested that differences in macroinvertebrate communities were primarily driven by sites rather than seasonal changes.

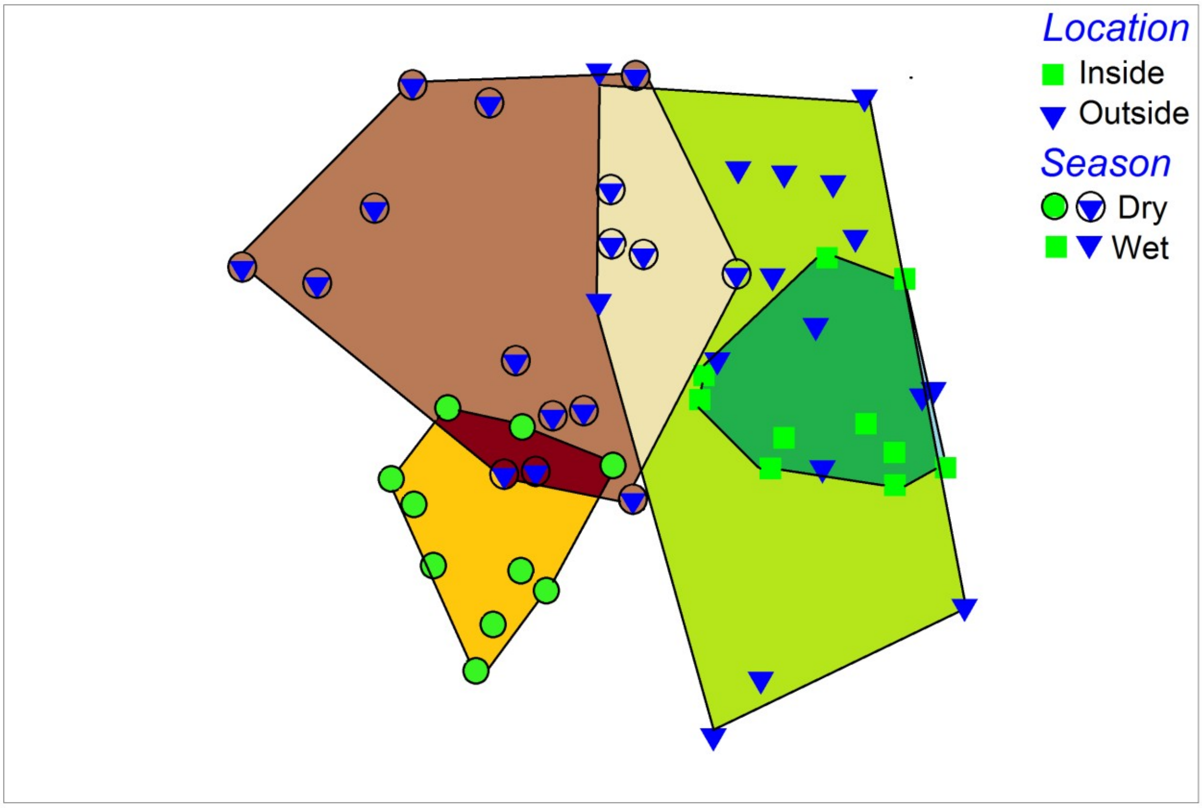

Figure 4.3. A non–metric multidimensional scaling highlighting the relationship among samples across locations and seasons.

Relationship between macroinvertebrate communities and environmental variables

Thepredictive performance of boosted regression trees (BRT) models relating macroinvertebrate taxa richness to environment variables was as follows: 16 300 number of trees fitted (boosted models), with a mean total and residual deviance of 9.56 and 0.106, respectively. The estimated cross–validated deviance ± standard error was 1.214 ± 0.413 and the cross–validated correlation of 0.93 ± 0.03 (with a training data correlation of 0.995). All variables were retained as significant terms in the BRT model relating macroinvertebrate taxa richness to environmental variables, with the first 10 variables accounting for ~60.7 % of macroinvertebrate taxa richness variation, with high predictor contributor to model fit being observed for sediment (i.e., Mn, Mo, As) and water (i.e., conductivity, K, Fe) chemistry variables (Table 4.3). The predicted macroinvertebrate taxa richness had a sigmoid shaped (i.e., positive) relationship with sediment (i.e., As, Ca, Sn) and water (i.e., Fe, Mg, pH) (Figure 4.4). Most of the variables, for example sediment (i.e., Mn, Mo, P, Na, Fe) and water (i.e., conductivity, K, Na, Mn) chemistry variables, showed a negative relationship with macroinvertebrate taxa richness (Figure 4.4).

Table 4.3. Contributions of predictor variables to boosted regression tree (BRT) models relating macroinvertebrate taxa richness to environmental variables tree complexity of 5 and learning rate of 0.001. Variables are sorted in order of decreasing contribution to phytoplankton richness. Abbreviations: .W – water chemistry variable, .S – sediment chemistry variable.

Predictor	Relative contribution (%)	Predictor	Relative contribution (%)
Mn.S	13.14	Se.S	1.78
Mo.S	12.91	Hg.S	1.74
Conductivity	7.90	Si.S	1.66
As.S	5.00	Sb.S	1.62
K.W	4.43	Co.S	1.32
Fe.W	4.26	K.S	1.32
Mg.W	3.50	V.S	0.92
Sn.S	3.45	Ca.S	0.91
Ca.S	3.10	B.S	0.69
P.S	3.03	Cd.S	0.65
Na.S	2.72	Ni.S	0.62
Fe.S	2.64	Pb.S	0.60
Na.W	2.64	Cr.S	0.54
pH	2.57	Mg.S	0.52
Mn.W	2.24	Cu.S	0.48
P.W	2.20	Al.S	0.40
Temperature	2.04	Sr.S	0.30
TDS	2.02	Zn.S	0.21
Ba.S	2.00	Salinity	0.13
ORP	1.81		

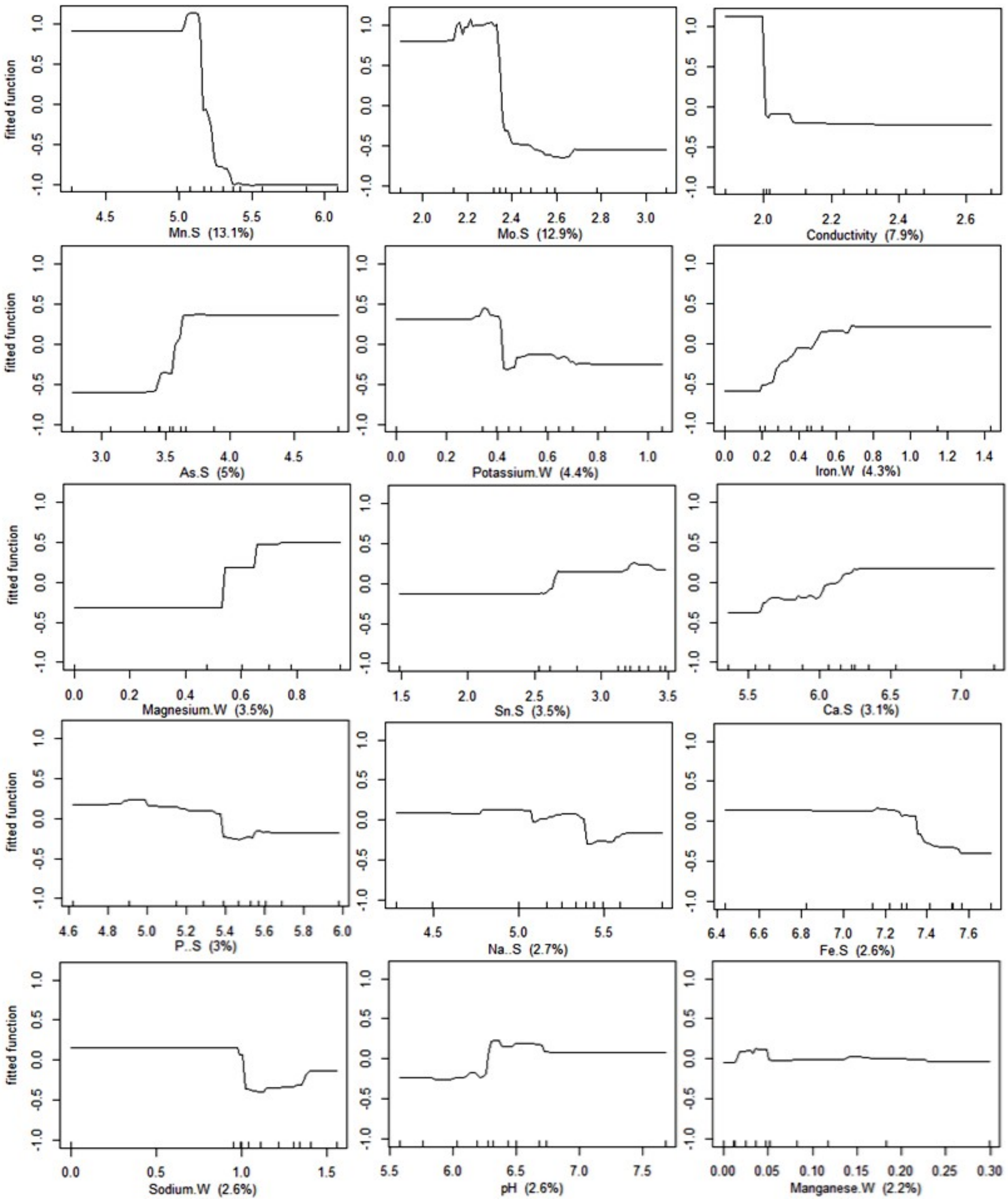

Figure 4.4. Macroinvertebrate taxa richness variation predicted by a boosted regression trees (BRT) model highlighting the first 15 sediment and water chemistry variables. Abbreviations: .W – water chemistry variable, Mn.S – manganese (sediment chemistry variable), Mo.S – molybdenum (sediment), As.S – arsenic (sediment), Sn.S – selenium (sediment), Ca.S – calcium (sediment), P.S – phosphorus (sediment), Na.S – sodium (sediment), Fe.S – iron (sediment), Al – aluminium, Na – sodium, As – arsenic, Mg – magnesium.

Discussion

In this study we managed to achieve our main objective which was to compare the taxonomic and functional characteristics of benthic macroinvertebrate communities in protected *versus* non–protected freshwater sites. However, our hypothesis (protected sites would exhibit high taxonomic richness and diversity, a large proportion of sensitive taxa, distinct community assemblages, greater functional traits diversity and elevated biological integrity compared to non–protected sites) was not fully supported as non–protected sites exhibited higher diversity and richness, although the sites were most dominated by tolerant taxa' rather than sensitive taxa'. The variation in physicochemical parameters observed between the dry and wet seasons in this study reflects the strong hydrological seasonality typical of the Nyl River system. Elevated TDS and conductivity levels during the dry season are mostly explained by reduced discharge and evaporative concentration of solutes, whereas lower values during the wet season reflect dilution due to increased flow and runoff.

Comparable seasonal concentration dilutions have been reported elsewhere in the region, for example Greenfield et al. (2012), documented high sediment metal loads and ionic concentrations during low flow periods in parts of the Nyl River system, and Khoza et al. (2012) found similar conductivity and ion–flux responses to flow variability in the Olifantspruit River catchment, a tributary system with comparable land–use pressures. These findings strengthen the interpretation that hydrology, regulated by seasonality and local water–use, is a primary driver of short–term water chemistry change in Limpopo catchments (Greenfield et al., 2012; Khoza et al., 2012). The observed water temperature dynamics during the wet season are characterised by elevated mean temperatures and broader daily ranges, particularly in shallow and slow–flowing reaches (Dallas, 2008; Smit et al., 2024). These variations are primarily driven by increased solar radiation and the inflow of thermally enriched runoff from adjacent heated surfaces (Dallas, 2008). Additionally, elevated temperatures influence dissolved oxygen concentrations through reduced solubility and enhanced biological demand, and can accelerate metabolic and decomposition rates, thus altering community stability and species tolerance thresholds (Thirion, 2016; Baker, 2018). In South African streams and pans, seasonal warming has been tied to shifts in species phenology and the timing of emergent life stages, which mostly affect food–web interactions and sampling interpretation if seasonality is not accounted for (Thirion, 2016).

Macroinvertebrate assemblages in our study displayed a clear spatial and seasonal variations between protected and non–protected areas, reflecting the influence of habitat diversity, hydrological seasonality, and anthropogenic pressures across the catchment. The community was dominated by taxa characteristic of lentic and slow–flowing environments, including *Hydrocanthus* sp., *Pseudagrion* sp., *Ceriagrion glabrum*, *Helisoma duryi*, and *Anacaenini* sp. These groups are typical of vegetated, warm, low–oxygen habitats that represent the Nylsvley floodplain (Dallas, 2008; Dalu et al., 2017). During the dry season, *Hydrocanthus* sp. dominated the protected area, while *Tanypus* sp. and *Pseudagrion* sp. were most abundant in non–protected areas. The dominance of *Hydrocanthus* sp. and other aquatic beetles under low–flow conditions reflect their tolerance to low oxygen levels and their ability to utilise fine sediment and detritus habitats (Ollis et al., 2006). In contrast, *Tanypus* sp. (Chironomidae) are opportunistic taxa that thrive in nutrient–enriched or organically polluted conditions (Dalu and Froneman, 2016). In the wet season, *Ceriagrion glabrum* and *Pseudagrion* sp. were notably abundant in both protected and non–protected sites. These odonates are sensitive to habitat complexity and flow regime, and their dominance during the wet season suggests a recovery of more sensitive taxa as flow and oxygen conditions improved (Chakona et al., 2020). The increase in molluscs such as *Helisoma duryi* (Planorbidae) and *Pila occidentalis* (Ampullariidae) further indicates the increase of tolerant grazers in nutrient–enriched sedimented microhabitats, as observed in other South African floodplain wetlands (Dallas, 2008; Greenfield et al., 2012).

Diversity indices reflected clear differences in macroinvertebrate community structure between sites and seasons. Taxa richness high in non–protected sites than in protected sites. Similarly, Shannon–Wiener diversity was high in non–protected areas compared to protected areas. These findings suggest that while non–protected areas support a greater number of taxa, this richness may be driven by the presence of pollution–tolerant taxa, resulting in unsteady diversity (Dalu and Froneman, 2016; Dalu et al., 2017). Evenness values were slightly high in the wet season than the dry season, indicating a more balanced community structure following seasonal floods that temporarily reduced dominance by tolerant taxa. The dominance index supports this notion, being high in the dry season at the protected sites, reflecting a strong influence of a few dominant taxa such as *Hydrocanthus* sp. and *Pseudagrion* sp., and low in non–protected sites, where assemblages were more evenly distributed among chironomids, odonates, and aquatic beetles. The Simpson's and Shannon–Wiener indices patterns together suggest moderate to high diversity, but with affected dominance and compositional shift. Similar patterns have been reported in the Okavango Delta and other South African riverine wetlands, where fluctuating hydroperiods and spatial habitat variability maintain intermediate diversity levels (Bird et al., 2019; Chakona et al., 2020).

Functional group indicators supported the interpretation of differential ecological condition between sites. The %EPT was high in the non–protected sites compared to the protected sites. This pattern might seem unexpected, but it can be explained by temporary colonisation of EPT taxa in areas that are slightly disturbed and experience high water flow and oxygen levels during the wet season (Dallas, 2007). The EPT/Chironomidae ratio showed a similar pattern, being high in the non–protected sites. In contrast, the proportion of Oligochaeta and Chironomidae was noticeably high in disturbed or downstream reaches, indicating excessive organic pollution or metal enrichment (Greenfield et al., 2012; Dahms et al., 2016). For example, *Tubifex* sp. reached high abundances in protected sites during the wet season, suggesting sediment–bound contamination and insufficient oxygen conditions consistent with observed sediment metal levels in the Nyl River system (Dahms et al., 2016).

The SASS scores mean values were slightly high in the non–protected sites compared to protected sites, while ASPT values were relatively stable across sites and seasons. The lack of significant difference between seasons supports previous studies suggesting that SASS and ASPT may not fully capture the influence of sediment contamination or hydrological alterations in wetland–dominated systems (Thirion, 2008; Dalu et al., 2017). The observed spatial and temporal trends reflect complex interactions among flow variability, habitat type and contamination exposure. The high abundance of tolerant taxa such as *Tanypus* sp., *Clogmia* sp. and *Tubifex* sp. during the dry season corresponds with low flows, high conductivity, and reduced oxygen levels, conditions that favour taxa capable of resisting sedimentation and organic enrichment (Dallas, 2008; Dalu and Froneman, 2016). In contrast, the wet season promoted recolonisation by odonates and mayflies, which prefer flowing, oxygenated water and structurally complex habitats.

Protected sites such as the Nylsvley Nature Reserve maintained assemblages dominated by relatively sensitive taxa such as *Pseudagrion* sp., *Ceriagrion glabrum*, and *Hydrocanthus* sp., reflecting better habitat integrity. However, the presence of tolerant groups and moderate SASS scores indicate that even protected sites are influenced by external pressures. Jewitt (2015) and Bailey et al. (2015) highlighted, agricultural encroachment and catchment–scale runoff near protected boundaries threaten nature reserves (protected sites) water quality through sediment and nutrient loading through a process contributing to the contaminant hotspots detected in this system. Protection alone, therefore, does not guarantee ecological resilience if catchment connectivity, upstream contamination, and hydrological alterations persist (Le Roux et al., 2019; Smit et al., 2024). Sustaining the ecological integrity of the Nylsvley floodplain requires managing upstream land use, limiting metal and nutrient inputs, and maintaining seasonal flow connectivity to support recolonisation by sensitive taxa.

The results demonstrate that the Nylsvley Wetlands (protected area) support moderately diverse macroinvertebrate assemblages shaped by both natural seasonal hydrology and anthropogenic disturbance. Diversity metrics and functional indicators highlight the importance of habitat diversity, sediment quality, and flow regime in structuring aquatic macroinvertebrate communities. These findings highlights that monitoring frameworks combining SASS/ASPT indices with diversity measures (i.e., Shannon–Wiener, Simpson, and evenness) provide a more delicate understanding of ecological condition in complex systems (Ollis et al., 2006; Dalu et al., 2017; Chakona et al., 2020).

Conclusion

This study demonstrated that both environmental variables and sites play an important role in shaping benthic macroinvertebrate communities within the Nyl River system. Although the Nylsvley Nature Reserve maintained relatively good ecological integrity, evidence of diffuse pollution and trace metal accumulation in sediments suggests that anthropogenic activities in surrounding catchments continue to exert pressure on water and habitat quality. Non–protected sites supported high overall richness but were dominated by tolerant taxa, indicating moderate ecological stress rather than true biodiversity enhancement. The observed relationships between macroinvertebrate assemblages and environmental variables confirm that these organisms are reliable indicators of ecosystem condition. The overall, findings highlight that while protection reduces severe degradation, integrated catchment management beyond reserve boundaries remains essential to maintain the ecological functioning and biodiversity of the Nyl River floodplain.

CHAPTER FIVE: GENERAL SYNTHESIS

GENERAL DISCUSSION

The present study assessed macroinvertebrate communities and environmental dynamics in relation to land use activities along the Nyl River, South Africa, with two main objectives which made our chapter 3: (i) to evaluate the spatio-temporal patterns of metal pollution in water and sediments of a semi–arid river basin in South Africa: Protected and non–protected areas and chapter 4: (ii) to compare the taxonomic and functional characteristics of benthic macroinvertebrate communities in protected *versus* non–protected areas. It should be noted that rivers function as integrative systems where physical, chemical, and biological components interacts continuously, making them responsive to both natural variation and human–induced pressures (Dalu et al., 2025; Gavrilaș et al., 2025; Chen et al., 2025). Their significance within freshwater ecosystems is evident when considering their dynamic nature and the multiple roles they fulfil across ecological and human dimensions (Weigelhofer et al., 2021; Gavrilaș et al., 2025).

Rivers serve as natural pathways that facilitate the movement of water, nutrients, and metals across landscapes (Dalu et al., 2025; Gavrilaș et al., 2025). Moreover, they provide a wide range of ecosystem services such as water provision, food production, flood regulation, nutrient cycling, and habitat provision (Weigelhofer et al., 2021; Gavrilaș et al., 2025). Therefore, any alteration in river condition whether through pollution, habitat modification, excessive water abstraction, or climate–related variability has consequences that extend beyond ecological boundaries (Johnson et al., 2024; Gavrilaș et al., 2025; Chen et al., 2025; Sultana and Jabeen, 2025). River disturbances are among the most significant threats to freshwater ecosystems worldwide, driven by both natural factors (e.g., floods, drought, geological processes) and human activities (e.g., pollution, habitat degradation) (Weigelhofer et al., 2021; Johnson et al., 2024; Gavrilaș et al., 2025; Chen et al., 2025). Human activities often release pollutants such as heavy metals, nutrients, pesticides, plastics, and untreated wastewater into river systems, altering water chemistry and degrading ecological integrity (Johnson et al., 2024; Dalu et al., 2025; Gavrilaș et al., 2025; Chen et al., 2025).

Contaminants released into river systems tend to accumulate in sediments, which act as both sinks and potential sources of these substances over time (Liu et al., 2018; Yin et al., 2022; Custodio et al., 2022; Muñoz–Arcos et al., 2024). This accumulation disrupts nutrient cycles by altering the composition and function of sediment microbial communities, which are essential for processes such as nitrogen and phosphorus cycling (Yin et al., 2022; Custodio et al., 2022; Chen et al., 2024). For example, microplastics and heavy metals can shift microbial community structure, reduce microbial diversity, and impair key metabolic functions, ultimately affecting nutrient transformation and biogeochemical cycling in sediments (Liu et al., 2018; Yin et al., 2022; Custodio et al., 2022; Chen et al., 2024).

The presence of these contaminants also degrades habitat quality, making conditions less suitable for sensitive aquatic organisms. Elevated concentrations of heavy metals and other pollutants in sediments have been shown to reduce the richness, diversity, and evenness of benthic macroinvertebrate communities, which are important indicators of ecosystem health (Bere et al., 2016; Qu et al., 2017; Rico–Sánchez et al., 2022; Dalu et al., 2025; Adubor et al., 2025). Sensitive taxa may decline or disappear, while more tolerant species become dominant, leading to a loss of biodiversity and altered food web dynamics (Bere et al., 2016; Erasmus et al., 2021; Rico–Sánchez et al., 2022; Dalu et al., 2025).

Managing and protecting river systems requires integrated catchment approaches that address anthropogenic disturbances, improved wastewater treatment, promote sustainable land use, and monitor chemical and biological indicators (Weigelhofer et al., 2021; Gavrilaș et al., 2025). Hence, the current study aimed to integrate the spatiotemporal variation of metal concentrations in water and sediments alongside the responses of macroinvertebrate communities. In chapter 3 the results of the study showed significant differences in water and sediment chemistry between protected and non–protected areas, as well as between dry and wet seasons. Water variables such as TDS, conductivity, Na, and Ca were consistently high in non–protected areas, especially during the dry season, indicating greater anthropogenic influence and reduced dilution (Dalu et al., 2025; Gozzi, 2025). Sediment metals including K, Al, Si, Zn, Ba, and Pb showed strong spatial variation, with protected areas generally exhibiting high concentrations, particularly during the dry season. Seasonal effects were most pronounced for Si, Na, and Cd in sediments, reflecting hydrological changes and potential remobilization of contaminants (Dalu et al., 2025; Ozseker and Terzi, 2025; Gozzi, 2025).

As continued in Chapter 3 risk assessment indices (i.e., CF, Igeo, EF) highlighted aluminium as the most significant contaminant, with values indicating moderate to strong pollution and moderate enrichment. Zinc and copper also showed moderate to considerable contamination, while lead and manganese remained low across sites and seasons. Cadmium and arsenic were generally low, with only minor enrichment observed for arsenic in the protected wet season. These patterns align with findings from other river systems, where spatial and seasonal variability in metal contamination is often linked to both natural processes and anthropogenic activities such as agriculture, urban runoff, and industrial discharges (Ali et al., 2021; Hossain et al., 2024; Wang and Chadwick, 2024; Ozseker and Terzi, 2025; Sanad et al., 2025). The comparison on Canadian sediment quality guidelines showed that metals in this study fell within the no effect levels and the low effect levels with no observed severe effect levels. This suggests that, while contamination is present, severe ecological risks may be limited under current conditions (Ali et al., 2021; Hossain et al., 2024; Dalu et al., 2025; Ozseker and Terzi, 2025).

While the Nyl River shows moderate contamination and generally low ecological risk, studies from more heavily impacted systems (i.e., urban or industrial rivers) report high levels of metals such as Cd, Pb, and Zn, often exceeding severe effect thresholds and posing significant risks to both aquatic life and human health (Ali et al., 2021; Proshad et al., 2021; Wang and Chadwick, 2024; Hossain et al., 2024; Sanad et al., 2025). Conversely, some rivers with effective management and lower anthropogenic input demonstrate lower contamination and healthier ecological status (Kim and An, 2015; Calattini et al., 2025). This contrast underscores the importance of local context, land use, and management practices in shaping riverine chemical and ecological conditions.

Chapter 4 findings on macroinvertebrate communities of the Nyl River floodplain showed high taxonomic richness and clear seasonal and spatial variation in composition and diversity. Dominance by a few taxa (i.e., *Pseudagrion* sp., *Hydrocanthus* sp.) was observed across sites and seasons, while certain families were restricted to specific periods, reflecting dynamic community turnover. Diversity indices (i.e., Simpson, Shannon–Wiener) and taxa richness were generally higher at non–protected sites and during the dry season, indicating that both protection status and hydrological regime shape community structure. PERMANOVA and ANOVA analyses confirmed significant differences in macroinvertebrate assemblages across sites and seasons, consistent with findings from other river systems where environmental variables such as water chemistry, habitat structure, and seasonal hydrology drive macroinvertebrate diversity and composition (Baker et al., 2023; Sun et al., 2024; Munyai et al., 2025).

The ANOSIM and SIMPER analyses reveal that macroinvertebrate community structure in the Nyl River floodplain is primarily shaped by spatial differences between protected and non–protected areas, rather than by seasonal variation. The high Global R–value for locations and substantial average dissimilarity between protected and non–protected areas indicate that protection status is a key driver of community composition. Protected areas were dominated by sensitive taxa such as *Pseudagrion* sp. and *Ceriagrion glabrum*, while non–protected areas had a higher proportion of pollution–tolerant taxa such as *Tanypus* sp., reflecting the influence of anthropogenic pressures and water quality degradation (Souleymane et al., 2020; Dalu et al., 2022; Majdi et al., 2022; Munyai et al., 2025; Dalu et al., 2025). In contrast, the low Global R–value for seasons and overlapping n–MDS clusters suggest that seasonal changes have a relatively minor effect on overall community structure, a pattern also observed in other subtropical and temperate river systems where spatial factors and local environmental gradients outweigh seasonal effects (Dalu et al., 2017; Dube et al., 2017; Dalu et al., 2022; Munyai et al., 2025). However, SIMPER analysis still showed notable shifts in dominant taxa between dry and wet seasons, indicating that certain species respond to hydrological changes, even if the overall assemblage structure remains stable.

In Chapter 4 boosted regression tree modelling further supported the strong link between macroinvertebrate richness and environmental variables, with sediment metals (i.e., Mn, Mo, As) and water chemistry (i.e., conductivity, K, Fe) emerging as key predictors. Positive relationships were observed with some variables (i.e., sediment: As, Ca, Sn; water: Fe, Mg, pH), while other water and sediment variables (i.e., sediment: Mn, Mo, P, Na, Fe; water: conductivity, K, Na, Mn) showed negative associations, highlighting the complex interaction between chemical conditions and biological diversity (Dalu et al., 2017; Brysiewicz et al., 2022; Dalu et al., 2022; Munyai et al., 2025; Dalu et al., 2025). These findings are consistent with broader research showing that macroinvertebrate communities are sensitive indicators of habitat quality, water and sediment chemistry, and land use impacts. Protected areas tend to support higher diversity and more sensitive taxa, while non–protected or impacted areas are characterised by greater dominance of tolerant species and reduced community evenness (Souleymane et al., 2020; Dalu et al., 2022; Majdi et al., 2022; Munyai et al., 2025; Dalu et al., 2025). Effective river management should therefore prioritise the maintenance of habitat quality and chemical integrity, especially in non–protected areas, to support diverse and resilient macroinvertebrate assemblages

GENERAL CONCLUSIONS

This study demonstrated that macroinvertebrate community structure and diversity in the Nyl River are strongly influenced by both spatial and seasonal factors, with protection status playing a more significant role. The analysis of water and sediment chemistry revealed notable spatial and temporal variability in metal concentrations, with certain metals showing strong concentrations particularly in protected areas during the dry season. The findings likely suggest complex interactions between environmental factors and contamination sources. Protected areas supported more stable and less polluted macroinvertebrate assemblages, while non–protected areas exhibited higher dissimilarity and a great presence of pollution–tolerant taxa, indicating anthropogenic impacts such as sewage effluent runoff which can degrade community integrity. The relationships between macroinvertebrate richness and environmental variables highlighted the importance of both sediment and water chemistry, with specific metals and physicochemical parameters acting as key drivers of community patterns. To improve river health and biodiversity, it's important to focus on safeguarding and fixing natural environments, keeping or boosting the variety of habitats, and regularly checking water and sediment quality in order to detect pollution and its ecological effects early. Additionally, managing anthropogenic inputs such as sewage effluent and encouraging habitat complexity can help create stronger and more diverse communities of macroinvertebrates, which serve as vital bioindicators for ecosystem health in semi-arid river systems.

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APPENDICES

Table S1: The calculated ecological risk assessment from the collected sediment samples along the Nyl River: from the protected and non–protected sites during the dry and wet season.

Metals	**Season**	**Sites**	**Measured concentration**	**Background Value (Dahms, 2015)**	**CF**	**Pollution Level (contamination)**	**Igeo value**	**Igeo Class**	**EF**	**EF Levels**
Al	dry	Protected	55953.1	7897.82	7.1	Highly	2.2	Moderately to strong polluted	5.0	Moderate
		Non–protected	30731.6		3.9	Considerable	1.4	Moderately polluted	3.2	Moderate
	wet	Protected	49218.9		6.2	Highly	2.1	Moderately to strong polluted	4.5	Moderate
		Non–protected	25401.6		3.2	Considerable	1.1	Moderately polluted	3.2	Moderate
Pb	dry	Protected	42.6	35.0	1.2	Moderate	–0.3	Unpolluted	0.9	No enrichment
		Non–protected	20.5		0.6	Low	–1.4	Unpolluted	0.5	No enrichment
	wet	Protected	52.4		1.5	Moderate	–5.5	Unpolluted	1.1	Minor enrichment
		Non–protected	28.3		0.8	Low	–0.9	Unpolluted	0.8	No enrichment
Zn	dry	Protected	88.1	24.3	3.6	Considerable	1.3	Moderately polluted	2.5	Minor enrichment
		Non–protected	49.6		2.0	Moderate	0.5	Unpolluted to moderately	1.7	Minor enrichment
	wet	Protected	89.3		3.7	Considerable	1.3	Moderately polluted	2.7	Minor enrichment
		Non–protected	45.7		1.9	Moderate	0.3	Unpolluted to moderately	1.9	Minor enrichment
As	dry	Protected	3.8	5.9	0.7	Low	–1.2	Unpolluted	0.5	No enrichment
		Non–protected	2.8		0.5	Low	–1.7	Unpolluted	0.4	No enrichment

		Protected	12.7		2.2	Moderate	0.5	Unpolluted to moderately	1.6	Minor enrichment
	wet	Non–protected	3.4		0.6	Low	–1.4	Unpolluted	0.6	No enrichment
Cu	dry	Protected	29.2	10.7	2.7	Moderate	0.9	Unpolluted to moderately	1.9	Minor enrichment
		Non–protected	26.4		2.5	Moderate	0.7	Unpolluted to moderately	2.1	Minor enrichment
	wet	Protected	43.5		4.1	Considerable	1.4	Moderately polluted	2.9	Minor enrichment
		Non–protected	24.4		2.3	Moderate	0.6	Unpolluted to moderately	2.3	Minor enrichment
Mn	dry	Protected	246.5	530.9	0.5	Low	–1.7	Unpolluted	0.3	No enrichment
		Non–protected	421.2		0.8	Low	–0.9	Unpolluted	0.7	No enrichment
	wet	Protected	201.3		0.4	Low	–2.0	Unpolluted	0.3	No enrichment
		Non–protected	240.8		0.5	Low	–1.73	Unpolluted	0.5	No enrichment
Cr	dry	Protected	105.6	34.4	3.1	Considerable	1.0	Moderately polluted	2.2	Minor enrichment
		Non–protected	94.3		2.7	Moderate	0.9	Unpolluted to moderately	2.3	Minor enrichment
	wet	Protected	119.4		3.5	Considerable	1.2	Moderately polluted	2.5	Minor enrichment
		Non–protected	69.0		2.0	Moderate	0.4	Unpolluted to moderately	2.0	Minor enrichment
Cd	dry	Protected	0.1	2.1	0.0	Low	–5.7	Unpolluted	0.0	No enrichment
		Non–protected	0.1		0.0	Low	–6.0	Unpolluted	0.0	No enrichment
	wet	Protected	0.0		0.0	Low	–6.3	Unpolluted	0.0	No enrichment
		Non–protected	0.0		0.0	Low	–6.3	Unpolluted	0.0	No enrichment