ENRICHMENT AND GEO-ACCUMULATION OF HEAVY METALS IN SUPERFICIAL SEDIMENTS, MACROPHYTES AND EDIBLE LEAFY VEGETABLES

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (BIOLOGICAL SCIENCE)

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ENRICHMENT AND GEO-ACCUMULATION OF HEAVY METALS IN SUPERFICIAL SEDIMENTS, MACROPHYTES AND EDIBLE LEAFY VEGETABLES

A thesis submitted to the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, for the degree of Doctor of Philosophy.

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Format 2: As a set of papers which are published, in the press, submitted, or intended for submission.

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

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Supervisor: Date. 16 July 2025

Prof Caswell Munyai

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Supervisor: Date 16 July 2025

Dr Tatenda Dalu

ABSTRACT

Wetlands play a vital role in maintaining ecological balance, supporting biodiversity, and providing essential ecosystem services such as flood control, water purification, and carbon sequestration. However, increasing levels of metal contamination threaten these ecosystems and public health. This study investigated the concentration, accumulation, and translocation of trace and heavy metals in wetland sediments, aquatic macrophytes, and edible leafy vegetables, with a focus on assessing phytoremediation potential.

Field samples of sediments (n = 2) were collected across three seasons (hot-dry, cool-dry, and hot-wet) from urbanised riverside wetlands in Mbombela, South Africa. The metal pollution indices, such as contamination factor, enrichment factor, geo–accumulation factor and pollution load index were used to determine the pollution of metals in the sediments across different sites among seasons. Results showed that wetland sediments were highly contaminated, particularly with Mn, Co, Cu, Pb, Zn, Al, and Fe most significantly during the cool-dry season.

The four aquatic macrophytes (*Typha capensis*, *Cyperus papyrus*, *Cyperus dives*, and *Phragmites australis*) collected from same location as sediments were divided into roots, stems and leaves. The study used the bioconcentration and translocation factors to determine the accumulation and translocation of metals within macrophyte species. The study indicated that different macrophytes accumulated different metals within the roots and leaves of all species during the cool–dry followed by hot–dry and hot–wet seasons. Among the macrophytes studied, *C. dives* and *T. capensis* demonstrated strong phytoremediation potential, indicated by high bioconcentration and translocation factors.

Additionally, edible vegetables (cabbage, rape, pumpkin leaves, and spinach) were obtained from local markets in Tonga town for metal analysis and human health risk assessment. Vegetables exhibited elevated metal concentrations, with several exceeding permissible intake limits, posing potential health risks as indicated by bioconcentration factors and target hazard quotients.

To validate the phytoremediation of aquatic macrophytes ,*Dryopteris pentheri*, Penther’s wood fern was grown in water amended with metals which showed the ability to tolerate and accumulate metals,

confirming its suitability for phytoremediation applications. The study hypothesise that sediments, macrophytes, edible vegetables and ferns will accumulate and translocate the contaminants.

Overall, this study highlights the urgent need for stricter regulation of industrial emissions into wetland systems and the importance of using plant-based remediation strategies to mitigate metal pollution. These findings contribute valuable insights for environmental management and public health protection in peri-urban and rural communities.

In conclusion, to minimise the heavy metal contamination in wetlands. The findings of this study generally reveal that the concentrations of the analysed metals exceeded the permissible limits established by the World Health Organisation and the Food and Agricultural Organisation. Furthermore, since this study showed that the four aquatic macrophyte species can accumulate metals, it is recommended to also evaluate other plant species growing around the wetlands, not just aquatic macrophytes. Policymakers from Government Departments should develop clear frameworks to improve regulations for wetland protection against metal pollution and establish safety standards for agricultural practices and environmental protection, especially to safeguard vulnerable peri-urban and rural communities.

DECLARATION 1 – PLAGIARISM

I, **Elizabeth Kola,** declare that

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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DECLARATION 2 – PUBLICATIONS

This thesis is comprised of a general introduction (Chapter 1), a review chapter (Chapter 2), a series of data chapters (Chapters 3, 4, 5 and 6) and a general synthesis and conclusion (Chapter 7). The data chapters are written as scientific papers with references at the end of each chapter, some of which have been published, with the remaining chapters under review (see below).

Journal articles

- **Kola, E**., Munyai, C. and Dalu, T., 2025. A review of macrophyte phytoremediation in Africa: current research and challenges. ***Chemistry and Ecology***, 41(5):710–728.
- **Kola, E**., Munyai, C., Munyai, L.F., Mpopetsi, P., Dondofema, F., and Dalu, T. 2025. Enrichment and geo–accumulation of trace and heavy metals in superficial sediments along urbanised Austral wetlands. ***Journal of Freshwater and Ecology,*** 40(1):2509572.
- **Kola, E**., Munyai, C., Munyai, L.F., Dondofema, F., Moyo, S., Naicheng, W. and Dalu, T. Metal concentrations in edible leafy vegetables and their potential risk to human health. *Submitted in* ***International Journal of Environmental Research and Public Health***. Accepted.
- **Kola, E**., Munyai, C., Mpopetsi, P., Munyai, L.F., Dondofema, F. and Dalu, T. Metal concentrations, accumulation, and translocation within selected aquatic macrophytes from urban wetlands. ***Water, Air and Soil Pollution***. Minor revision.
- **Kola, E**., Munyai, C., Mpopetsi, P., Munyai, L.F., Dondofema, F. and Dalu, T. Metal tolerance and phytoremediation potential of *Dryopteris pentheri* (Krasser) C. Chr. ***Applied Ecology and Environmental Research,*** 23 (5): 10031-10050.

Conferences

- **Kola, E.**, Munyai, C. and Dalu, T. 2025. Enrichment and geo–accumulation of trace and heavy metals in surface sediments along urbanised Austral wetlands. Mpumalanga Wetlands Forum, SANBI Lowveld National Botanical Garden, Mbombela. 30 May 2025.

- **Kola, E.,** Munyai, C., Munyai, L.F., Mpopetsi, P., Dondofema, F., and Dalu, T. 2025. Seasonal variation, enrichment and geo–accumulation of trace and heavy metals in superficial sediments along urbanised Austral wetlands in Mpumalanga. 73rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), jointly with Italian Society of Phytochemistry (SIF) Naples, Italy. 31 August to 03 September 2025. (Accepted, will be attending).

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1.
GENERAL INTRODUCTION

1.1 Background

Wetlands form about 5-8% of the Earth's surface, which is water table above the land, where some in places are seasonal or permanent (Salimi et al., 2021). Wetlands are among the most important ecosystems on Earth, providing well-balanced ecological maintenance and plant protection (Sinthumule, 2021). Apart from being important, they are neglected and poorly protected. Moreover, once they are destroyed, it is very difficult to resurrect them because they are very sensitive ecosystems (Rojas et al., 2024).

Human activities like agriculture, rapid urban expansion, and urbanisation often lead to ecosystem damage or alteration. These activities release some heavy metals into wetlands after the disposal of fertilisers, domestic sewage and other pollutants (Xin et al., 2020). Due to the fact that heavy metals cannot be broken down by any biological or chemical process, they persist in the environment and pose a significant risk to human health over time by biomagnifyingand accumulating in the human body (Ali et al., 2020a; Heisi et al., 2023). Excess inflow of ammonia, phosphorus, and other nutrients into the wetlands causes unpleasant water colour, tasteand smell (Alavaisha et al., 2019). However, most researchers are losing interest in conventionaltreatment technologies for removing heavy metals from the environment because these methodsare time-consuming, costly, environmentally unfriendly, and often unreliable (Ali et al., 2020a).These heavy metals have the potential to accumulate in different parts of vegetables (Fatai et al.,2024). Moreover, continuous consumption of such vegetables may lead to chronic cardiovascular, kidney, and liver diseases in humans (Ramachandra and Narayan, 2021).

Phytoremediation is considered more effective and less costly as a remedy to remove the heavy toxic metals from the wetlands and environment (Ali et al., 2020b). If these heavy metals accumulate in the sediments, they can be absorbed by plant roots and subsequently transferred to the shoots.

It is also reported that irrespective of low or high concentrations of cobalt (Co), cadmium (Cd), nickel (Ni), lead (Pb) are very toxic (Abdelaal et al., 2021). Due to their capacity to absorb high concentrations of metals from their surroundings (water or sediment) via their below-ground tissues/roots and transfer them to their above-ground tissues (stem or leaf, etc.) without impairing plant growth, aquatic macrophytes and other wetland plants are ideal tools in phytoremediation approaches (Abdelaal et al., 2021). Thus, the current study will focus on phytoremediation using the selected six macrophytes and edible vegetables.

1.2 Rationale for research

Metal ions have been associated with biological systems for many years. Still, their significance gained widespread scientific attention only in the last century (Jomova et al., 2022; Maret, 2021; Wang et al., 2024). This study examines the spatiotemporal dynamics of heavy metals and nutrients in superficial sediments, edible leafy vegetables, and aquatic macrophytes from riverside wetlands located about 4 km north of the Central Business District in Mbombela, South Africa. Pollution and land degradation are increasing worldwide and are having a significant impact on the environment. This study will be the first report to document data on heavy metal spatiotemporal dynamics and bioavailability, especially from Tonga town of Mpumalanga Province in South Africa from leafy edible vegetables, which are irrigated using water from the wetlands. Henceforth, addressing spatiotemporal dynamics in heavy metals and their effects is of global concern (Rao et al., 2021; Taghizadeh-Mehrjardi et al., 2021). For better environmental strategies and management, sufficient data about heavy metals must be documented.

The research will provide knowledge on source-receptor relationships. Information from this study provides insights into the potential health risks of metal pollution to rural communities that depend on Tonga aquatic ecosystems for domestic water use, especially irrigation of vegetables. This study will highlight the possible hazards that might be caused by the consumption of vegetables which polluted by heavy metals and also to alert the communities on how to use wetlands water safely and protection of wetlands. As a result, the center of interest for this research is the spatiotemporal dynamics of heavy metals and nutrients in biotic (macrophytes) and abiotic (sediments). In this situation, absolute data will be provided by the study of spatiotemporal dynamics in heavy metals and nutrients in biotic and abiotic environments. Such data can be used by local, provincial, and national environmental departments in South Africa, as well as other relevant state institutions, to develop mitigation measures aimed at reducing heavy metal concentrations in both aquatic and terrestrial ecosystems.

The selection of wetland plants depends on the locally available plant species or their suitability for the wetland, the frequency and depth of inundation and the ability of the plants to remove nutrients. In general, the primary role of wetland plants is to remove metals and purify water. Common reed (*Phragmites* spp.) and cattail (*Typha* spp.) are typical wetland vegetation species (Ju and Bohrer, 2022). *Phragmites australis* (Cav.) Trin. Ex. Steudel (Ferrario et al., 2022) has been widely used worldwide for phytoremediation purposes and is known to perform well in the treatment of wetlands (Lei et al., 2023; Mohsin et al., 2023; Rezania et al., 2019; Wu et al., 2023).

Although emergent macrophytes are less efficient in lowering nitrogen and phosphorus contents by direct uptake due to their lower growth rates as compared to floating and submerged plants, their ability to uptake nitrogen and phosphorus from sediment sources through rhizomes is higher than from the water (Yi et al., 2021). Similarly, recent studies have shown that the plant species to be used should have a high capacity to absorb nutrients (Barco and Borin, 2020; Mostofa et al., 2022). Barco et al. (2021) reported the use of *Phragmites australis, Lythrum salcicari, Juncus effusus, Carex* spp. and *Lemna* spp. in laboratory and showed high removal of pollutants.

Moreover, *Pistia stratiote* L. has a remarkable capacity to build up biomass, an added interest for producing methane (Sutaryo et al., 2022) and/-or green leaf manure (Alam et al., 2021).Similarly, the dead *P. stratiote* biomass can be used as a biosorbent material for removing heavymetals (Garcia et al., 2021) and also used for water treatment (Samal et al., 2021) or polluted ponds (Lakra et al., 2019) or monitoring water quality in rivers (Thongam and Meitei, 2021). Recent reports have shown that emergent macrophytes such as *P. australis*, free-floating hydrophytes - *Azolla filiculoides* Lam. and *P. stratiotes* were efficient in removing nutrients from domestic wastewater (Akowanou et al., 2023). It can be concluded that *P. australis, A. filiculoides, Lemna minor* L. and *P. stratiotes* have more pollutant removing potential than otherplants such as *Iris pseudacorus* L., which were used in floating treatment constructed wetlands. In addition, these plants are commonly available in most parts of the world with aquatic ecosystems.

1.3 Problem statement

Knowledge of the evolution of metal ions in biological systems is limited. However, it is clear that anthropogenic activities have significantly contributed to the widespread distribution of heavy metals (Jiang et al., 2021; Wu et al., 2021; Xiao et al., 2021), which affect aquatic ecosystems and are toxic to the environment in high concentrations (Kolarova and Napiórkowski, 2021; Singh et al., 2023). This trend of heavy metal distribution challenged organisms to either adapt or die. Heavy metals are toxic to biological systems at high concentrations; therefore, organisms regulate their distribution and concentration to maintain internal balance. Metal pollution affects not only aquatic ecosystems but also the environment in general. Most rural communities depend on these aquatic ecosystems for water and food, and this tends to affect their health because heavy metals are not bio-degradable (Dasharathy et al., 2022).

1.4 Aim of the study

To investigate the accumulation of heavy metals and nutrients in sediment and aquatic macrophytes within the Riverside Wetlands of the City of Mbombela across different seasons, assess their phytoremediation potential, and analyse leafy edible vegetables from Tonga town in Mpumalanga Province, with a focus on implications for human health.

1.5 Research objectives

- To assess the spatio-temporal sediment heavy metal and nutrient dynamics in the subtropical Riverside, Mbombela city wetlands in Mpumalanga Province.
- To determine the heavy metal concentrations and nutrient content in the under-field conditions with implications for human health in the case of edible leafy vegetables from Tonga, Nkomazi Local Municipality of Mpumalanga Province.
- To measure the heavy metals and nutrient content in the shoots, stems and roots of macrophytes under field conditions and assess the phytoremediation potential of selected macrophytes across different seasons.
- To validate phytoremediation potential towards heavy metals and nutrient uptake among the selected dominant macrophytes under experimental conditions.

1.6 Research hypotheses

- There will be spatio-temporal sediment heavy metal and nutrient dynamics in the subtropical Riverside, Mbombela city wetlands in Mpumalanga Province.
- The heavy metal concentrations and nutrient content in the under-field conditionswill have implications for human health in the case of edible leafy vegetables from Tonga, Nkomazi Local Municipality of Mpumalanga Province.

- The heavy metals and nutrient content will differ in the shoots, stems and roots of macrophytes under field conditions and assess the phytoremediation potential of selected macrophytes across different seasons.
- The selected macrophyte will confirm the phytoremediation potential and nutrient uptake among under experimental conditions.

1.7 Outline of dissertation/thesis structure

Chapter 1: This chapter covers the general introduction and background on the impacts of heavy metals on wetlands. The focus is on wetlands and the role of macrophytes and edible vegetables in phytoremediation. The rationale, aim and objectives of the study are also included in this chapter.

Chapter 2: This chapter is a systematic review based on current research and challenges of phytoremediation in Africa.

Chapter 3: This chapter deals with assessing spatiotemporal sediment, heavy metal and nutrient dynamics in Riverside wetlands in Mpumalanga Province.

Chapter 4: This chapter investigates the concentrations of heavy metals and nutrient content in macrophytes under field conditions, highlighting the implications for human health, particularly regarding edible leafy vegetables.

Chapter 5: This chapter represents data on the accumulation of heavy metals and nutrients in the leaves, stems and roots of macrophytes collected under field conditions.

Chapter 6: This chapter validates the phytoremediation potential of macrophytes in chapter 5 by using *Dryopteris pentheri* under experimental conditions.

Chapter 7: General conclusion and synthesis recommendation on the interconnectivity of all the results and findings are interpreted and discussed, and suggestions for further research are provided.

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CHAPTER 2.
A REVIEW OF PHYTOREMEDIATION IN AFRICA: CURRENT RESEARCH AND CHALLENGES

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2.1 Abstract
Aquatic ecosystems face a growing threat from pollution by heavy metals, nutrients, and organic contaminants from anthropogenic stressors such as rapid urban, industrial and mining development and agriculture. Therefore, conventional remediation methods can be expensive and disruptive once a system has been impacted. Phytoremediation, the use of macrophytes (i.e., large aquatic plants) to remove pollutants, offers a promising alternative and this review aims to explore the potential and extent of use of macrophytes for phytoremediation in aquatic environments within Africa, highlighting the challenges, knowledge gaps and future research directions to optimise this technology in the region. We further explore factors influencing phytoremediation efficiency, such as macrophyte species, heavy metal accumulation, and environmental conditions within the continent. This review identified a total of 11 African studies focusing on phytoremediation in aquatic environments using macrophytes within freshwater systems, with *Typha, Cyperus* and *Phragmites* being the most used species. Metals accumulated mostly in the roots and followed by the leaves. The most quantified metals were Cu, Cd and Cr. Most studies were conducted in South Africa (n = 7), Egypt (n = 5) and Ethiopia (n = 4) while only Botswana, Uganda, Zambia, Rwanda, Cameroon and Zimbabwe had just one study conducted for each country. Therefore, more studies using various macrophytes, funding for some projects and policies for phytoremediation are recommended in Africa and also to focus on other different macrophyte species to assess the phytoremediation potential.

Keywords: *Macrophytes, phytoremediation, aquatic ecosystems, pollution, heavy metals, nutrients, bioaccumulation, biomagnification*

2.2 Introduction

Conventional water treatment methods, often reliant on expensive infrastructure, may be impractical or unavailable in many rural areas of Sub-Saharan Africa (Schnabel et al., 2022; Wang et al., 2014) Hence, phytoremediation emerges as a cost-effective and environmentally friendly alternative (Odoh et al., 2019; Vymazal, 2011). Conventional remediation methods, such as chemical precipitation and dredging, can be expensive, energy-intensive, and disruptive to the environment (Singh et al., 2023; Vymazal, 2011). Phytoremediation, a technology harnessing the natural ability of plants to remove pollutants from water and soil, presents a promising alternative (Salt et al., 1998; Vymazal, 2011). Phytoremediation is a bioremediation technique that utilises plants to remove, degrade, or immobilise contaminants from soil, water, and air (Kristanti and Hadibarata, 2023).

Macrophytes, with their extensive root systems and high surface area, are particularly well-suited for aquatic phytoremediation (Das et al., 2021). Macrophytes, large aquatic plants that are either submerged, emergent, and/or floating, are particularly well-suited for phytoremediation in aquatic ecosystems (Madzivanzira et al., 2023). They play a crucial role in maintaining healthy aquatic ecosystems by providing habitat for aquatic organisms, stabilising shorelines, and filtering pollutants (Madzivanzira et al., 2023; Vymazal, 2011). Macrophytes remove contaminants from water through various mechanisms such as (i) phytoremediation where they directly absorb pollutants through their roots and store them in their tissues (i.e., stems and leaves) (Salt et al., 1998; Vymazal, 2011), (ii) rhizofiltration where the roots exudates released by macrophytes such as *Arundo donax* L. which immobilise metals by forming complexes and/or enhancing the growth of beneficial bacteria with metal-binding capabilities (Mench et al., 2010; Oustriere et al., 2017), (iii) phytodegradation, where certain macrophyte taxa such as *Scirpus grossus* L. and *Phragmites australis* Trin. Ex. Steudel harbour microbes in their root zone (i.e., rhizosphere) that degrade organic pollutants(Al-Baldawi et al., 2015; Newman and Reynolds, 2004), and (v) sedimentation where the macrophytes create a physical barrier, reducing water flow velocity and promoting the settling of suspended particles containing pollutants (Barko and James, 1998; Schulz et al., 2003). All these processes are supported or enhanced by several characteristics that make them effective in pollutant removal such as high biomass production (Craft, 2013; Saralegui et al., 2021), extensive root systems (Ali et al., 2019; Brix, 2020) and tolerance to pollutants (Kochi et al., 2020; Mesquita et al., 2022).

Water is a finite resource vital for human health, food security, and ecosystem function

(Valhondo and Carrera, 2019). Therefore, freshwater aquatic ecosystems such as lakes, reservoirs, rivers, and wetlands, are vital for maintaining global biodiversity and providing essential services such as water purification, flood control, and recreation (Barbier, 2017; Flotemersch and Aho, 2021). However, these ecosystems are increasingly threatened by pollution from various sources which are degrading water quality and posing a significant threat to human health, aquatic biodiversity, and ecosystem (Reid et al., 2019; Bashir et al., 2020; Dalu and Tavengwa, 2022; Reid et al., 2019). Therefore, rapid population growth, urbanisation, and agricultural intensification in Sub-Saharan Africa is exerting immense pressure on freshwater aquatic resources (Fouchy et al., 2019). These pollutants can disrupt ecological processes, harm aquatic biota, and render water unsuitable for drinking, irrigation, or other uses (Dalu and Tavengwa, 2022; Vymazal, 2011) Thus, restoring polluted aquatic ecosystems is crucial for ensuring clean water supplies and healthy ecosystems (Prajapati et al., 2017).

The deterioration of aquatic ecosystems has severe consequences, as it diminishes access to clean water for drinking, sanitation, and irrigation (Dalu and Tavengwa, 2022). It also disrupts aquatic biodiversity, impacting fisheries and tourism, crucial economic sectors in many Sub-Saharan African nations (Jackson et al., 2016). We discuss the mechanisms by which macrophytes accumulate pollutants, the factors influencing phytoremediation efficiency, and the advantages and challenges associated with this green remediation approach within African continent. The current study review studies in Africa on phytoremediation potential by different types of macrophyte, examining different plant parts accumulating heavy metals and the concentrations of accumulated metals using aquatic ecosystems within Sub-Saharan Africa, which utilises aquatic plants (i.e., macrophytes) to clean polluted water bodies.

2.3 Materials and methods

This systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) tool (Moher et al., 2009) (Figure 2.1). Six scholarly search engines and databases were used and these include Scopus, Google Scholar, Web of Science, JSTOR, Science Direct and Sabinet. We therefore searched for a wide variety of literature related to phytoremediation, heavy metals and macrophytes in Africa mostly targeting peer-reviewed publications and avoiding grey literature, with the following key words: "phytoremediation", "heavy metals", "macrophytes", "bioaccumulation", "rivers", "lakes", "wetlands", "metal uptake” and “aquatic ecosystems” being used including their combinations was used during the search to increase article hits. About 26 studies conducted in African studying macrophytes, heavy metals and/or phytoremediation were used in the current review. Furthermore, articles published in English, from Africa and those that reported phytoremediation of heavy metals by macrophytes were included in this review, whereas those which were not relevant to African countries (Type 1), articles which were not including the aquatic macrophytes (Type 2) and those without phytoremediation studies (Type 3) were excluded.

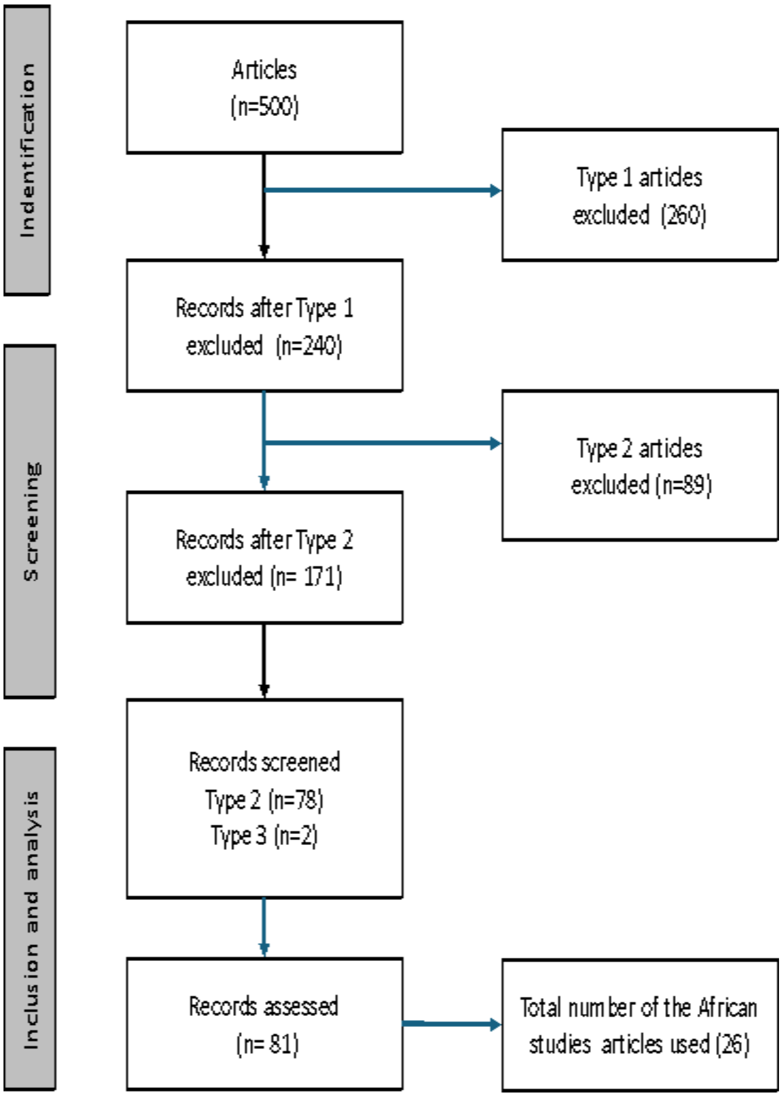

Figure 2.1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram for the exclusion and inclusion of articles in the current review.

2.4 Results and discussion

2.4.1 Phytoremediation studies in African countries

Most studies were conducted in South Africa (*n* = 7), Egypt (*n* = 5) and Ethiopia (*n* = 4) while only Botswana, Uganda, Zambia, Rwanda, Cameroon and Zimbabwe had just one study conducted for each country (Table 2.1; Figure 2.2). These African countries indicated that there is metal pollution and have shown that most of aquatic macrophytes sowed to be good phytoremediation candidates. These plant species have shown that roots are the main accumulator of heavy metals.

2.4.2 Phytoremediation in Botswana

Rhizofiltration was adopted in Botswana as a mechanism to remediate the contaminants from the mining activities. Rhizofiltration is defined as the process by which contaminants in a solution next to the root zone are assimilated, concentrated, and precipitated onto or into plant roots by biotic or abiotic means (Awa and Hadibarata, 2020; El-Liethy et al., 2022; Khan et al., 2023). *Baumea rubiginosa* (Spreng.) Boeckeler*, Cyperus papyrus* L., *Phragmites australi*s and *Schoenoplectus tabernaemontani* were shown to have a good potential for rhizofiltration mechanism in the country (Ishmael et al., 2024). These macrophytes accumulated above-average concentrations of Cr, Cu, and Ni in their roots and shoots. Roots accumulated high metal concentrations compared to the shoots, with the exception of *B. rubiginosa* which accumulated high metal concentrations in the shoots. Ishmael *et al.* (Ishmael et al., 2024) further found that these macrophytes accumulate different heavy metal, for example *B. rubiginous* and *P. australis* accumulated Cu only in the roots and shoots, whereas *C. papyrus* accumulated Cr, Cu and Ni in the shoots and *S. tabernaemontani* had Ni only in the shoots. Thus, a study by Ishmael *et al.* (Ishmael et al., 2024) highlights the importance of selecting the best macrophytes for phytoremediation.

2.4.3 Phytoremediation in Uganda

In Uganda, the phytoremediation study indicated that most metals accumulated in the leaves and roots of macrophytes, and their accumulation varied among species (Nabulo et al., 2008). It was observed that Cu concentrations ranged from 6.5 to 1.89 mg kg^{-1} in *Dryopteris* sp. and *Commelina benghalensi*s L. with *Pontederia crassipes* (Mart) Solms having high Zn concentration. Uganda wetlands are being contaminated by heavy metals from nearby industries, such as smelter and battery assembling facilities, which serve as the source of metal pollutants. Hence, it is recommended that there must be intervention of policies waste management strategies (Nabulo et al., 2008).

2.4.4 Phytoremediation in Ghana

Gbogbo and Otoo (2015) found Cd, arsenic (As), mercury (Hg), Cu and lead (Pb) in water, sediments, green algae, aquatic macrophytes (i.e., *Typha domingensis* Pers. and *Pistia stratiotes*), arthropods and fish, with only As and Cd being present in aquatic macrophytes. The Cd levels in Ghana were reported to be high, exceeding the recommended levels by United States of America Environmental Protection Agency guidelines, which made water to be unsafe for consumption (Gbogbo and Otoo, 2015). Ghana has many gold mining activities that use cadmium, which could suggest that heavy metal Cd is leaching into the aquatic and terrestrial ecosystems. *T. latifolia* accumulated similar heavy metals (i.e., Fe and Pb) in the shoots and roots. However, high Fe concentration was found in the roots (Anning and Akoto, 2018). Anning and Akoto (2018) suggested that *T. latifolia* be regarded as a helpful filter species for filtering water irrigation.

2.4.5 Phytoremediation in Ethiopia

Tadese and Seyoum (2015) study in Ethiopia, *Cyperus alternifolius* and *Typha domingensis* reduced Cr concentrations by 99.3 % in wastewater from leather processing industries in constructed wetlands. Roots accumulated high Cr concentrations in *T. domingensis*. Tadese and Seyoum (2015) suggested that constructed wetlands be used but highlighted their high costs. Furthermore, in other studies in Ethiopia conducted from natural wetlands using macrophytes, zinc (Zn) was accumulated in high leaf concentrations (Table 2.1). However, *C.alopercuroides* accumulated high manganese (Mn) concentrations, followed by Zn. *Schoenoplectus corymbosus* L. and *Equisetum ramosissimum* L. accumulated high Zn concentrations in their shoots and roots, whereas *Cyperus distans* L. accumulated high Zn concentrations in their roots. Fitamo and Leta (2010) highlighted that all the macrophytes analysed had good potential for use in phytoremediation studies as they had shown high tolerance to heavy metals from the mine soils.

2.4.6 Phytoremediation in Rwanda

Cyperus papyrus accumulated heavy metals in the roots, whereby lead (Pb) (56.1 mg kg^{-1}) was the highest among others (e.g., Cd (4.2 mg kg^{-1}), Cr (45.8 mg kg^{-1}) and Cu (29.7 mg kg^{-1}) (Table 2.1) (Sekomo et al., 2011). These results have alarmed that there is a serious health concern for people using products from the Nyabugogo Wetland in Rwanda. Thus, more strategies are needed to be implemented to remediate or restore these systems (Sekomo et al., 2011).

2.4.7 Phytoremediation in Nigeria

Pontederia crassipes was observed to accumulate Mn in both its roots and shoots in high concentrations and had a translocation factor ability from roots to shoots. High Mn concentrations followed by Zn were found to have accumulated in *Pontederia crassipes* shoots. Macrophyte species with high biomass and broad leaves, such as *P. crassipes*, considered weeds in aquatic ecosystems, stand a good chance of having high phytoremediation potential (Agunbiade et al., 2009).

Ipomoea aquatica shoots had high Pb concentrations (1.12 mg kg^{-1}), whereas *Ludwigia adscendens* had low Pb concentrations (1.12 mg kg^{-1}) in their shoots. The roots of P. *crassipes* had high Pb concentrations (5.69 mg kg^{-1}). High Cr concentrations were high in roots (5.41 mg kg^{-1}) in *Commelina benghalensis,* whereas Cr concentration was low in shoots (0.04 mg kg^{-1}) of *Althernathera philoxerrides*. Low Ni concentration (0.01 mg kg^{-1}) was observed in *L. adscendens* roots. High Cu concentrations (4.21 mg kg^{-1}) were found in *P. crassipes* shoots, whereas *I. aquatica* had a low Cu concentration (0.23 mg kg^{-1}) in its shoot. *Paspalum vaginatum* roots had a high Cu concentration (12.32 mg kg^{-1}), whereas a low Cu concentration (0.69 mg kg^{-1}) was observed in *Sagittaria sagittifolia* roots. Macrophyte species having similar ecological exposures tend to respond differently in their accumulation of heavy metals (Adesuyi et al., 2018).

2.4.8 Phytoremediation in Cameroon

Different macrophyte species accumulated different heavy metals at varying concentrations in Cameroon due to differences in macrophyte morphologies (Fonkou et al., 2005). For example, marsh and shallow aquatic macrophyte species such as *Cyperus papyrus* and *Echinochloa* *pyramidalis* with thick rhizomes and strong roots have long-term absorption and accumulation of heavy metal capabilities. High concentrations of Cd (600 mg kg^{-1}) and Zn (2 620 mg kg^{-1}) were found in *E. pyramidalis*, whereas Cu (10 700 mg kg^{-1}) and Pb (10 150 mg kg^{-1}) had high concentrations in *Enydra fluctuans* and *C. papyrus*, respectively. The Cd and Cu concentrations in *E. pyramidalis* and *I. aquatica* significantly different from *C. papyrus* and *E. fluctuans* concentrations (Fonkou et al., 2005).

2.4.9 Phytoremediation in Egypt

Egypt aquatic environments are mainly polluted by industrial activities such as mining, manufacturing, improper waste disposal, application of pesticides and fertilisers (Taha et al., 2004). The study revealed the accumulation of heavy metals (i.e., Cd, Co, Cu, Ni, Pb and Zn) in the different tissues of aquatic macrophytes, and some of the macrophytes with phytoremediation potential were *P. crassipes, Ceratophyllum demersum, P. australis, Myriophyllum spicatum, T. domingensis* and *Lemna gibba* (Kamel, 2013).

Seasonal changes were found to be important in heavy metal accumulation, particularly for Cr, Cu and Pb by roots, whereas Zn accumulated in the shoots of *Vossia cuspidata* (Galal et al., 2017). During the spring season, *V. cupsidata* had a high accumulation of heavy metals compared to other seasons (Galal et al., 2017). In the Kitchener drain, the system was highly contaminated by Cd, Ni and Pb, which were accumulated in the stems and leaves of *P. australis*, *Ludwiga stolonifera*, *Echinchloa stagnina* and *P. crassipes* (Eid et al., 2020).Abdelaal et al. (2021) reported that *Ranunculus scleleratus* accumulated Fe and Cd in the leaves, whereas *P. crassipes* accumulated Cd, Co, Fe and Ni in the roots, whereas *P. australis* accumulated Fe and Cd, in the roots and *Cyperus alopecuroides* accumulated only Cu in the roots. All *Ceratophyllum* and *Limna gibba* plant parts accumulated Pb and Cu, whereas*Potamogeton pectinatus* accumulated on Pb in all plant parts (Younis and Nafea, 2015). If theenvironment has different types of macrophytes, it can receive a better phytoremediation thanhaving a single species (Abdelaal et al., 2021). Furthermore, Abdelaal et al. (2021) recommended that government and other stakeholders take care of the environment, and further experimental studies should be conducted to understand phytoremediation processes within the African context better.

2.4.10 Phytoremediation in South Africa

South Africa is big producer of Cr, coal, diamonds (C), gold (Au), Cu, Pb, iron (Fe) ore, emerald, fluorspar, ilmenite, manganese (Mn), Ni, vanadium (V), Zn, tin (Sn), silica, uranium (U), zirconium (Zr), and phosphate ($PO^{3-}4$) (Heisi et al., 2023). Blesbokspruit wetland showed that

P. crassipes had accumulated heavy metals in the following order, Cr > Pb > Ni > As in the roots. Similarly, the roots of *P. australis* and *T. capensis* also accumulated heavy metal (i.e., As) (Heisi et al., 2023). From these results, Heisi et al. (2023) recommended *Pontederia crassipes* to be a good candidate for phytoremediation. *Typha capensis* and *Arundo donax* have both accumulated Zn, Mn, Ni ad Fe in their tissues, where Fe being the highest in both plants from Elsburgspruit River tributaries (van der Merwe et al., 1990). It is recommendedto plant or conserve *A. donax* in Elsburgspruit and prevent burning of this plant, because if it gets burned its ash with heavy metals return to the aquatic ecosystem (van der Merwe et al., 1990).

Bolboschoenus maritimus from Diep River had accumulated higher concentrations of Zn, Fe and Cu in the roots as compared to the leaves and stems. The concentration recorded in the roots were more than 1 200 mg kg^{-1} from different sites (Shuping et al., 2011). Shuping et al. (2011) further stated that there is a need for further investigation into if *Bolboschoenus maritimus* can be used as a biomarker and toxicological endpoint.

Phragmites australis and *S. corymbosus* have accumulated different metals (i.e. B, Na, Ca and Mg) in the roots, stem and leaves, whereas Na, Mg and Ca were accumulated in the leaves of *T. capensis.* These results suggest that combination of macrophytes need to be tested for phytoremediation for proper aquatic ecosystem treatment (Munyai and Dalu, 2023). Similarly, macrophyte (*P. australis*) accumulated Mn, Fe, Cu, Zn and B in the roots, and only K was found in the stem, whereas leaves accumulated N, P, Ca and Mg (Netshiongolwe et al., 2020). Moreover, *P. australis* has accumulated Cr, Pb, Zn and Pb in all plant parts, where Shibambu et al. (2017) reported that the accumulation of these metals in the wetland is affected by water flow. The whole plant of *P. crassipes, T. capensis, Cyperus sexangularis* and *P. australis* were reported to have accumulated heavy metals (i.e., As, Cd, Cr, Cu, Fe, Hg, Pb and Zn), whereas, *Stuckenia pectinata* has accumulated As, Cd, Cr and Cu in all the parts (stem, leaves and roots). It is suggested that these macrophytes stand to be good candidates for phytoremediation (Tshithukhe et al., 2021).

2.4.11 Phytoremediation in Zambia

Phragmites australis and *Typha* spp. have accumulated Cu, Co and Pb from the wetlands in Zambia and they are highly recommended to be used in phytostabilisation of the pollutants in the wetlands, as they are best solution for the old mining activities and improve the water flow and decreasing floods in the Zambian wetlands (Nabuyanda et al., 2022).

2.4.12 Phytoremediation in Zimbabwe

The plant species found was *Typha domingensis* for the study of heavy metals (i.e., Fe, Cu, Cr, Pb, Cd, Mn and Zn). The findings were in the following order root > stem > leaves for most metals with the exception of Pb, Cd and Mn, where high metal accumulation was observed in the roots followed by leaves and stems (Dube et al., 2019). This study recommended further studies focusing on the bioavailability of metals by other aquatic macrophytes. The poor state of aquatic ecosystems, particularly wetland and river ecosystems, is one of the most pressing world challenges currently, and most of these systems are above the recommended levels of pollutants, which results in toxic effects on living organisms within the aquatic ecosystems (Dalu and Tavengwa, 2022). Aquatic ecosystems can be beneficial to various human and biotic communities, however they have been destroyed or altered by anthropogenic activities (Fluet-Chouinard et al., 2023; Wasserman and Dalu, 2022). Anthropogenic activities such as habitat degradation, agriculture, pollution and invasive species affect the state of wetland in urban areas which results in the addition of heavy metals (Adeeyo et al., 2022). Heavy metals are a major cause of pollution and have been reported to be removed by aquatic macrophytes through the process of phytoremediation (Milke et al., 2020). The current review highlights some of the most commonly used macrophytes (e.g., *Typha, Phragmites*, *Cyperus*) for phytoremediation purposes within aquatic ecosystems (Schück and Greger, 2020; Wani et al., 2020).

The findings of the current study have indicated that the accumulation of heavy metals differently by macrophytes is due to the morphology of macrophyte. Cardwell et al. (2002) reported that accumulation of metals by aquatic macrophytes depends on the plant morphology, whereby *Phragmites*, *Typha* and Cyperus are recommended macrophyte for phytoremediation due to their rapid growth and high biomass (Milke et al., 2020). Additionally, several studies (El-Amier et al., 2020; Munyai and Dalu, 2023; Nguyen et al., 2021) reported that *Typha* and *Phragmites* were amongst the most popular aquatic macrophytes used for phytoremediation. Furthermore, studies have highlighted that emergent aquatic macrophytes usually accumulate lower metal concentrations than submerged aquatic macrophytes (Cardwell et al., 2002).

Metals such as Cd, Cr, Cu and Ni were the most frequently studied metals in African countries due to their potential toxic effects (Table 2.1). The current review further showed that different macrophytes accumulate different heavy metal concentrations due to various factors, such as, plant species, plant parts and location within the aquatic ecosystem (El-Amier et al., 2020). *Phragmites australis* has been highlighted to be good potential phytoremediation taxa for heavy metals such as Cd, Cr, and Cu due to its wide tolerance and adaptations to a wide variety of hazardous environments (Nabi, 2021).

Roots and leaves were recorded as the main macrophyte parts that accumulate heavy metals compared to other parts such as shoots and stems (Table 2.1). Similarly, Azizi et al. (2020) and Ebrahimi et al. (2014) observed that different macrophytes accumulate heavy metals in different macrophyte parts, with roots being the part where metals accumulate as it is the point of absorption. Furthermore, roots accumulate higher quantity of heavy metals due to the thick parenchyma cells comprising wide intercellular spaces (El-Amier et al., 2020). This suggests that the translocation of metals to other macrophyte parts might be limited by factors such as macrophyte species, properties and specific metals (Nguyen et al., 2021) and growing season, were mature macrophytes are expected to accumulate metals within their leaves (Kassaye et al., 2016).

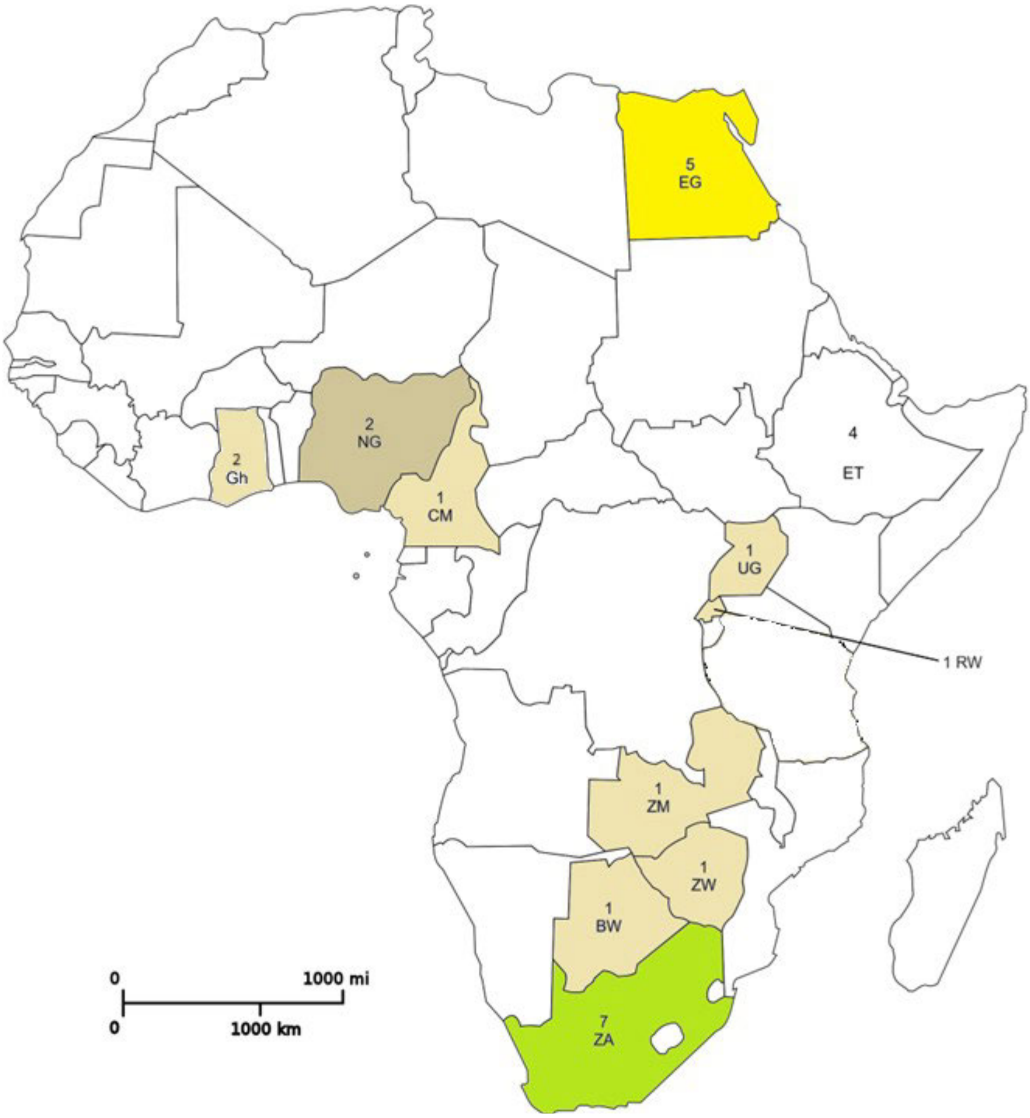

Figure 2.2 Heavy metal accumulation by macrophyte studies (i.e., in terms of numbers) in Africa. Abbreviations: BW (Botswana), CM (Cameroon), EG (Egypt), ET (Ethiopia), Gh (Ghana), NG (Nigeria), RW (Rwanda), UG (Uganda), ZA (South Africa), ZM (Zambia), ZW (Zimbabwe).

Table 2.1. Overview of macrophytes with potential for phytoremediation of heavy metals in Africa. Abbreviation: (-) not mentioned

Country	Type	Plant part	Metals assessed (concentration mg kg^{-1})	Plant species	References
Botswana	Natural wetland	Shoots	Cr (6080.86) Cu (1231.12) Ni (1163.42)	*Cyperus papyrus*	Saralegui et al. (2012)
		Roots	Cr (5047.15) Cu (2166.43) Ni (949.97)		
		Roots	Ni (1015.88)	*Schoenoplectus tabernaemontan*	
		Shoots	Cu (927.36)	*Baume rubiginosa*	
		Roots	Cu (401.70)		
		Roots	Cu (7416.42)	*Phragmites australis*	
		Shoots	Cu (221.71)		
Uganda	Natural wetland	Leaves	Cu (9.7±1.2) Ni (6.1 ± 1.1) Zn (41.9± 3.5)	*Pennisetum clandestinum*	Craft (2013)
		Roots	Cu (23.1±5.7) Ni (17.5 ± 8.1) Zn (266.3 ± 109.3)		
		Leaves	Cu (11.3±3) Zn (31.2 ± 0.0) Ni (9.9±0.0)	*Colocasia esculenta*	
		Roots	Cu (7.7±0.0) Ni (6.4 ±0.0) Zn (47.1± 0.0)		
		Leaves	Cu (18.9±3.6) Ni (16.3±5.2)	*Commelina benghalensis*	
		Roots	Zn (114.9 ± 23.7) Ni (195.3 ± 61.4)		
		Leaves Roots	Cu (13.5 ± 4.7) Ni (5.1 ± 0.9) Zn (53.5 ± 11.7) Cu (13.9 ± 1.9) Ni (14.8 ± 3.7) Zn (90.2 ± 11.2)	*Cyperus papyrus*	
		Leaves Roots	Cu (12.2 ± 2.3) Ni (23.6 ± 9.3) Zn (26.0 ± 58.9) Cu (22.3 ± 51.7) Ni (25.1 ± 11.8) Zn (239.1 ± 46.4)	*Pontederia crassipes*	

		Leaves Roots	Cu (6.5 ± 0.) Ni (6.5 ± 0.9) Zn (32.1 ± 3.6) Cu (8.1 ± 1.8) Ni (10.5 ± 4.7) Zn (6.5 ± 5.7)	*Dryopteris* sp.	
Ghana	Natural wetland	Shoots	Cd (0.69 ± 0.12)	*Typha domingensis*	Ali et al. (2019)
			As (0.12 ± 0.2)	*Pistia stratiotes*	
	Constructe d wetland	Roots Shoots	Fe (456 – 1549) Pb (1.2 – 7.6) Fe (2.0 – 183) Pb (1.55 – 3.95)	*Typha latifolia*	Heisi et al. (2023)
Ethiopia	Natural wetland	Shoots	Cu (12.60) Ni (16.14) Co (13.69) Zn (95.11) Pb (16.31 Cd (2.73) Cr (44.52)	*Cyperus distans*	Mesquita et al.(2022)
		Roots + roots	Cu (26.30) Ni (22.65) Co (21.69) Zn (54.41) Pb (23.54 Cd (3.73) Cr (58.68)	*Equisetum ramosissimum*	
		Shoots + roots	Cu (14.73) Ni (16.83) Co (11.81) Zn (114.07) Pb (28.13) Cd (2.95) Cr (4.7)	*Schoenoplectus corymbosus*	
Ethiopia	Lake	Leaves	Cr (1.7) Cu (1.9) Zn (19) As (0.3) Cd (0.01) Pb (0.15)	*Nymphaea nouchali*	Tshithuke et al. (2021)
		Leaves	Cr (3.3) Cu (8.4) Zn (16) As (0.34) Cd 0.13) Pb (0.28)	*Potamogeton schweinfurthii*	
		Leaves	Cr (5.2) Cu (2.5) Zn (32) As (0.94) Cd (0.04) Pb (0.53)	*Potamogeton thubergii*	
		Leaves	Cr (1.9) Cu (1.3) Zn (12) As (0.13)	*Setaria geminata*	

			Cd (0.01) Pb (0.2)		
		Leaves	Cr (-) Cu (-) Zn (-) As (-) Cd (-) Pb (-)	*Schoenoplectiella corymbosa*	
		Roots	Cu (8.2) Cr (2.5) Co (0.13) Ni (1.5) Zn (48) As (0.11) Se (0.28) Cd (0.02) Pb (0.19) Mn (204)	*Cyperus alopecuroides*	
Ethiopia	Natural wetland	Leaves	Cr (0.080.90), Pb (0.037.30) Cd (0.025.98)	*Arundo donax*	Munyai and Dalu (2023)
Ethiopia	Constructe d wetland	Root and shoots	Cr (-)	*Cyperus alternifolius* *Typha domingensis*	Kochi et al. (2020)
Rwanda		Roots	Cd (4.2) Cr (45.8) Cu (29.7) Pb (56.1)	*Cyperus papyrus*	Jackson et al. (2016)
Nigeria	Natural wetland	Shoots	Cd (0.050 ± 0.006) Cu (56.58 ± 0.098) Cr (10.12 ± 0.39) Fe (636.17 ± 29.10) Mn (681.88 ±34.05) Ni (1.041± 0.001) Pb (0.065 ± 0.006) V (3.35± 0.06) Zn (223.09± 15.62) As (0.038 ± 0.002)	*Pontederia crassipes*	Moher et al. (2009)
Nigeria	Natural wetland	Shoots	Cr (2.23)	*Commelina benghalensis*	Ishmaelm et al. (2024)
		Roots	Cr (5.41)		
		Shoots	Pb (1.12)	*Ipomoea aquatica* *Ludwigia adscendens*	
		Roots	Pb (5.69)	*Pontederia crassipe*s	
		Roots	Cr (0.18)	*Althernathera philoxerrides*	
		Shoots	Cr (0.04)		
		Roots	Cu (12.32)	*Paspalum vaginatum*	
		Roots	Cu (0.69)	*Sagittaria sagittifolia*	
Cameroon	Natural wetland	Whole plant	Cd (600 ± 22 Cu (1125 ± 100) Zn (2620 ± 138)	*Echinochloa pyramidalis*	Nabulo et al. (2008)

			Pb (8840 ± 203)		
			Cd (590 ± 56) Cu (1125 ± 100) Zn (2200 ± 98) Pb (6800 ± 203) Cd (438 ± 12) Cu (9250 ± 361) Zn (2000 ± 231) Pb (10.150± 780) Cd (465 ± 30) Cu (10.700 ± 90) Zn (2000 ± 231) Pb (6580 ± 418)	*Impomoea aquatica* *Cyperus papyrus* *Enydra fluctuans*	
Egypt	Natural wetland	Stem	Cd (1.79) Co (17.35) Cu (74.97) Ni (30.63) Pb (163) Zn (903) Cd (1.8) Co (32.5) Cu (53.8) Ni (125.0) Pb (37.26) Zn (1677) Cd (2.35) Co (23.5) Cu (96.3) Ni (48.09) Pb (208.71) Zn (1172) Cd (1.34) Co (9.0) Cu (36.4) Ni (25.23) Pb (104.0) Zn (531.5) Cd (3.21) Co (43.10) Cu (129.21) Ni (151.6) Pb (42.4) Zn (843.2) Cd (1.38) Co (42.0) Cu (153.2) Ni (91.55) Pb (23.87) Zn (862.4)	*Myriophyllum spicatum* *Pontederia crassipes* *Ceratophyllum demersum* *Lemna gibba* *Phragmites australis* *Typha domingensis*	Van der Merwe et al. (1990)
Egypt	Natural wetland	Roots	Cr (-) Cu (-)	*Vossia cuspidata*	Fitamo and Leta (2010)

		Shoots	Pb (-) Cr (-) Zn (-)		
Egypt	Natural wetland	Stems and leaves	Pb (-) Cd (-) Ni (-)	*Ludwigia stolonifera* *Phragmites australis* *Echinochloa stagnina* *Pontederia crassipes*	Kasaye et al. (2016)
Egypt	Natural wetland	Leaves Roots Roots Roots	Fe (71.96) Cd (4.66) Fe (2034.83) Co (57.69) Cd (137.30) Ni (149.40) Fe (-) Cd (-) Cu (6.34)	*Ranunculus sceleratus* *Pontederia crassipes* *Phragmites australis* *Cyperus alopecuroides*	Mekonnen and Fantu (2016)
Egypt	Natural wetland	Whole plant	Cu (07, 01) Pb (0.09)	*Ceratophyllum demersum*	Tadesa and Seyoum (2015)
			Pb (0.15)	*Potamogeton pectinatus*	
			Cu (0.19) Pb (0.15)	*Lemna gibba*	
			Cd (0,11)	*Pistia stratiotes*	
South Africa	Natural wetland	Roots	As (17.23) Pb (116.6) Cr (342.9)	*Pontederia crassipes*	Sekomo et al. (2011)
			As (1.5)	*Typha capensis*	
			As (9.9)	*Phragmites auastralis*	
South Africa	River	whole plant	As (-) Pb (-) Cu (-) Fe (-) Hg (-) Zn (-) Cr (-) Cd (-) Cu (-) Cr (-) Cd (-) As (-)	*Pontederia crassipes* *Typha capensis* *Cyperus sexangularis* *Phragmites auastralis* *Stuckkenia pectinata*	Eid et al. (2020)
South Africa	River	Roots, stems and leaves	B (-) Na (-) Mg (-) Ca (-) N (-) Na (-) Ca (-)	*Phragmites australis*, *Schoenoplectus corymbosus* *Typha capensis*	Fonkou et al. (2005)

		Roots, stems and leaves	Mg (-)		
South Africa	River	Whole plant	Zn (301.22) Mn (1241.55) Ni (23.67) Fe (2025.75) Zn (175.2) Mn (1153.2) Ni (151.21) Fe (1690.12)	*Typha capensis* *Arundo donax*	Angunbiade et al. (2009)
South Africa	River	Roots, stems and leaves	Cu (-) Zn (-) Al (-) Fe (-)	*Bolboschoenus maritimus*	Adesuyi et al. (2018)
South Africa	River	Roots	Fe (-) Mn (-) Cu (-) Zn (-) B (-)	*Phragmites australis*	Kamel (2013)
		Stem Leaves	K (-) N (-) P (-) Ca (-) Mg (-) Mg (-)		
South Africa	Natural wetland	Whole plant	Cr (-) Pb (-) Zn (-) Cd (-)	*Phragmites australis*	Galal et al., (2017)
Zambia	Wetland	Whole plant	Co (105 ± 130) Cu (27 ± 33) Pb (111 ± 87-) Co (19 ± 3) Cu (39 ± 39) Pb (122 ± 150)	*Phragmites mauritianus* *Typha* spp.	(Abdedal et al. (2021)
Zimbabwe	Natural wetland	Roots	Cu (-) Pb (-) Zn (-)	*Typha domingensis*	Younis and Nafea (2015)

2.5 Conclusions

Africa is now home to numerous industries and mining activities, resulting in increased discharges of partially treated waste containing heavy metals being deposited into the aquatic environment. The conventional methods or remediation seem not to be very effective. Hence, several African countries are now exploring or starting to use phytoremediation to filter waste laden with contaminants and pollutants from aquatic ecosystems (Odoh et al., 2019). Phytoremediation has been noted as an environmentally friendly alternative and very cheap (Ighovie and Ikechukwu, 2014). This study has shown few phytoremediation studies within Africa, which could be attributed to:

i. Research funding in many African countries is often limited, with priorities sometimes focusing on more immediate economic and/or health-related issues, potentially leading to less investment for environmental research such as phytoremediation studies.

ii. In many African countries, environmental issues such as water pollution might not be prioritised as highly as other concerns such as defense, mining and education, and/or the regulatory frameworks supporting environmental research might not be robust enough to encourage extensive studies within the field of phytoremediation.

iii. Economic development and industrialisation pressures are leading to prioritising projects that have immediate economic benefits over those perceived as more long-term and/or preventive, such as phytoremediation.

iv. The political instability, conflicts, and governance challenges in some African parts, particularly in Western, Central and Eastern Africa, can hinder environmental research activities.

v. Phytoremediation is a specialised field that requires a deep understanding of the biology of the plant species. Hence, there may be a lack of expertise and/or awareness about the potential effectiveness of phytoremediation among researchers and policymakers in Africa, resulting in fewer studies. Furthermore, phytoremediation studies often require extensive field data and a good understanding of macrophyte species that can effectively remove contaminants and pollutants. Therefore, a lack of comprehensive environmental data and understanding of macrophyte biology, limited access to necessary resources, and the availability of appropriate macrophyte species could hinder research efforts.

Africa's diverse climate and ecosystems mean that phytoremediation strategies effective in one region may not be applicable in another. The complexities of designing studies that account for diverse climate and ecosystem variations may discourage research efforts and hinder collaborations with other countries. Within Africa, there are limited collaboration opportunities between African researchers and those in regions where aquatic phytoremediation is more established, significantly limiting the exchange of knowledge, techniques, and technologies that could support more research within this area (Odoh et al., 2019).

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CHAPTER 3.

ENRICHMENT AND GEO–ACCUMULATION OF TRACE AND HEAVY METALS IN SURFACE SEDIMENTS ALONG URBANISED AUSTRAL WETLANDS

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3.1 Abstract

Urban wetlands are often polluted by heavy and trace metals being discharged from industries, urbanisation and agriculture, which tend to alter the sediment quality. Sediments are important as they act as a sink for contaminants in aquatic environments. Wetlands play an important role in maintaining ecological balance, preventing floods and droughts, providing shelter for various animals and plants, and supporting human well-being through essential services such as carbon sequestration and water purification. The present study aimed to assess the spatiotemporal distribution and sediment pollution indices of trace and heavy metals in surface sediments and identify potential sources of contamination in urbanised Austral wetlands. Sediment samples were collected within the Riverside's Wetlands, City of Nelspruit, South Africa, across three seasons (i.e., hot–dry, hot–wet and cool–dry seasons). A multivariate principal component analysis (PCA) was used to determine the metal relationship. The metal pollution indices, such as contamination factor, enrichment factor, geo–accumulation factor and pollution load index were used to determine the pollution of metals in the sediments across different sites among seasons. The study found that Na concentration observed during the cool-dry season was lower compared to the concentrations of other metals. The pollution indices indicated that wetland sediments were highly contaminated, mainly with Mn, Co, Cu, Pb, Zn, Al and Fe, especially during the cool–dry season. The current study highlights an urgent need to come up with immediate measures to control severe heavy metal pollution from the industrial emission into the wetlands. These immediate measures should be carried out to minimize the rate of contamination and extent of future metal contamination.

***Keywords**: Contamination, heavy metals, pollution index, sediments, spatial distribution, wetlands*

3.2 Introduction

Wetlands, whether permanent or seasonal, are dynamic ecosystems characterised by periodic or permanent flooding, where anaerobic process often dominate (Balwan and Kour, 2021). They occupy about 5 – 10% of Earth's terrestrial space and serve as a productive supporting ecosystem for both aquatic and terrestrial biodiversity (Aryal et al., 2021; Balwan and Kour, 2021; Chakraborty et al., 2023). Furthermore, wetlands are even known as the kidneys of the earth because of their water purification potential (Bernal-González et al., 2022; Pratap et al., 2023; Talukdar et al., 2022). Apart from this, wetlands have various functions and values, such as flood management (Acreman and Holden, 2013) and cycling of nutrients (Marschner, 2021). However, these functions are altered by heavy metals being discharged into the wetlands through various environmental activities.

Anthropogenic activities contribute to the enrichment of nutrients and heavy metals in wetlands (Afzaal et al., 2022; Zhang et al., 2021). These activities include urbanisation, industrialisation and agricultural farming (Munizaga et al., 2024). Increases in urbanisation and industrialisation are posing serious global threat on wetland health (Ballut-Dajud et al., 2022). Once heavy metals are introduced into the wetlands, their distribution depends on several factors such as climate, temperature, water and soil pH, and dissolved oxygen (Zhao et al., 2024; Zheng et al., 2024). They are in the form of elements – including metalloids – and are different from other chemical compounds by having high atomic weight and a density greater than that of water (Appenroth, 2010; Bharti and Singh, 2013).

Heavy metals are released into the wetlands to settle in the underlying sediments (Afzaal et al., 2022). Once deposited in sediments, heavy metals tend to persist and are not subject to biological degradation, though they may undergo chemical transformation affecting bioavailability. Some studies (Afzaal et al., 2022; Zhang et al., 2021) reported essential metals (i.e. copper (Cu), zinc (Zn arsenic (As), cobalt (Co) and toxic metals (i.e cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb) which might pose hazardous effects in sediments. These are the metals which were outlined by The Environmental Protection Agency (EPA) as non–degradable hazardous metals (Munyai et al., 2023). This calls for a serious monitoring of heavy metal accumulation in the wetland sediments. Furthermore, the study of assessing heavy metal accumulation in sediments is essential for understanding contamination patterns and potential ecological risks. This suggests that assessing the metal contamination in the sediments can serve as reliable indicators for identifying pollution sources and informing management interventions (Dalu et al., 2020). The pollution indices are the essential tools used to assess the contamination from heavy metals in the sediments. These pollution indices can be classified as standalone and integrated. Geo–accumulation index (I_{geo}), enrichment factor (EF), pollution load index (PLI) and contamination factor (CF) are classified as standalone indices, whereas modified degree of contamination (mC_{deg}) is commonly used as an integrated index (Ferreira et al., 2022). Given their proximity to urban and industrial areas, the Riverside wetlands may be at elevated risk of contamination; however, systematic data on metal dynamics in the Mbombela region remain limited.

The present study focuses on the Riverside Wetlands in the Mpumalanga province of South Africa, and similar to many urbanised wetlands, they are vulnerable to contamination by heavy metals from industrial and urban activities. Studies (Githaiga et al., 2021; Xia et al., 2022; Xiong et al., 2024; Yang et al., 2023) have shown that urbanised wetlands act as sinks for pollutants (Dalu et al., 2020), including trace and heavy metals, which can accumulate in sediments and pose ecological and human health risks (Essien et al., 2022; Panda et al., 2023). Assessing enrichment and geo–accumulation in these systems is critical for understanding contamination levels and informing remediation efforts. Therefore, the current study aimed to assess the spatiotemporal distribution and sediment pollution indices of trace and heavy metals in superficial sediments and identify potential sources of contamination in urbanised Austral wetlands. We hypothesised that trace and heavy metal enrichment levels in superficial sediments will vary significantly across different locations, with high concentrations observed near heavy industrial areas. (i.e., areas with vehicle workshops).

3.3 Materials and methods

3.3.1 Study area

The study was carried out within the Riverside's wetlands (Figure 3.1), City of Nelspruit, South Africa, and these wetland systems range in size between 1 500 and 38 000 m^2. Riverside is located within the Lowveld region of Mpumalanga province, in the northeastern part of the country (Nkosi and Mathivha, 2025). The elevation ranges from 632 m to 688 m above sea level. The wetlands are located within the Riverside City Improvement District area, with the catchment dominated by automotive industries. The area has a mild subtropical climate, with its temperatures during summer ranging between 16–19°C and 25–27 °C, to 10–16 °C and 22–26 °C in winter. The study area receives a mean annual rainfall of 667 mm per year (Nkosi and Mathivha, 2025).

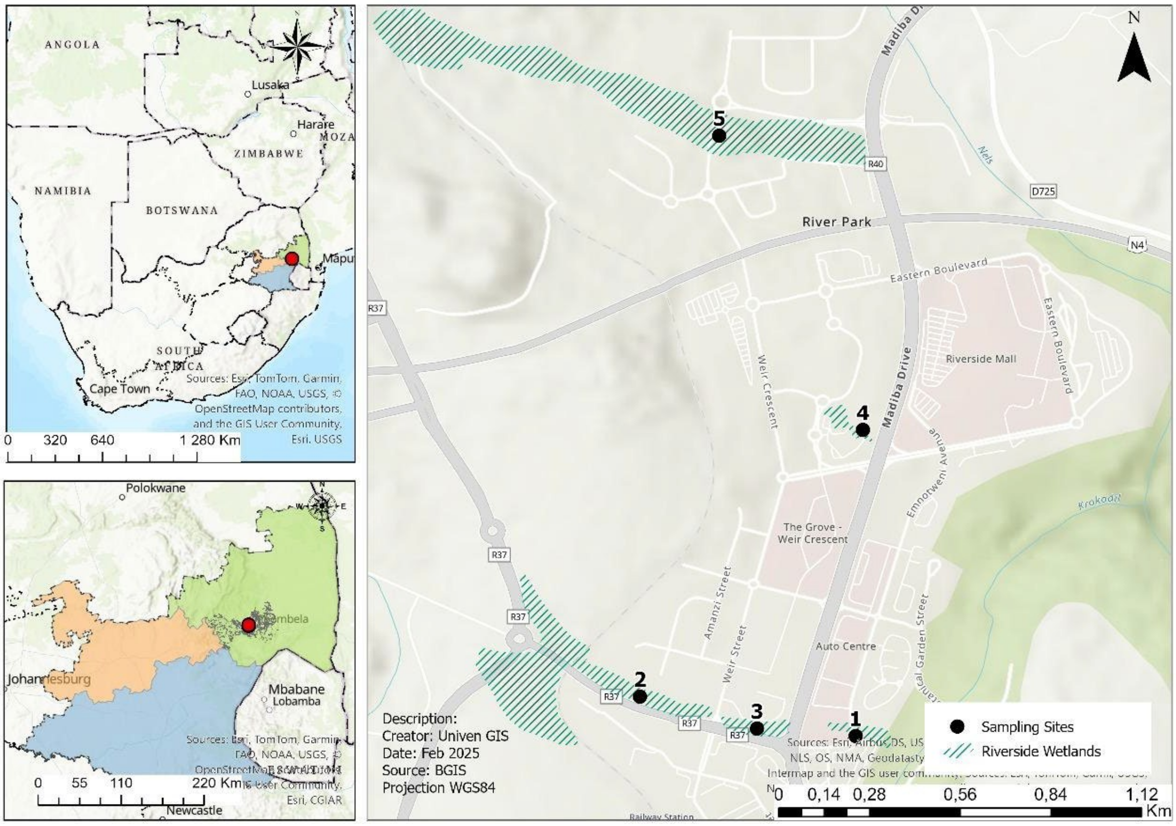

Figure 3.1. Location of the five study sites with the Riverside Wetlands in the City of Nelspruit, South Africa.

3.3.2 Sediment sampling

Five sites (wetlands) were randomly selected from the five Riverside Wetlands due to the closeness to the motor industries and roads, each with 6 points where sediments were collected. The sampling was conducted in the cool–dry (15 June 2023), hot–dry (12 September 2023) and hot–wet (11 December 2023) seasons, and the same sites were revisited in each season. The climate type between January and May is warm and normally dry. Before sample collection, debris or organic matter was removed, and thereafter, about recent deposition of 5 cm into the sediment layer sample was collected. Sediment samples were collected from 6 points per site (i.e., wetland system). Thereafter, 3 samples (i.e., localities) each were combined to create an integrated superficial sediments sample of 1.0 kg using a plastic shovel. Thus, for each site, 2 integrated sediment samples were collected and placed in clean polyethylene–labelled Ziplock bags before transportation to the laboratory for further analysis.

In the laboratory, the samples were oven–dried at 70 °C using an EcoTherm oven for 3 days. After drying the samples, the soil was ground to powder using a pestle and mortar and sieved through a 2 mm sieve. The oven–dried ground sediment samples were then sent to a South African National Accreditation System (SANAS) certified laboratory, i.e., Stellenbosch University Central Analytical Facility, for further processing of metals (i.e., sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), boron (B), chromium (Cr), aluminum (Al), manganese (Mn), iron (Fe), cobalt (Co), molybdenum (Mo), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) 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). Before analysis, samples were microwave digested as described by Kuss (1992) and Koprus et al. (2022). Each digestion batch included duplicate samples and quality control standards with 1 573a tomato leaf (~0.3 g) as a reference standard. Certified Reference Material (National Institute for Science and Technology, Gaithersburg, USA) were used as plant control. The accuracy range was 92.7–114.0 %, with a recovery range of 88.2–113.0 % was recorded.

3.3.3 Sediment metal pollution indices

Five sediment metal pollution indices were used for the assessment of the degree of contamination in the Riverside wetlands due to the trace metal accumulation: contamination factor (CF), enrichment factor (EF), geo–accumulation index (*I*geo), pollution load index (PLI), and modified degree of contamination (mCdeg).

3.3.3.1 Contamination factor

The contamination factor was used to measure the levels of sediment contamination by metals against background values to determine the extent to which a site is polluted with a particular metal element, i.e., it is the ratio of the metal concentration to the background metal value (Barbieri, 2016). The contamination factor is given in the equation below:

$$\text{Contamination factor} = C_m / C_b$$

Where C_m is the sediment metal concentration and C_b background metal value. The classification of CF output values is as follows: CF < 1 – low contamination, 1 ≤ CF < 3 – moderate contamination, 3 ≤ CF < 6 – considerable contamination, and CF > 6 – very high contamination (Salah et al., 2012).

3.3.3.2 Geo–accumulation index

Geo–accumulation (*I*geo) quantifies metal contamination in sediments by comparing current metal concentrations to background metal levels to assess whether the presence of metals is due to natural or anthropogenic activities providing valuable information for environmental monitoring and management (Müller, 1969). It is classified into seven classes and is a quantitative measure of the degree of pollution in aquatic sediments, calculated as follows:

$$\text{Igeo} = \log_2\left(\frac{Cn}{1.5 \times Bn}\right)$$

Where Cn is the concentration of the sediment metal, and Bn is the geochemical metal background. Seven classes of *I*geo are used: <0 practically unpolluted, >0–1 unpolluted to moderately polluted, >1–2 moderately polluted, >2–3 moderately to strongly polluted, >3–4 strongly polluted, >4–5 strong to very strongly polluted and >5 very strongly polluted (Müller, 1979).

3.3.3.3 Enrichment factor

Enrichment factor (EF) is a quantitative measure used to assess the origin (i.e., natural or anthropogenic) and the degree of contamination or level of metals in sediments (Emmerson et al., 1997). It compares the concentration of a target metal to that of a reference element (e.g., Fe) in the sample relative to its background metal concentration. The equation is as follows:

$$EF = \frac{\left(\frac{Cx}{Cref}\right) sample}{\left(\frac{Bx}{Bref}\right) background}$$

Where: Cx is the concentration of the metal in the examined environment, Cref is the concentration of the reference metal (i.e., Fe) in the sediment sample, Bx is the concentration of the reference metal in the examined environment, and Bref is the concentration of the reference metal (i.e., Fe) in the background environment. The enrichment factor categories (Odat, 2015) are as follows: <2 is a deficiency to minimal enrichment, >2–5 is moderate enrichment, >5–20 is significant enrichment, >20–40 is very high enrichment and >40 is extremely high enrichment (Barbieri, 2016).

3.3.3.4 Pollution load index

Pollution load index (PLI) is a quantitative tool used to assess the overall contamination level of sediments or soils by heavy metals to help evaluate potential impacts of metals on ecosystem and human health (Tomlinson et al., 1980). The equation is as follows:

$$PLI = \frac{(Cf1 \times Cf2 \times Cf3 \times \ldots..Cfn)}{n}$$

Where n is a number of metals in the sample, and Cf is the contamination factor of metal. A PLI value of ≤ 1 means no metal pollution, whereas a PLI value >1 indicates that metal pollution exists (Tomlinson et al., 1980).

3.3.3.5 Modified degree of contamination

The modified degree of contamination (mC_{deg}) assists in assessing overall metal contamination in sediment samples. The degree of contamination is a cumulative index calculated by the sum of individual metal CF values. The modified degree of contamination can be used for better assessment value (Mazurek et al., 2017). The modified degree of contamination can be calculated using the following relation:

$$mC_{deg} = \frac{\sum CF}{n}$$

Where, CF is the contamination factor for individual metals, and *n* is the number of analysed metals. The mC_{deg} classification is as follows: <1.5 indicates nil to a very low degree of contamination, >1.5–2 low degree of contamination, >2–4 a moderate degree of contamination, >4–8 a high degree of contamination, >8–16 a very high degree of contamination, >16–32 an extremely high degree of contamination, and >32 ultrahigh degree of contamination (Mazureket al., 2017).

3.3.4 Data analysis

The sediment metal data were first tested for normality (i.e., using Shapiro–Wilk test) and homogeneity of variances (i.e., Levene's test) and were found to meet all assumptions of parametric analysis. A two–way analysis of variance (ANOVA) was used to determine the differences in metal concentrations among the sites (i.e., sites 1–5) and seasons (i.e. cool–dry, hot–dry, hot–wet) using SPSS version 25. A principal component analysis (PCA) with the varimax rotation method was used to determine the natural and/or anthropogenic sources of sediment metals across study sites and seasons. Furthermore, a two–way cluster analysis, using Ward's average group linkage method and correlation as a distance measure, was used for metal source identification in PC–ORD version 5.10 (McCune and Mefford, 1999).

3.4 Results

3.4.1 Metal concentration dynamics

Sodium concentrations were only recorded during the cool–dry season at relatively low concentrations (30.3–87.3 mg kg^{-1}), with Cr and Fe concentrations being relatively high among sites and seasons (Figure 3.2). Furthermore, Co, Mo, Cu, Zn, As, Cd and Pb had low metal concentrations among sites and seasons (Figure 3.2). Based on two–way ANOVA analysis, all metals were significantly ($p < 0.01$) different among sites, except for Na which was found to be similar ($p > 0.05$) across sites (Table 1). Significant ($p < 0.05$) seasonal differences were observed for Na, P, Al, Cr, Mo, Cu, Zn, Cd and Pb (Table 2.1).

Based on the sediment quality guidelines for metals concentrations (Persaud et al., 1993), Mn (519.5 mg kg^{-1}, site 2, hot-wet season; Lowest effect level (LEL) – 460 mg kg^{-1}), Cu (18.9 mg kg^{-1}, site 2, cool-dry season; 37.3 mg kg^{-1}, site 3, cool-dry season; 16.0 mg kg^{-1}, site 4, cool-dry season; LEL – 16 mg kg^{-1}), Zn (143.6 mg kg^{-1}, site 2, hot-dry season; LEL – 120 mg kg^{-1}), As (6.1 mg kg^{-1}, site 3, cool-dry season; LEL – 6 mg kg^{-1}), Cd (0.6 mg kg^{-1}, site 2, hot-dry season; LEL – 0.6 mg kg^{-1}) and Pb (32.0 mg kg^{-1}, site 2, hot-dry season; LEL – 31 mg kg^{-1}) were above the lowest effect level. Metals not exceeding the permissible levels suggested to have no effects or impacts on aquatic organisms.

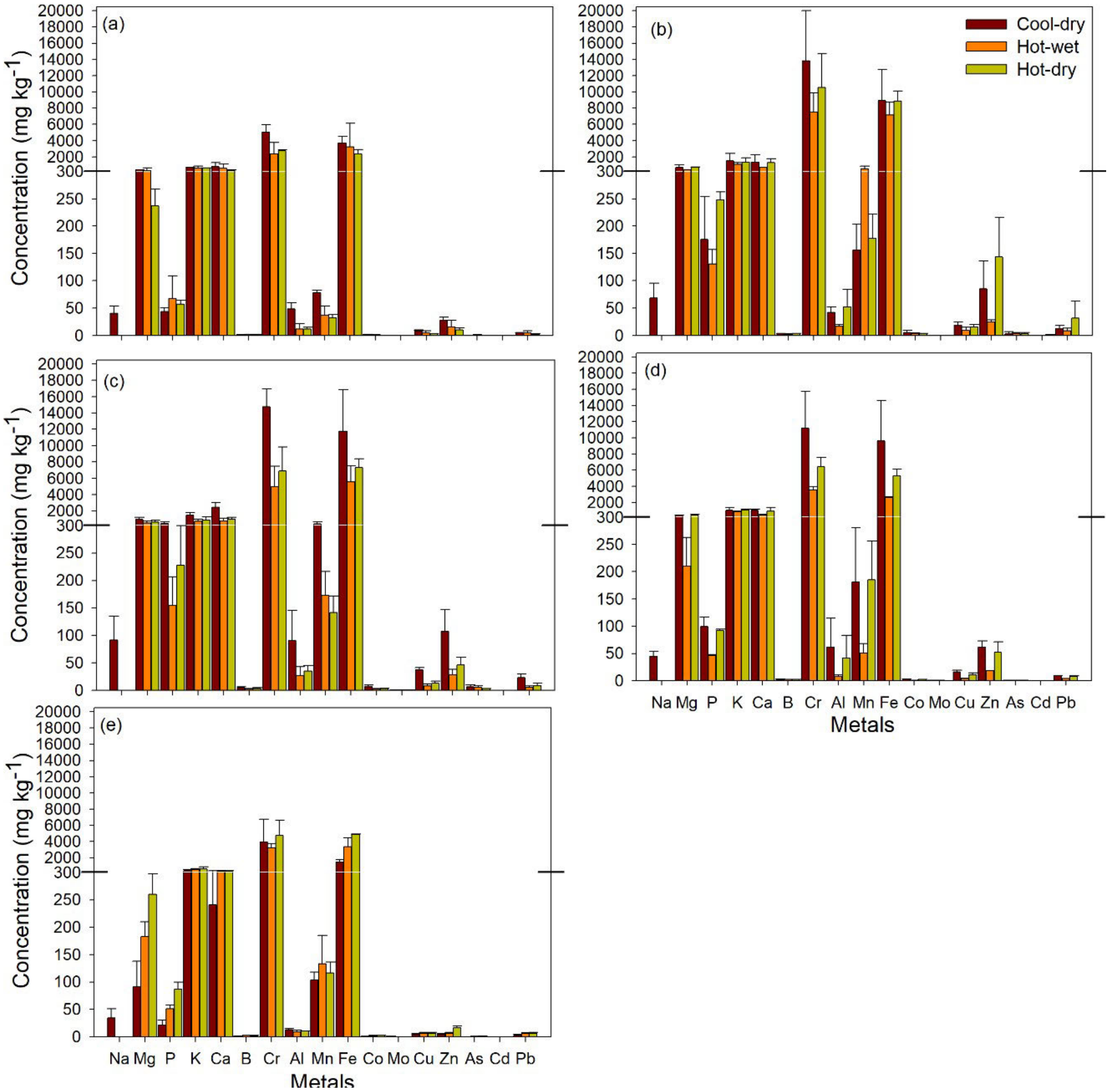

Figure 3.2: Mean metal concentrations (± standard deviation) variation across sites and seasons: (a) site 1, (b) site 2, (c) site 3, (d) site 4, and (e) site 5, within Riverside Wetlands.

Table 3.1: Two–way analysis of variance for metals among sites and seasons recorded along the Riverside Wetlands

Variable	Sites			Seasons			Sites × Season		
	df	F	*P*	*Df*	F	*P*	*df*	F	*P*
Na	4	1.908	0.161	**2**	**74.009**	**<0.001**	8	1.908	0.134
Mg	**4**	**10.806**	**<0.001**	2	2.193	0.146	8	1.431	0.262
P	**4**	**33.871**	**<0.001**	**2**	**5.071**	**0.021**	**8**	**4.387**	**0.007**
K	**4**	**6.543**	**0.003**	2	0.991	0.394	8	0.678	0.704
Ca	**4**	**6.805**	**0.002**	2	2.353	0.129	8	1.330	0.302
B	**4**	**8.838**	**0.001**	2	0.655	0.534	8	2.343	0.074
Al	**4**	**8.954**	**0.001**	**2**	**8.799**	**0.003**	8	0.806	0.607
Cr	**4**	**4.894**	**0.010**	**2**	**9.990**	**0.002**	8	0.946	0.510
Mn	**4**	**15.523**	**<0.001**	2	1.715	0.213	**8**	**3.717**	**0.014**
Fe	**4**	**10.409**	**<0.001**	2	2.408	0.124	8	2.590	0.053
Co	**4**	**14.290**	**<0.001**	2	4.536	0.029	**8**	**4.966**	**0.004**
Mo	**4**	**8.691**	**0.001**	**2**	**28.850**	**<0.001**	8	2.074	0.106
Cu	**4**	**8.458**	**0.001**	**2**	**11.454**	**0.001**	8	1.917	0.132
Zn	**4**	**31.258**	**<0.001**	**2**	**14.427**	**<0.001**	**8**	**4.326**	**0.007**
As	**4**	**21.926**	**<0.001**	2	2.441	0.121	**8**	**2.845**	**0.039**
Cd	**4**	**7.872**	**0.001**	**2**	**5.862**	**0.013**	8	1.482	0.243
Pb	**4**	**6.257**	**0.004**	2	2.769	0.095	8	1.816	0.152
Hg	**4**	**16.442**	**<0.001**	**2**	**75.017**	**<0.001**	**8**	**42.039**	**<0.001**

3.4.2 Relationship of metal variables among sites and seasons

We employed multivariate principal component analysis (PCA; Figure 3.3, Table 3.2), cluster analysis (Figure 3.4) and Pearson correlations (Table 3.3) to characterise metal dynamics. The PCA revealed that, except for Na, all metals in wetland sediments exhibited strong positive correlations, indicating a high degree of association among them (Figure 3.3, Tables 3.2 and 3.3). The PCA results suggest that apart from Na, all other metals in the studied wetland sediments likely originated from a common source and this was supported further by the cluster analysis (Figure 3.4).

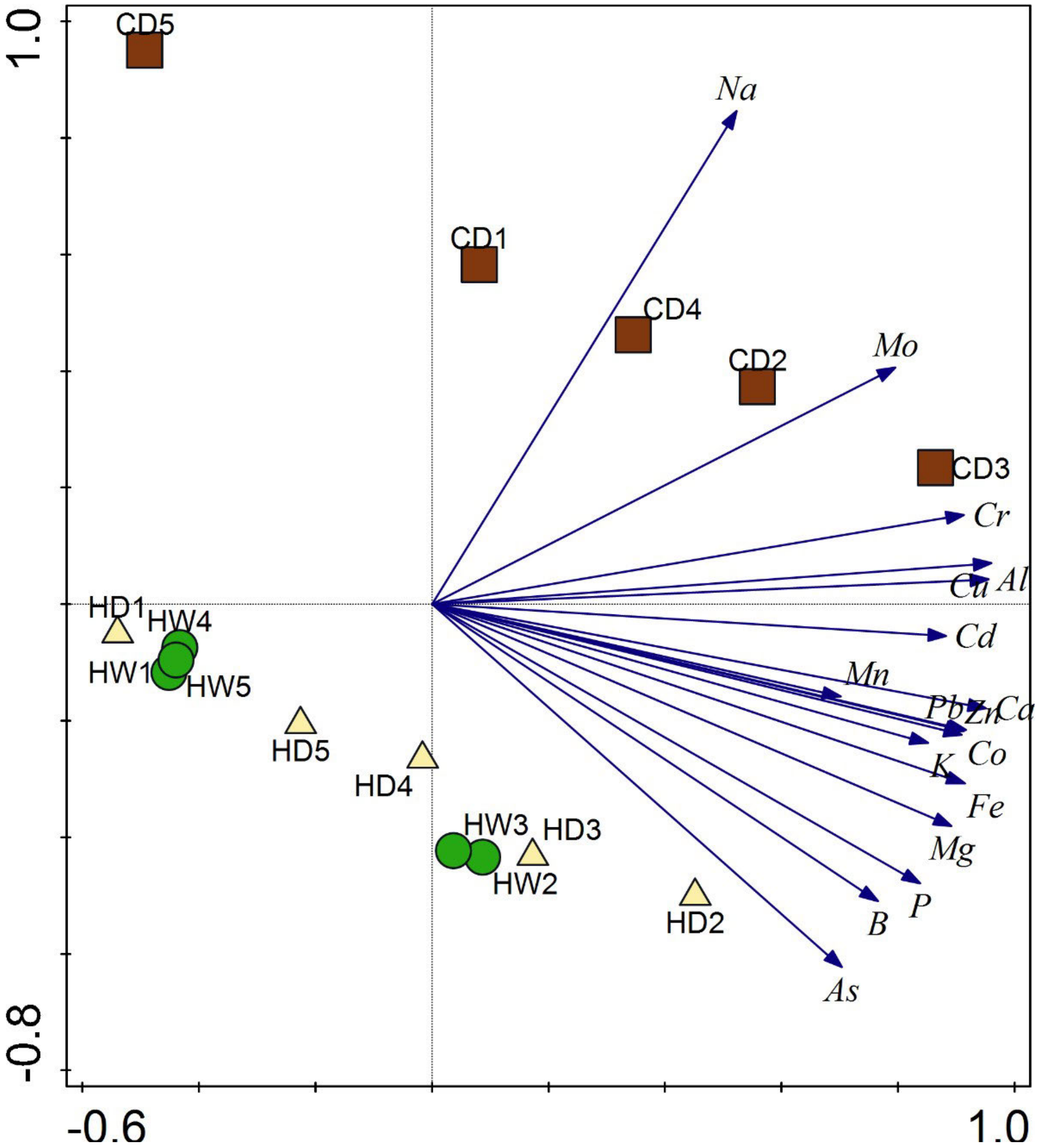

Figure 3.3: Principal component analysis (PCA) biplot assessing the relationship of metals among sites and seasons within the Riverside wetlands.

Using PCA, the first two principal components (PC) explained 87.2 % of the total variance, with PC1 and PC2 explaining 75.4 % and 11.8 % of the total variance, respectively. Eigenvalues of the two extracted PCs were both > 1.0, accounting for 3.4 and 2.4 for PC1 and PC2, respectively (Table 3.2). Cluster analysis results identified three distinct groups for seasons and sites: *group 1* included cool–dry season sites 1–4, *group 2* included hot–wet season sites 2 and 3, and hot–dry season sites 2–4, and *group 3* had the cool–dry season site 5, hot–wet season sites 1, 4 and 5, and hot–dry season site 1 (Figure 3.4). Similarly, the other cluster analysis identified 2 groups based on metals: *group 1* had Na and *group 2* had the rest of the metals (Figure 3.4).

Table 3.2: Principal component analysis (PCA) results assessing the relationship of metals among sites and seasons within the Riverside wetlands

Factors	**PC1**	**PC2**	**PC3**	**PC4**
Eigenvalue	12.817	2.002	0.756	0.385
% of variance	75.397	11.777	4.449	2.267
Cum.% of variance	75.397	87.173	91.623	93.89
Eigenvalue	3.44	2.44	1.94	1.606
Metals	***Factor loadings***			
Na	–0.353	**–0.886**	–0.034	–0.16
Mg	**–0.941**	0.215	0.159	–0.149
P	**–0.906**	0.341	0.073	0.058
K	**–0.899**	0.082	0.083	–0.143
Ca	**–0.972**	0.014	0.104	–0.145
B	**–0.851**	0.388	0.125	0.132
Cr	**–0.864**	–0.342	0.136	–0.126
Al	**–0.942**	–0.226	–0.141	–0.017
Mn	**–0.728**	0.025	–0.642	0.122
Fe	**–0.958**	0.146	–0.117	–0.075
Co	**–0.939**	0.074	–0.248	–0.115
Mo	**–0.700**	–0.595	0.214	0.198
Cu	**–0.941**	–0.227	–0.143	–0.045
Zn	**–0.941**	0.019	0.25	–0.035
As	**–0.788**	0.513	0.077	–0.042
Cd	**–0.871**	–0.179	0.146	0.401
Pb	**–0.953**	0.030	–0.101	0.146

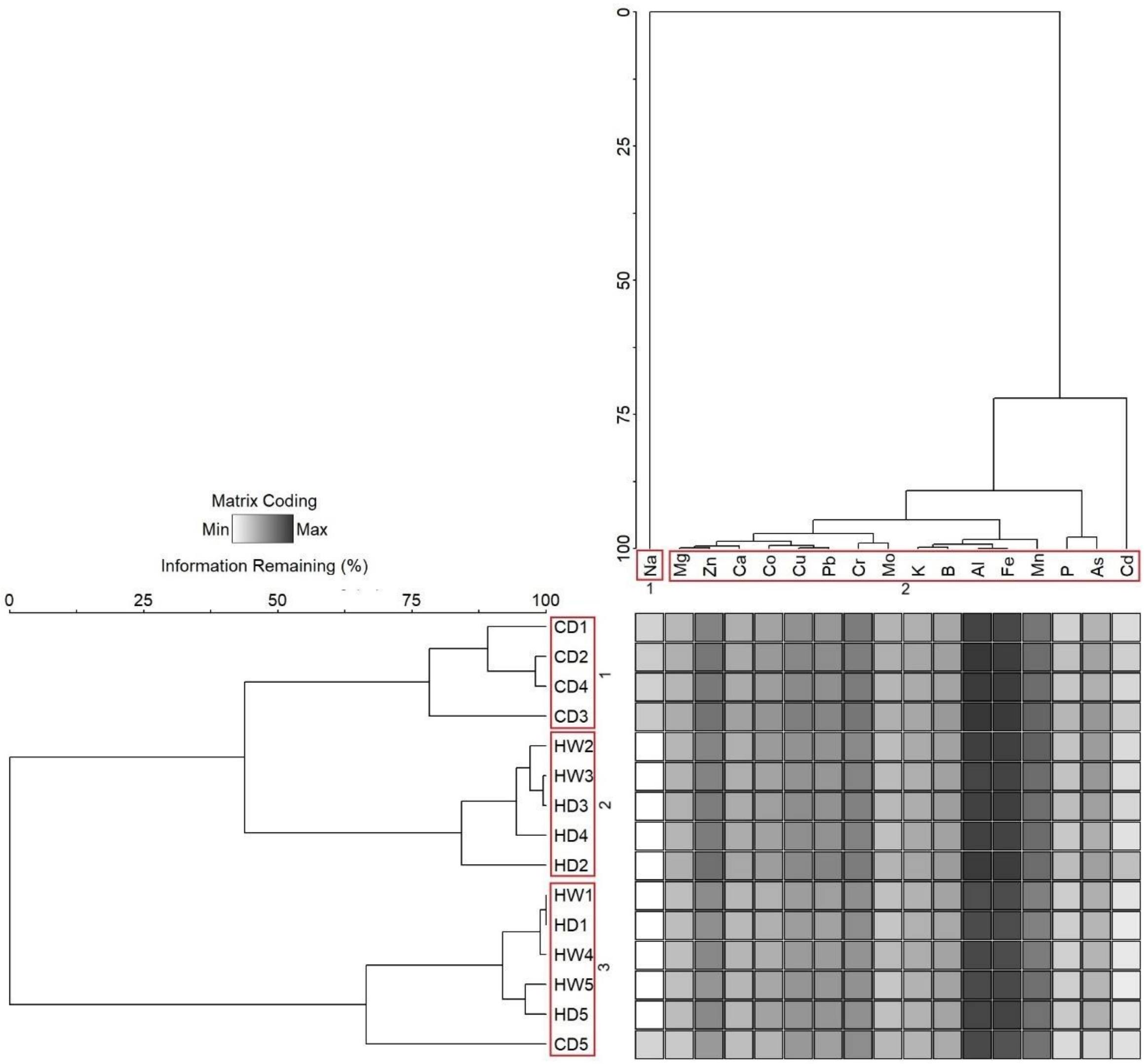

Figure 3.4: Two–way cluster analysis highlighting the relationship among metals, sites and seasons.

In Pearson's correlation analysis (Table 3.3), the values were taken from − 1 to + 1, where – 1 and +1 indicate a perfect negative and positive correlation, respectively, and 0 indicates no correlation. In the present study, correlation coefficient values that were significant ($p < 0.05$) were considered, and significant positive correlations were observed for most of the metals (Table 3.3). Sodium did not show any significant correlations with Mg, P, K, Ca, B, Cu, Zn, As, Cd and Pb. Furthermore, Na showed weak positive and significant ($p < 0.05$) correlations with Al, Mn, Fe and Co. The metal B *vs* Mo and Cr, and As *vs* Mo were found not to be significantly ($p > 0.05$) correlated (Table 3.3).

Table 3.3: Pearson correlation matrix for the studied metal found in the Riverside wetlands. The values shaded in grey are the r values and the ones in white are the p values. The p values in bold indicate significance at $p < 0.05$

	Na	Mg	P	K	Ca	B	Cr	Al	Mn	Fe	Co	Mo	Cu	Zn	As	Cd	Pb
Na	1	0.470	0.800	0.270	0.100	0.930	**<0.001**	**0.010**	0.250	0.260	0.120	**<0.001**	**<0.001**	0.170	0.570	0.070	0.202
Mg	0.14	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.01**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
P	0.05	**0.91**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.02**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
K	0.21	**0.81**	**0.75**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.01**	**<0.001**	**<0.001**	**<0.001**	**0.01**	**<0.001**
Ca	0.30	**0.94**	**0.84**	**0.80**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
B	−0.02	**0.76**	**0.86**	**0.77**	**0.73**	1	**0.01**	**<0.001**	**0.010**	**<0.001**	**<0.001**	0.070	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Cr	**0.51**	**0.76**	**0.63**	**0.53**	**0.80**	0.45	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.01**	**<0.001**	**<0.001**
Al	**0.47**	**0.79**	**0.74**	**0.88**	**0.83**	**0.68**	**0.68**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mn	0.22	**0.58**	**0.63**	**0.54**	**0.61**	**0.50**	**0.56**	**0.69**	1	**<0.001**	**<0.001**	**0.04**	**<0.001**	**0.01**	**<0.001**	**0.01**	**<0.001**
Fe	0.21	**0.90**	**0.88**	**0.75**	**0.87**	**0.66**	**0.73**	**0.81**	**0.75**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Co	**0.29**	**0.88**	**0.85**	**0.79**	**0.87**	**0.71**	**0.68**	**0.87**	**0.79**	**0.94**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Mo	**0.70**	**0.50**	**0.43**	**0.44**	**0.58**	0.34	**0.71**	**0.65**	**0.38**	**0.51**	**0.51**	1	**<0.001**	**<0.001**	0.16	**<0.001**	**<0.001**
Cu	0.51	**0.81**	**0.77**	**0.71**	**0.88**	**0.64**	**0.84**	**0.87**	**0.73**	**0.86**	**0.88**	**0.72**	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Zn	0.26	**0.86**	**0.81**	**0.73**	**0.84**	**0.67**	**0.76**	**0.78**	**0.49**	**0.83**	**0.77**	**0.67**	**0.78**	1	**<0.001**	**<0.001**	**<0.001**

As	–0.11	**0.86**	**0.91**	**0.72**	**0.75**	**0.82**	**0.45**	**0.63**	**0.56**	**0.79**	**0.79**	0.26	**0.57**	**0.66**	1	**0.01**	**<0.001**
Cd	0.34	**0.64**	**0.67**	**0.45**	**0.66**	**0.56**	**0.71**	**0.64**	**0.49**	**0.64**	**0.62**	**0.77**	**0.73**	**0.80**	**0.45**	1	**<0.001**
Pb	0.24	**0.84**	**0.81**	**0.71**	**0.87**	**0.73**	**0.72**	**0.81**	**0.69**	**0.85**	**0.87**	**0.61**	**0.90**	**0.82**	**0.63**	**0.83**	1

3.4.3 Sediment metal pollution indices

3.4.3.1 Contamination factor

In general, the cool–dry season had high contamination factor (CF) values, during the hot–dry seasons shown least CF values among sites 1–4, at site 5 during the cool–dry season had the least CF values (Figure 3.5). For site 1 CF values, most metals fell within the low to moderate contamination for the hot–dry and hot–wet seasons, with the cool–dry season exhibiting moderate to considerable contamination for metals such as Al and Co (Figure 3.5a).In sites 2 and 3, most metals showed moderate to very contamination across all seasons (Figure3.5b, c). Site 3 had high CF values across all seasons. Site 4 ranged from low (mostly hot–dry)to very high contamination (mostly cool–dry and hot–wet), with Mn, Al, Fe, Cu and Zn havingelevated CF values (Figure 3.5d). Site 5, cool–dry season showed low to moderate contamination for most metals, with Mn having high CF values (considerable contamination) for that site (Figure 5e). Based on ANOVA analysis, Mg ($F = 4.542$, $p = 0.013$), K ($F = 3.869$,$p = 0.024$) and As ($F = 9.844$, $p = 0.001$) were found to be significant among sites, whereas nosignificant ($p > 0.05$) seasonal differences were found (Table 3.4).

3.4.3.2 Enrichment factors

Enrichment factors were generally within background levels for most metals, with a few metals such as Mg, P, Ca, Cr, Al, Mn, Fe, Co, Cu, Zn, As and Pb for mostly in sites 2 and 3 across all seasons, and site 4 (cool–dry) showed low to moderate enrichment (Figure 3.6). The cool–dry season showed more enrichment compared to other seasons. Manganese (site 2, hot–wet; site 3, cool–dry), Co (site 3, cool–dry), Cu (site 3, cool–dry), Zn (site 3, cool–dry; site 2, hot–dry), As (site 3, cool–dry and hot–wet) and Pb (site 3, cool–dry; site 2, hot–dry) showed significant enrichment (Figure 6). Magnesium ($F = 10.818$, $p < 0.001$), P ($F = 13.7108$, $p < 0.001$), K ($F = 4.720$, $p = 0.011$), Ca ($F = 6.801$, $p = 0.002$), B ($F = 9.639$, $p < 0.001$), Al ($F = 7.443$, $p = 0.002$), Mn ($F = 3.625$, $p = 0.029$), Fe ($F = 6.842$, $p = 0.002$), Co ($F = 7.733$, $p = 0.001$), Mo ($F = 6.410$, $p = 0.003$), Cu ($F = 16.094$, $p < 0.001$), Zn ($F = 8.279$, $p = 0.001$) and As ($F = 7.153$, $p = 0.002$) were significantly different among sites, whereas Ca ($F = 5.777$, $p = 0.014$), Al ($F = 9.719$, $p = 0.002$), Cr ($F = 5.388$, $p = 0.017$), Co ($F = 5.227$, $p = 0.019$), Mo ($F = 22.804$, $p < 0.001$), Cu ($F = 23.609$, $p < 0.001$) and Zn ($F = 6.626$, $p = 0.009$) were significantly different among seasons (Table 3.4).

3.4.3.3 Geo–accumulation index

During the hot–dry season, all metals showed moderate to strong pollution for site 1, with–the hot–wet season showing to be practically unpolluted (Figure 3.7a). Unpolluted to strongly polluted was observed for all metals among season in sites 2 and 3, except Mo and Cd (Figure 3.7b,c). Similar patterns to site 1 were observed for site 5 across all seasons, where most metals showed to be practical unpolluted, except for Mn, Co, Cu, and Pb for hot–wet andhot–dry seasons (Figure 3.7e). No significant *I*geo value differences based on the ANOVA analysis were observed for P (F = 2.370, $p = 0.099$), Cr (F = 2.366, $p = 0.099$) and As (F = 1.142, $p = 0.375$), with significant ($p < 0.05$) differences being observed for the rest of the metals (Table 4). Seasonal *I*geo value differences were observed for Mg (F = 5.803, $p = 0.014$),P (F = 6.977, $p = 0.007$), K (F = 5.491, $p = 0.016$), B (F = 4.754, $p = 0.025$), Co (F = 5.155, $p= 0.020$), Zn (F = 5.605, $p = 0.015$), As (F = 4.604, $p = 0.028$) and Pb (F = 5.174, $p = 0.020$) (Table 3.4).

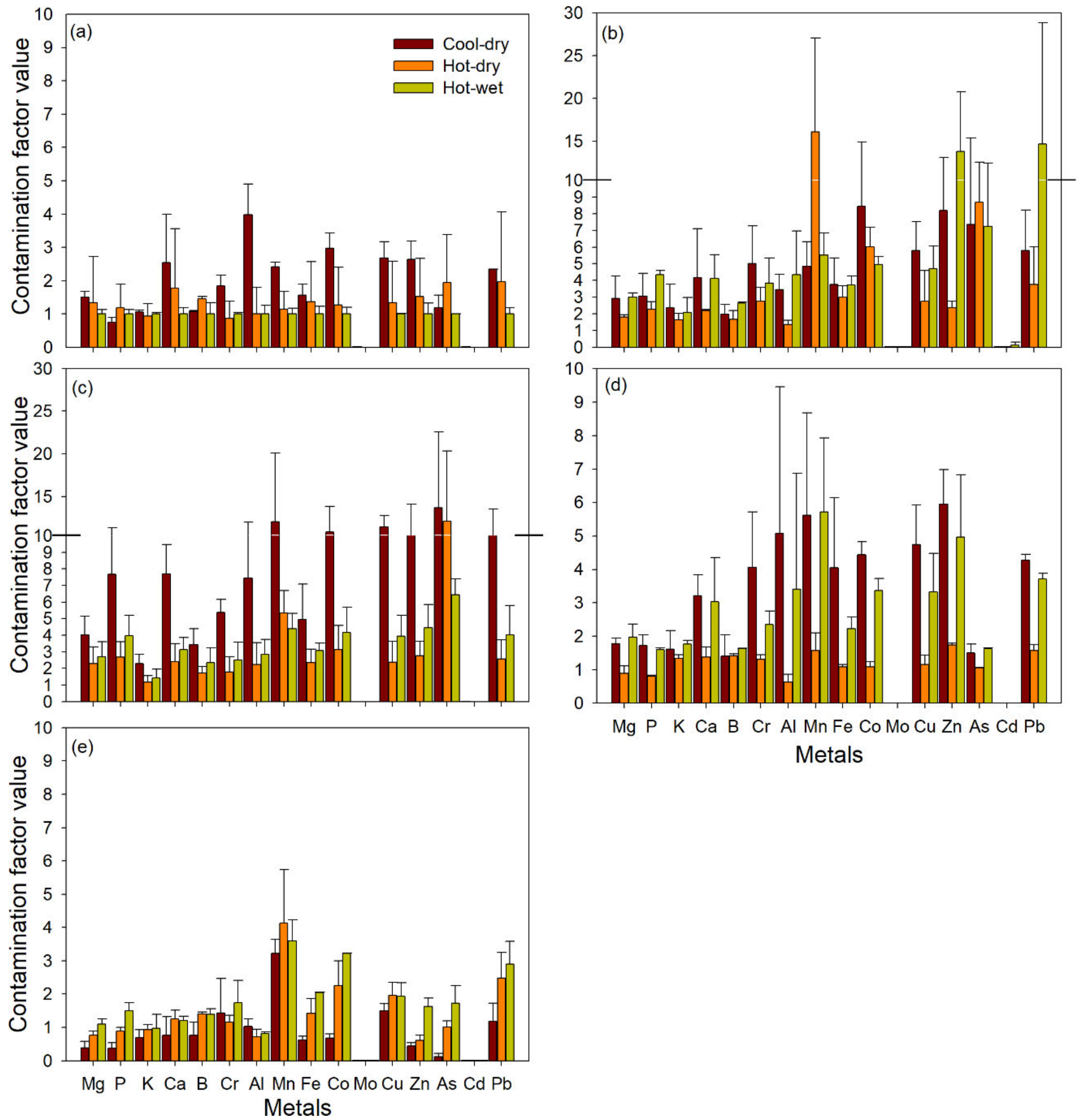

Figure 3.5: Variation in contamination load factor values among sites and seasons: (a) site 1, (b) site 2, (c) site 3, (d) site 4, and (e) site 5. Note differences in scales for panels b and c.

Table 3.4: Two–way ANOVA analysis for the sediment metal pollution indices

Variable	Sites			Season			Sites × Season		
	Df	**F**	***P***	***df***	**F**	***P***	***df***	**F**	***P***
Contamination factor									
Mg	**4**	**4.542**	**0.013**	2	1.731	0.211	8	2.620	0.051
P	4	2.812	0.063	2	0.839	0.451	**8**	**2.847**	**0.038**
K	4	**3.869**	**0.024**	2	1.679	0.220	8	0.673	0.708
Ca	4	2.813	0.063	2	0.553	0.586	8	1.215	0.354
B	4	1.688	0.205	2	1.344	0.290	8	1.450	0.255
Al	4	1.470	0.261	2	0.201	0.820	8	1.393	0.276
Cr	4	1.712	0.200	2	0.772	0.480	8	0.797	0.614
Mn	4	0.594	0.672	2	1.441	0.268	8	2.039	0.112
Fe	4	2.281	0.109	2	0.497	0.618	**8**	**3.096**	**0.028**
Co	4	1.365	0.293	2	0.300	0.745	8	1.634	0.196
Mo	4	1.138	0.376	2	1.150	0.343	8	0.620	0.749
Cu	4	1.421	0.275	2	0.453	0.644	8	1.231	0.347
Zn	4	2.211	0.117	2	0.908	0.424	8	0.942	0.512
As	**4**	**8.844**	**0.001**	2	0.762	0.484	**8**	**8.781**	**<0.001**
Cd	4	1.083	0.400	2	0.893	0.430	8	1.022	0.461
Pb	4	1.083	0.400	2	1.097	0.359	8	1.230	0.347
Enrichment factors									
Mg	**4**	**10.818**	**<0.001**	2	2.865	0.088	8	1.171	0.377
P	**4**	**13.710**	**<0.001**	2	2.941	0.084	8	2.617	0.052
K	**4**	**4.720**	**0.011**	2	1.415	0.273	8	0.586	0.775
Ca	**4**	**6.801**	**0.002**	**2**	**5.777**	**0.014**	8	2.160	0.095
B	**4**	**9.639**	**<0.001**	2	0.876	0.437	8	2.563	0.055
Al	**4**	**7.443**	**0.002**	**2**	**9.719**	**0.002**	8	1.058	0.439
Cr	4	2.229	0.115	**2**	**5.388**	**0.017**	8	0.839	0.584
Mn	**4**	**3.625**	**0.029**	2	0.605	0.559	8	2.106	0.102
Fe	**4**	**6.842**	**0.002**	2	3.243	0.068	8	1.575	0.213
Co	**4**	**7.733**	**0.001**	**2**	**5.227**	**0.019**	8	2.061	0.108

Mo	**4**	**6.410**	**0.003**	**2**	**22.804**	**<0.001**	8	1.340	0.297
Cu	**4**	**16.094**	**<0.001**	**2**	**23.609**	**<0.001**	8	6.454	0.001
Zn	**4**	**8.279**	**0.001**	**2**	**6.626**	**0.009**	8	2.623	0.051
As	**4**	**7.153**	**0.002**	2	0.313	0.736	8	0.393	0.907
Cd	4	1.226	0.341	2	0.926	0.418	8	1.023	0.460
Pb	4	2.765	0.066	2	1.469	0.261	8	1.412	0.269
Geo–accumulation index									
Mg	**4**	**3.844**	**0.024**	**2**	**5.803**	**0.014**	**8**	**4.806**	**0.004**
P	4	2.370	0.099	**2**	**6.977**	**0.007**	**8**	**6.552**	**0.001**
K	**4**	**12.720**	**<0.001**	**2**	**5.491**	**0.016**	8	2.140	0.097
Ca	**4**	**5.266**	**0.007**	2	3.071	0.076	**8**	**3.197**	**0.025**
B	**4**	**6.994**	**0.002**	**2**	**4.754**	**0.025**	**8**	**2.896**	**0.036**
Al	**4**	**5.524**	**0.006**	2	1.377	0.282	**8**	**2.802**	**0.041**
Cr	4	2.366	0.099	2	0.779	0.477	8	1.445	0.257
Mn	**4**	**5.447**	**0.007**	2	2.231	0.142	8	2.042	0.111
Fe	**4**	**5.668**	**0.006**	2	2.686	0.101	**8**	**4.987**	**0.004**
Co	**4**	**4.079**	**0.020**	**2**	**5.155**	**0.020**	**8**	**4.836**	**0.004**
Mo	**4**	**33.012**	**<0.001**	2	2.185	0.147	**8**	**2.670**	**0.048**
Cu	**4**	**5.260**	**0.008**	2	2.175	0.148	**8**	**3.802**	**0.012**
Zn	**4**	**8.789**	**0.001**	**2**	**5.605**	**0.015**	**8**	**4.421**	**0.006**
As	4	1.142	0.375	**2**	**4.604**	**0.028**	**8**	**4.716**	**0.005**
Cd	**4**	**27.122**	**<0.001**	2	2.611	0.106	**8**	**2.981**	**0.033**
Pb	**4**	**5.095**	**0.009**	**2**	**5.174**	**0.020**	**8**	**3.229**	**0.024**

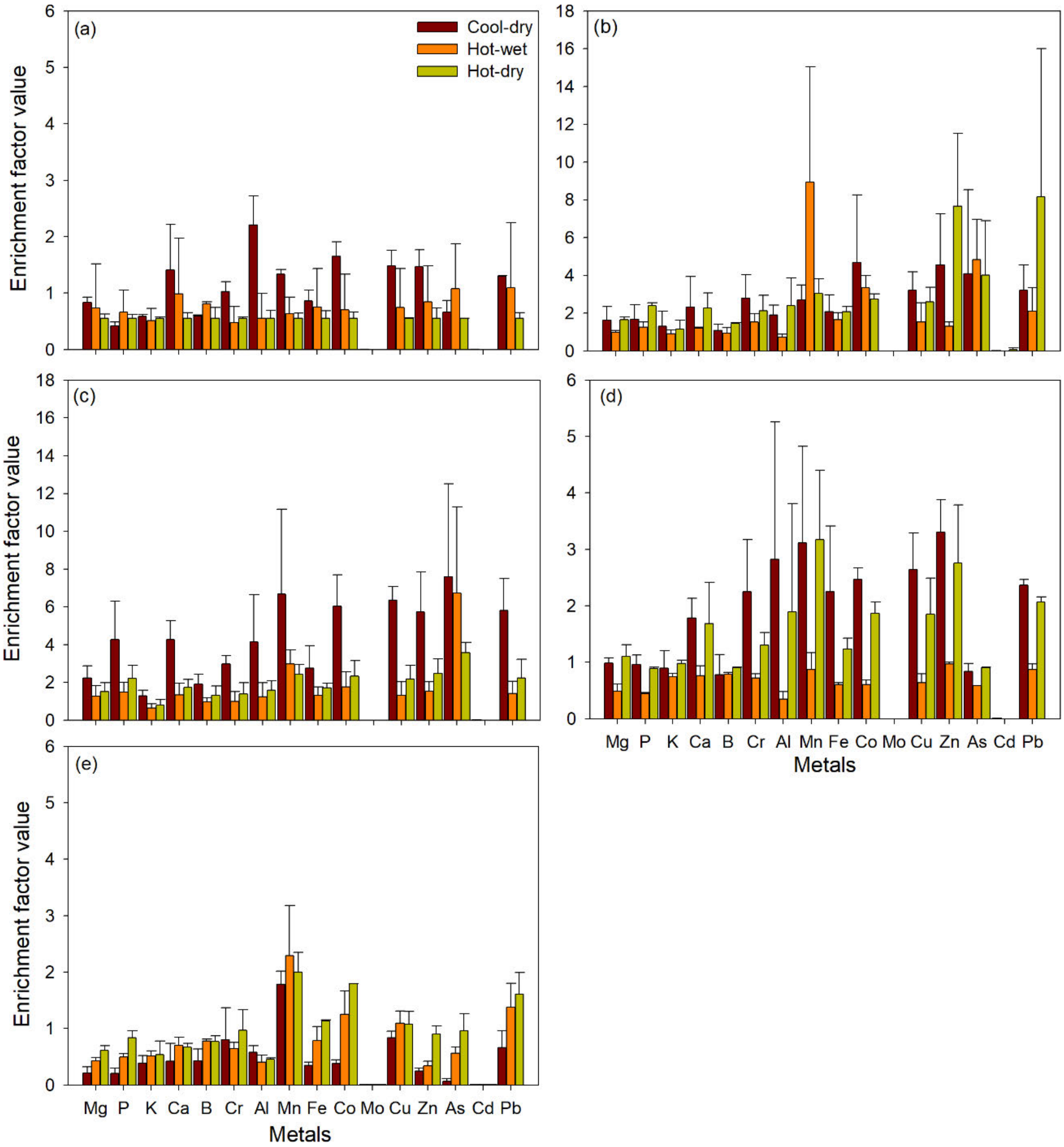

Figure 3.6: Variation in enrichment factor values among sites and seasons: (a) site 1, (b) site 2, (c) site 3, (d) site 4, and (e) site 5. Note differences in scales for panel b and c.

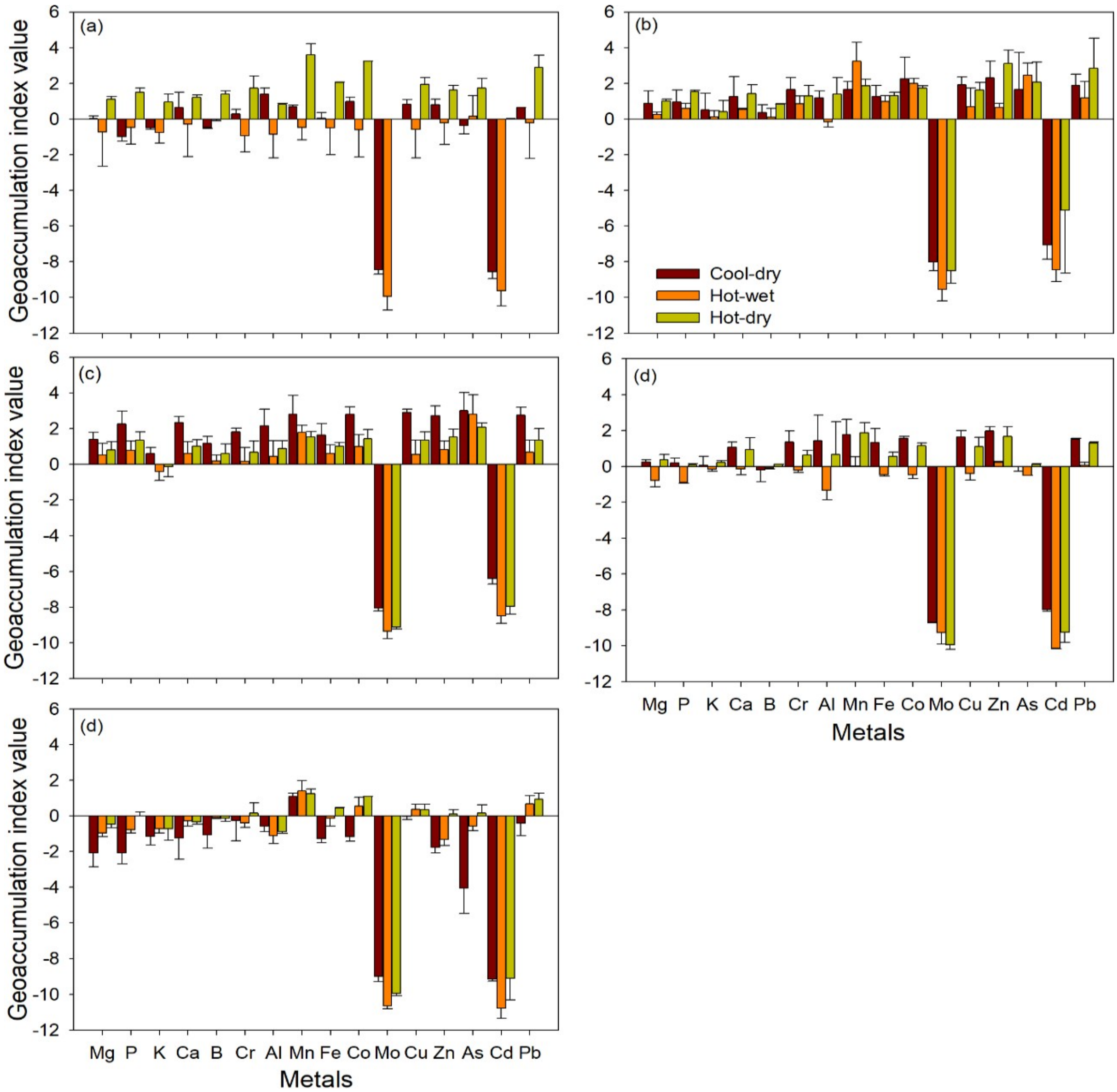

Figure 3.7: Geo–accumulation load index values for sites and seasons within the Riverside wetlands: (a) site 1, (b) site 2, (c) site 3, (d) site 4, and (e) site 5.

3.4.3.4 Pollution load index

Pollution load index values for sites 1 and 5 (all seasons) were < 0.02, suggesting no metal pollution; however, sites 2 and 3 (all seasons) had PLI values of >10, indicating the existence of metal pollution. Site 4 (cool–dry) had a PLI value of 10.5, during hot–wet and hot–dry season, site 4 had PLI values of < 0.5. The PLI was found not to be significantly different among sites($F = 1.001$, $p = 0.438$) and seasons ($F = 1.210$, $p = 0.391$). The mC_{deg} value showed nil to very low degree of contamination (< 1.5) for site 1 (hot–wet, mean 1.1; hot–dry, mean 0.8), site 4 (hot–wet, mean 1.0) and site 5 (cool–dry, mean 0.8; hot–wet, mean 1.3) (Figure 8). Low degree of contamination was observed for site 1 (cool–dry, mean 1.7) and site 5 (hot–dry, mean 1.5), and a high degree of contamination was observed for site 2 (cool–dry, mean 4.0; hot–dry, mean 4.8) and cool–dry (site 3, mean 6.7) (Figure 3.8). Based on ANOVA analysis, the mCdeg values were found to be significantly different among sites (F = 11.811, $p < 0.001$) and seasons (F = 4.253, $p = 0.034$). Tukey's posthoc analysis indicated significant differences between cool–dry *vs* hot–wet (p = 0.027), with all sites being significantly different amongst each other except site 1 *vs* 4 ($p = 0.545$), site 1 *vs* 5 ($p = 1.000$), site 2 *vs* 3 ($p = 1.000$), site 4 *vs* 5 ($p = 0.544$).

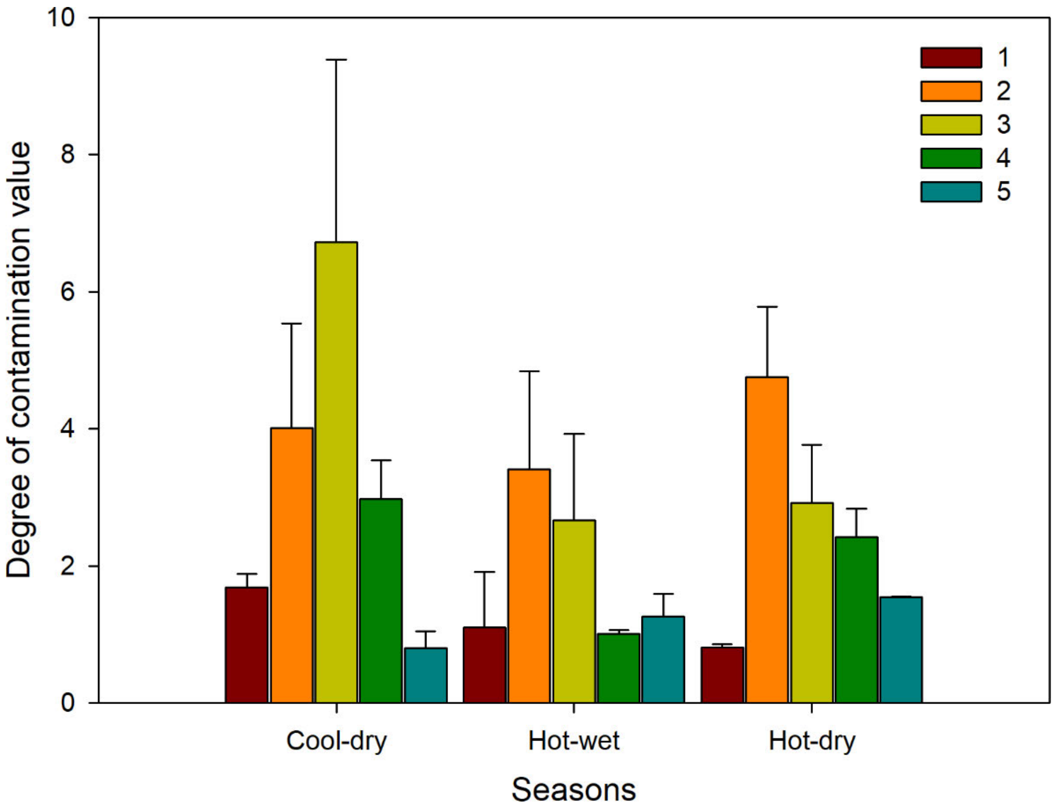

Figure 3.8: The degree of contamination of metals within the Riverside wetlands.

3.5 Discussion

Generally, high trace and heavy metal concentrations did vary significantly across different locations, which is consistent with our hypothesis. Additionally, it was observed that the primary sources of these metal concentrations in the sediments came from activities around these locations, such as workshop spillage runoff and industry discharge.

The current results indicate that low Na concentrations were only found during the cool–dry season among all metals found in the wetlands. In contrast, Netshiongolwe et al. (2020) reported high Na concentrations during the cool–dry season. Sodium might be influenced by various sources than other metals because it is more abundant in water bodies or is influenced by different geochemical processes, such as weathering of metals or anthropogenic activities (Ahmed et al., 2022; Akhtar et al., 2021). Furthermore, the current study showed high Cu, Mo, Zn, As, Cd, and Pb concentrations in all seasons and sites. Our results agrees with Oura et al. (2022) who reported high concentrations of Cd, Cr, Cu, Mn, Fe and Zn among hot seasons from different sites. Additionally, our results showed high Cr and Fe concentrations among sites and seasons. This suggests that Fe might be originating from the earth's crust (Oura et al., 2022), and runoff in agricultural fields, semi–urban areas, and untreated domestic wastewater discharged from housing and industrial regions (Tanjin et al., 2024) in these wetlands. Additionally, the current results observed high Cr concentrations. Similar results were reported by Mohajane and Manjoro (2022) where urban rivers had high concentrations of Cr.

Through PCA, our results showed that except for Na, metals in wetlands sediment were highly correlated with each other, signifying a strong positive association. These results suggest that all metals come from the same source being explained by their strong associations. Dalu et al. (2020) explained that the high correlations of metals are an indication of a common source and mutual dependence. Thus, the principal component analysis (PCA) of the current study suggests that all metal metals, with the exclusion of Na, come from the same source. The study suggests that Na might be coming from the salinisation from the road salt, wastewater and weathering infrastructure (Jia et. al., 2020).

Generally, high contamination factor (CF) values were observed during the cool–dry season. Our results confirm that during the cool–dry season, metals were not diluted instead they get conserved in the sediments due to less rainfall and water (Dalu et al., 2022). The cool–dry season is characterised by low humidity and cooler temperatures, which can influence the behaviour and distribution of heavy metals. Moreover, metals such as Al and Co were found in moderation in site 1. Thus, it might suggest that Al and Co are being released from the domestic waste and dust from the road and motor industry. This confirms that site 1 received these two metals related to the domestic wastes and industries around and further suggest that some metals might have been accumulated by plants around the site. Our results observed that site 4 ranged from low in the hot–dry season to very high contamination in cool–dry and hot–wet, with Mn, Al, Fe, Cu and Zn having elevated CF values. The current results suggest that the contamination at site 4 shows different seasonal patterns, with cool–dry and hot–wet seasons having the highest levels of pollution, particularly from metals like Mn, Al, Fe, Cu, and Zn. This indicates that during these seasons, the accumulation or mobilization of metals is more noticeable; thus, the CF for these metals is elevated, posing potential environmental and health risks. The metals observed at site 4 might be coming from metal–containing– wastes, and vehicle exhausts, as these activities are the main sources of Mn, Al, Fe, Cu, and Zn (Rashid et al., 2023). Our results indicate that there were significant differences in metals among the sites, which confirms that these sites might receive the same wastes from different industries and seasons did not have any significant differences.

The current study indicates highly moderate–strongly metal pollution and unpolluted during hot–dry season and hot–wet season in site 1, respectively. This might be because site 1 is downstream of other sites where it receives pollutants from other sites and conserves metals during the hot–dry season. During the hot–dry season, there is less water flow, whereas during the hot–wet season, there is more water flow, which results in metal accumulation in sediments (Durrieu et al., 2023). Netshiongolwe et al. (2020) reported different results–, such as sites being extremely polluted during hot–wet season.

Similar to site 1, site 5 was also unpolluted, except for Mn, Co, Cu and Pb during hot–wet and hot–dry seasons. Since forests surround these sites (i.e., 1 and 5), it might be that some terrestrialand aquatic macrophytes have accumulated the metals (Huang et al., 2021; Khellaf et al., 2022; Nguyen et al., 2021; Saha et al., 2022), those plants did act as excluders of Mn, Co, Cu and Pb. Unpolluted to strongly polluted sediments were observed from sites 2 and 3. However, they werenot polluted by Mo and Cd among seasons. Our results suggest that the industries around these sites do not have any source of Mo and Cd. Our study indicates that manganese (Mn) was one of the metals which were extremely polluted in sites 1 and 5 across the hot–dry and hot–wet seasons,this was also confirmed by Netshiongolwe et al. (2020) who reported that Mn was highly polluted insome sites. Generally, the current findings indicate that most metals accumulate during –the cool–dry season.

The current study's findings showed different enrichment factors (EF) in different seasons across the sites. The current study indicates that sites 2 and 3 had low to moderate enrichment of metals such as Mg, P, Ca, Cr, Al, Mn, Fe, Co, Cu, Zn, As and Pb. These two sides are located at the upper side of other sites, hence the enrichment of these metals across all seasons. The metal enrichment was observed more during the cool–dry season in site 3 due to less inflow (Durrieu et al., 2023). In contrast, high EF values were observed during the hot–wet season, thus suggesting that the wetland received high metal pollution from the catchment (Tendaupenyu and Magadza, 2019). Our results indicate that sites 2 and 3 had more metal enrichment, with metals such as Mn, Co, Cu, Zn, As and Pb being associated with transit roads with high traffic density (Świetlik et al., 2015). Świetlik et al. (2015) further explained that metals such as Cu, their enrichment is affected by road traffic, thus suggesting that brakes wear results in an increase in Cu mobility. Generally, EF > 5 metals indicate that they originate from the anthropogenic sources (Saleem et al., 2013).

Our study indicates that there was no pollution observed at sites 1 and 5, which was explained by a pollution load index (PLI) of < 0.02 among all seasons. Our results suggest that the metals might have accumulated in the plants than in the sediments, since forests surrounded these sites (1 and 5), and wetlands had some plant debris. Various studies have been done where plants are found to be good candidates for accumulating or removing heavy metals from the environment through a process called phytoremediation (Khan et al., 2021; Pouresmaieli et al., 2022; Rehman et al., 2024; Shi et al., 2023). Besides, the plants absorb metals, and the dead plant debris with microorganisms might have played a role in reducing pollution in the sediments. Microorganisms can enhance the phytoremediation efficiency of heavy metals (Sharma, 2021). The current findings showed high metal pollution being explained by PLI > 10 during the cool–dry season at site 4. Our results are consistent with the results of Netshiongolwe et al. (2020), who reported high PLI during the cool–dry season. The current results suggest that there is suggest suggesting that there is a deterioration of site 4 sediment quality due to the high PLI. The current results indicate that PLI was not affected by either site or season. The cool–dry season appeared to be the season with a high degree of contamination. During cool–dry season, rainfall and precipitation are less with increased evapotranspiration, and higher saline levels (Serakalala and Mdletshe, 2024).

3.6 Conclusion

The present study, the concentration, spatial distribution, seasonal variation and pollution assessment of metals in wetland sediments were analysed. The study indicates high heavy metal pollution levels of contamination from different sites among seasons. Correlation analyses using PCAdemonstrated a positive association among metals, indicating common source for pollutants., withexception of Na. The use of pollution indices, such as geo-accumulation index, enrichment index, pollution load index and degree of contamination provided a comprehensive assessment of the wetlands impact. Pollution in Riverside Wetlands, particularly for Mn, Al, Fe, Cu, and Zn is concerning. They pose a significant threat to sediment quality. The current findings call for proper conservation and monitoring of Riverside Wetlands and further studies are essential to evaluate the effectiveness of these measures and ensure sustainable management. The call for policymakers to establish guidelines would benefit from specifying responsible stakeholders such as Government departments and concrete measures for wetland protection and agricultural safety. Additionally, there is a need for more assessments on the biological uptake and source of apportionment which are critical for making pollution prevention and control policies (Duan et al.,2020).

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CHAPTER 4.

METAL CONCENTRATIONS IN EDIBLE LEAFY VEGETABLES AND THEIR POTENTIAL RISK TO HUMAN HEALTH

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4.1 Abstract

Leafy green vegetables are a vital source of essential nutrients necessary for human growth. However, their nutritional benefits may be compromised upon consumption of edible leafy vegetables contaminated by heavy metals and trace metals. Therefore, the study aimed to investigate the bioaccumulation of heavy metals in five commonly consumed leafy green vegetables and soils from where these vegetables were grown and determine the human health risks that may arise from consuming those vegetables from Tonga town in Mpumalanga province, South Africa. The edible leafy vegetables included *Lactuca sativa* (lettuce), *Brassica oleracea* (cabbage), *Brassica napus* (rape), *Cucurbita pepo* (pumpkin leaves), and *Spinacia oleracea* (spinach). Soil and plant samples were collected from active cultivation sites and analysed to determine selected heavy and trace metals. The study further evaluated the bioconcentration factor (BCF), daily intake of metals (DIM), human health risk index (HRI) and target hazard quotient (THQ) to quantify potential health risks associated with dietary exposure. The Si, K, Na, Ca, Mg, Al and Fe concentrations were accelerated in the soils. In general, vegetables exhibited elevated Ca, Fe, Si, Al and Sr levels, although spinach had high Na concentrations. Bioconcentration factor showed the trend: Mg > B > Si > V for trace metals and Cr > Co > Mn > Ni > B for heavy metals in lettuce, spinach, and pumpkin leaves. The human risk index for all vegetables showed that all metals were not likely to induce any health hazards to humans, and the target hazard quotient for B, Si, V, Al, Cr, Mn, Fe, Ni, Zn and Pb showed potential for substantial health risk hazard. The findings of this study generally reveal that the concentrations of the analysed metals exceeded the permissible limits established by the World Health Organisation and the Food and Agricultural Organisation. Given the high levels of metals detected in the soil and vegetables within the study area, it is important to investigate their potential implications for human health and mitigate both acute and chronic health challenges associated with heavy metal exposure. Furthermore, this study will guide policymakers in developing improved regulations and safety standards for agricultural land/farming practices and environmental protection, particularly for vulnerable peri-urban and rural communities.

Keywords: *Bioaccumulation, carcinogenic, health risk, heavy metals, leafy vegetables*

4.2 Introduction

Metal contamination in leafy vegetables has become an increasing concern due to its potential risk to human health (Zakir et al., 2021). Leafy edible vegetables, such as *Lactuca sativa* (lettuce), *Brassica oleracea* (cabbage), *Brassica napus* (rape), *Cucurbita pepo* (pumpkin leaves), and *Spinacia oleracea* (spinach), are widely consumed due to their high nutritional value (Feseha et al., 2021; Zakir et al., 2021). However, they can also accumulate toxic heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), copper (Cu), and mercury (Hg) from contaminated soils and water (Akan, 2021; Dalu et al., 2022; Khatun et al., 2022; Fatai, 2024; Fatai et al., 2024). This contamination can occur through multiple pathways, including agricultural practices involving the application of pesticides, herbicides, fertilisers, wastewater irrigation, and proximity to urban or mining areas (Akhtar et al., 2021; Rashid et al., 2023; Sarkar et al., 2024). The accumulation (bioaccumulation) of metals in leafy vegetables presents a significant risk, as these metals can be transferred to animals and humans through consumption (Affum et al., 2020; Uddin et al., 2021; Najmi *et al.*, 2023). Certain metals such as zinc (Zn) and magnesium (Mg) are essential for human growth, but in small quantities. However, excessive exposure to these metals can lead to adverse health effects (Witkowska et al., 2021; Jomova et al., 2022; Khaliq et al., 2022). A notable example is that of cadmium (Cd), can cause kidney damage and bone complications (Olayiwola *et al.*, 2023; Umoren *et al.*, 2024). Lead (Pb) is known to negatively affect cognitive development in children and cause cardiovascular problems in adults (Collin et al., 2022; Nasab et al., 2022), due to its carcinogenic effects (Palma-Lara et al., 2020; Kumar et al., 2021), and mercury (Hg) toxicity can lead to neurological disorders (Kaur et al., 2021; Nabi and Tabassum, 2022).

The African continent is experiencing unplanned rapid urbanisation and industrialisation, which has led to increased pollution and contamination of water and soil (Darko et al., 2021; Chen et al., 2022; Swain, 2024). Many activities such as farming activities (Kookana et al., 2020; Carrilho andTrindade, 2022), human activities (Xiong *et al.*, 2023) especially in urban and peri-urban areas, occur near industrial zones, floodplain areas, wetlands and/or along highways, exposing vegetables to environmental pollutants. Farming activities often rely on irrigation from potentially contaminated water sources (Qadir et al., 2010; Gashaye, 2020; Singh et al., 2024) and contaminated soils (Tauqeer et al., 2022), increasing the likelihood of metal uptake by crops. Therefore, understanding the concentration of metal in these vegetables is essential to adhering to the developed international guidelines for safe consumption levels and potential threats to animal and human health. Some metals are suitable for human consumption. However, they must not exceed the permissible limits described by the World Health Organization (WHO) (Moyo et al., 2020). Regular dietary intake of contaminated vegetables could result in gradual bioaccumulation and biomagnification in the human body, leading to chronic health issues (Ahmed et al., 2022; Gupta et al., 2022).

Exposed vulnerable communities, especially those in rural areas that depend on subsistence farming, are at risk, making it imperative to assess the potential dangers of consuming contaminated vegetables. The studies (Bvenura and Afolayan, 2012; Lion and Olowoyo, 2013) indicated a high hazard quotient (HQ) of spinach in South Africa and further confirmed that it is risky if consumed. Hence, the current study aimed to evaluate metal concentrations in vegetables cultivated by small-scale farmers from their small gardens and sold by subsistence farmers in the town of Tonga at Ehlanzeni District Municipality of Mpumalanga in South Africa. In addition, we assessed the non-carcinogenic risk of vegetable consumption on human health in a rural area in the subtropical South African region. Thus, we anticipate this research will guide policymakers in developing improved regulations and safety standards for agricultural land/farming practices and environmental protection, particularly for exposed vulnerable urban and rural communities. Doing so will help safeguard public health and promote sustainable food production in South Africa.

4.3 Materials and methods

4.3.1 Study site

Soil and vegetable Samples were collected from Tonga (25.7097’ S, 31.7195’ E), Nkomazi Local Municipality in the Mpumalanga province of South Africa (Figure 4.1). This municipality covers 4 786.9 km^2, which is approximately 17 % of the land of the Ehlanzeni district (Nkomazi Local Municipality, 2020). This municipality has 43 villages, and Tonga is one of them (Nkomazi Local Municipality, 2020). Tonga was the site of interest, because they use water from floodplain wetlands and rivers to irrigate their leafy vegetables, with wetlands retaining some of the nutrients and metals in the water.

Tonga is a town in the Nkomazi Local municipality (Figure 4.1), which is dominated mainly by Shangaan/Xitsonga and SiSwati speaking ethnic groups due to its close proximity to Mozambique and Eswatini (Mkhonto, 2018). Tonga's population was 17 333 as of the 2011 census and is estimated to reach over 591 000 residents by 2022. In 2011 Census reported Tonga's population with relatively high density of about 2 300 people per square kilometre

(Morakinyo, 2016). On average, summer temperatures can reach 35 °C or higher during heatwaves, whereas winter can be 10 °C. This area receives summer rainfall between 750 mm and 860 mm, while winters are generally dry.

Geologically, Tonga is based on the Kaapvaal Craton, identified by granite, gneiss, and basalt, impacting the region's soil quality. This combination of geological features and soil types (clay, sandy and alluvial soils) supports agricultural activities, especially sugarcane farming. Alluvial soil is naturally found along the riverbanks, which supports most agricultural production (Khoza, 2023). The main biome within Tonga is the Savanna, which forms one-third of South Africa. It is characterised by an upper covering of shrubs, scattered trees and a ground covered by different types (Mucina *et al.*, 2006).

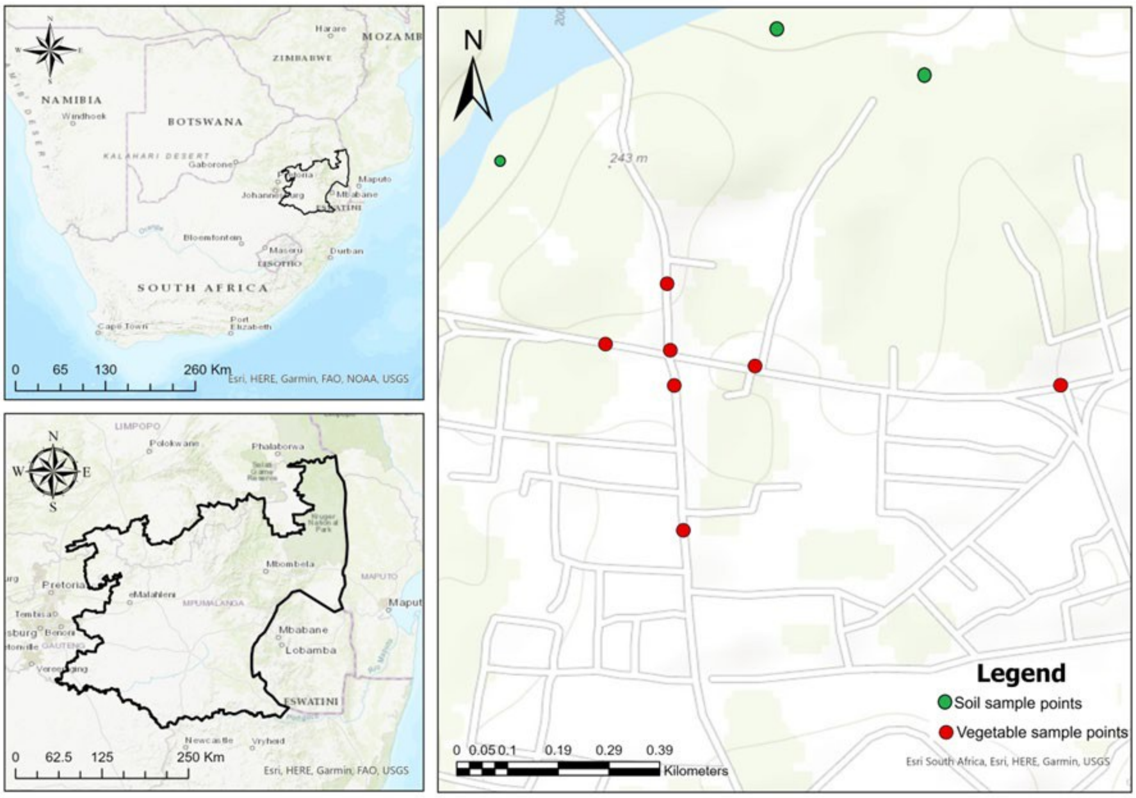

Figure 4.1. *Location of the study area in Tonga, Nkomazi Municipality, Mpumalanga Province of South Africa.*

4.3.2 Collection of soil and vegetables

Edible leafy vegetables (i.e., lettuce, cabbage, rape, pumpkin leaves, spinach, $n = 10 – 40$ leaves per vegetable type) were randomly purchased/bought from different traders within Tonga in

September 2023. Immediately after each purchase, each leafy bundle (*n* = 10) was placed individually in a labelled Ziplock bag and transported to the laboratory. Prior to purchasing the vegetables, the traders were asked where they got the vegetables from; furthermore, visitation of the cultivation areas/gardens was conducted to confirm the source of irrigation water, which was from the nearby river system (i.e., Nkomati). These edible leafy vegetables were not deposited in the herbarium; they are common local vegetables within the study area. Integrated soil samples (*n* = 2, 500 g) from different sites were collected using a plastic hand shovel and placed into polyethylene Ziplock bags.

4.3.3 Vegetable and soil sample preparation

In the laboratory, the edible leafy vegetables were washed with distilled water to remove any soil particles. Each vegetable leaf type and soil samples were then oven-dried at 60 °C for 72 hours. After drying, soil samples were ground using a porcelain pestle and mortar and sieved through a 63 µm sieve to remove any plant material (Bray and Kurtz, 1945). The dried vegetables were ground into a fine powder and homogenised using a kitchen blender (Summit ® Pro Bar Blend series BBS1200, Hamilton Beach Brands Inc., Glen Allan, VA) and 20 g powder of each vegetable was weighed and placed into an airtight Ziplock bag for the determination of trace metal concentrations dynamics: magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), phosphorus (P), boron (B), aluminum (Al), silicon (Si), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd), tin (Sn), barium (Ba), strontium (Sr), and lead (Pb) at the Central Analytical Facility, Stellenbosch University, South Africa.

About 0.5 g of plant and soil samples were digested with concentrated nitric acid (HNO3). The residue was further digested using aquaregia (1: 3, concentrated HNO3: HCl solution, by volume). Vegetable and soil samples were analysed for trace metals using inductively coupled plasma mass spectrometry (ICP-MS). After evaporation of the liquid part to near dryness, 2 % (w/v) of HNO3 was added and centrifuged. Then, trace metal concentrations in the clear solution were determined by ICP–MS. Blank digests were separately carried out for soil and plant samples. In the digestion procedures, pure 65 % HNO3 (Merck) and 37 % HCl (Merck) were used. Double-distilled water was used throughout the analytical work (Avci, 2012).

To determine metal concentrations, 0.5 g of each powdered vegetable was weighed and transferred into a Teflon digestion vessel, where 7 mL of high purity concentrated 65 % nitric acid (HNO_3) and 1 mL high purity concentrated 30 % hydrogen peroxide (H_2O_2) were added. Subsequently, the vessels were capped, fitted into rotor segments, and inserted into the microwave digestion system, where the samples were radiated for 20 minutes. Upon cooling, the solution was transferred into a 50 mL volumetric flask before analysis using an Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS) instrument with an Elan 9000 and Agilent 7500a (Fatai, 2024). All sample analysis was conducted at the Central Analytical Facility, Stellenbosch University, South Africa. The % accuracy of the internal quality control ranged from 89.4 % to 113.6 %, with the tomato leaf certified reference material having % recovery on certified reference of 86.2 % to 114.4 %.

4.3.4 Data analysis

4.3.4.1 Statistical analysis

The data were first tested for normality (i.e., using Shapiro–Wilk test) and homogeneity of variances (i.e., Levene's test) and were found to meet all assumptions of parametric analysis. A one–way analysis of variance (ANOVA) was used to determine the differences in metal concentrations among vegetable types (i.e., lettuce, cabbage, rape, pumpkin leaves, spinach) using SPSS version 25. A principal component analysis (PCA) with the varimax rotation method was used to determine the natural and/or anthropogenic sources of sediment metals among vegetable types, was used for metal source identification in PC–ORD version 5.10.

4.3.4.2 Bio-concentration factor of metals

Bio-concentration factor (BCF) of metals translocated from the soils to vegetables was measured by calculating the ratio of the concentration of each metal in vegetable leaves and the concentration of corresponding metal in the respective soil where these vegetables were grown (Ngoc Ba *et al.*, 2024). A BCF value of less than 1 means minimal translocation of metals from the soils to vegetables, and a value greater than 1 means a high translocation of metals from soils to vegetables.

4.3.4.3 Daily intake of metals

The daily intake of metals (DIM) is the potential risk associated with the ingestion of heavy metals via the consumption of vegetables (Ngoc Ba *et al.*, 2024) and was calculated using the equation:

$$\mathrm{DIM} = \frac{C_{\mathrm{metal}} \times C_{\mathrm{factor}} \times D_{\mathrm{food\ intake}}}{B_{\mathrm{average\ weight}}}$$

where, C_{metal} represent the metal concentrations in vegetables (mg kg^{-1}), C_{factor} is a conversion factor which is estimated at 0.085 (Obiora et al., 2016), the human average body weight (average weight) in South Africa is 60.7 kg (Walpole et al., 2012), and the intake of vegetables(Dfood intake) for South Africans is estimated at 200 g per person per day (Nel and Steyn, 2002; Shisana et al., 2014).

4.3.4.4 Health risk index

The risk to human health (HRI) by intake of metal through the consumption of vegetables in the study area was calculated using an equation described by Fatai (2024):

$$HRI = DIM/RfD$$

where, DIM is the daily exposure to metals and, RfD is the reference oral dose. The RfD values are used in this study for boron (B), aluminum (Al), silicon (Si), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd), tin (Sn), barium (Ba), and lead (Pb) were 0.2, 1, 0.005, 0.007, 1.5, 0.033, 0.7, 0.02, 0.02, 0.4, 0.003, 0.005, 0.6, 0.004, 0.2, 0.004), respectively (Feseha *et al.*, 2021; Nkosi and Msimango, 2022; Chowdhury *et al.*, 2024). If the ratio is lower than 1, then vegetables are not likely to induce any health hazard to humans and If HRI value is equal to or higher than 1, it is considered to be not safe for humans as a potential health risk may occur (Chauhan and Chauhan, 2014).

4.3.4.5 Target hazard quotient

The target hazard quotient (THQ) assesses the non-cancerous risks due to heavy metals intake through regular intake of contaminated vegetable (Gupta et al., 2022). The health risk hazard is enhanced with increased THQ and was calculated using the following equation:

$$\mathrm{THQ} = \frac{\mathrm{EDI}}{R\mathrm{fD}}$$

where, EDI refers to estimated daily intake of heavy metals (mg/person/day), and RfD is oral reference dose. The THQ values of less than 1 mean no non-carcinogenic risks, and greater than 1 value, there is a considered potential of a substantial health risk hazard.

4.4 Results

4.4.1 Soil and vegetable trace and heavy metal dynamics

4.4.1.1 Soil trace metal concentrations

The mean concentrations of trace metals in the soil were generally high for Si, K, Na, Ca, and Mg. In contrast, lower concentrations were observed for P, V, and B (Table 4.1). Similarly, for heavy metals in the soil, elevated levels were recorded for Al and Fe, while much lower concentrations were measured for Cu, Ni, Pb, Co, As, Cd, and Hg (Table 4.1).

4.4.1.2 Trace metal concentrations in vegetables

Cabbage exhibited notably high Ca, K, Na, Mg, and P concentrations, while low concentrations were observed for Si, B, and V (Table 4.1). Rape showed elevated concentrations of Ca, K, Na, Mg, and P, whereas low concentrations were recorded for Si, B, and V, indicating a similar trend as observed in cabbage (Table 4.1). On average, trace metal concentrations in lettuce were high in K, Ca, Na, P, and Mg, with distinctly lower concentrations of Si, B, and V (Table4.1). Concentrations of K, Ca, P, and Mg characterised pumpkin leaves. However, the concentrations of Na, Si, B, and V were generally lower in pumpkin leaves. Spinach leaves exhibited high Na, K, Mg, Ca, and P concentrations, coupled with low concentrations of Si, B, and V (Table 4.1). An Analysis of Variance (ANOVA) revealed significant differences ($p < 0.05$) in the concentrations of Na, Mg, P, K and Ca (Table 4.2).

4.4.1.3 Heavy metal concentrations in vegetables

For heavy metals, cabbage exhibited high concentrations of Fe, Si, Al and Sr, with low concentrations being observed for metals such as Ba, Cr, Ni, Mo, Sn, Pb, Cd, As, and Hg (Table 4.1). In rape, heavy metal concentrations were high in Sr, Fe, and Al, while low concentrations were recorded for Ni, Mo, Sn and Co (Table 4.1). Heavy metals such as As, Cd, Pb, As, and Hg were below the 0.1 mg kg^{-1} permissible levels (Table 4.1). Lettuce had high heavy metal concentrations of Al and Fe, with lower levels in Co, Sn, Mo, Pb, As, Cd, and Hg (Table 4.1). The mean concentration of heavy metals in pumpkin leaves was generally high for Al and Fe, with low concentrations recorded for Co, Mo, Sn, Pb, As, Cd, and Hg (Table 4.1). Spinach leaves were characterised by high levels of Al and Fe, with low concentrations of Co, Sn, Mo, Pb, As, Cd, and Hg (Table 4.1). Analysis of variance for various metals in leafy edible vegetables indicated that Al, Fe, Cu, Zn, As, Sr, Mo, and Cd showed significant differences ($p < 0.005$) among vegetable groups (Table 4.2), indicating variability in heavy metal accumulation across the studied vegetables (Table 4. 2).

Table 4.1: Concentration of metals (mean ± standard deviation) measured in soils (shaded in grey) and edible leafy vegetables from Tonga, South Africa

Metal	Unit	Soil	Cabbage	Rape	Lettuce		Pumpkin		Spinach	
					Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Na	mg/kg	344.6 ± 29.6	19505.2	10514.3	4350.9 – 13537.1	8698.9 ± 3863	486.5 – 1106.1	799 ± 282.1	52152 – 56542.9	54347.5± 3104.8
Mg	mg/kg	678.4 ± 237.3	7186.8	5945.6	3279 – 4936.1	4146.8 ± 714	4066.9 – 6952.7	6041.9 ± 1349.1	8122.4 – 8503.2	8312.8 ± 269.2
P	mg/kg	57.4 ± 11.6	4031.3	4409.5	2922 – 5616	447 ± 31126	7413 – 13689	10182 ± 2598	3124 – 4672	3898 ± 1095
K	mg/kg	641.5 ± 67.8	21746	19603	25270 – 60077	39940 ± 14811	39447 – 60354	51499 ± 8879	21325 – 26835	24080 ± 3896
Ca	mg/kg	318.7 ± 29.2	31268	23448	8253 – 11906	10097 ± 1878	10001 – 10994	10476 ± 409.2	8130 – 8323	8227 ± 136.5
B	mg/kg	1.4 ± 1.2	32.6	23.5	20.6 – 31.3	26.1 ± 4.7	32 – 33.7	32.8 ± 0.7	28.9 – 30.5	29.7 ± 1.1
Al	mg/kg	2753 ± 1301	328.6	93.3	675.1 – 6092	3165 ± 2233	1187 – 2075	1774 ± 410.3	1263 – 2108.3	1685 ± 598
Si	mg/kg	1706.5 ± 813.5	377.2	351.7	279.5 – 602.2	485.5 ± 142.4	386.9 – 538.6	439.3 ± 67.9	298.3 – 518.4	408.4 ± 155.6
V	mg/kg	6.7 ± 2.9	1.3	0.3	1.8 – 19.3	10.1 ± 7.3	1.8 – 5.7	3.1 ± 1.7	4.1 – 5	4.6 ± 0.7
Cr	mg/kg	12.1 ± 7.7	10.6	2.4	8.4 – 186.6	80.1 ± 76.1	21.1 – 47.9	36.3 ± 11.3	32 – 55.6	43.8 ± 16.6
Mn	mg/kg	32.3 ± 29.5	45.3	32.9	47.3 – 139.4	104.9 ± 39.8	46.9 – 75.9	55.8 ± 13.4	113.4 – 124.3	118.8 ± 7.6
Fe	mg/kg	2379 ± 1292	417.8	100.1	682 – 5764	3063 ± 2107	930.4 – 1928	1321 ± 427.4	1491 – 1737	1614 ± 174.3

Co	mg/kg	0.7 ± 0.4	0.9	0.3	0.7 – 17.2	7.2 ± 7	2 – 4.1	2.7 ± 0.9	2 – 2.1	2 ± 0.1
Ni	mg/kg	2.6 ± 2.2	3.9	1.8	4.4 – 63.5	31.3 ± 25.2	13.4 – 25.8	18.9 ± 5.1	13 – 22.8	17.9 ± 6.9
Cu	mg/kg	3.3 ± 1.4	4.5	2.3	7.9 – 20.4	16.3 ± 5.7	13.1 – 17.9	15.6 ± 1.9	12.4 – 16.9	14.7 ± 3.1
Zn	mg/kg	10.4 ± 7.4	28.8	19.9	34.6 – 58.9	48.7 ± 10.8	42.8 – 69.2	52.3 ± 11.6	27.9 – 32.7	30.3 ± 3.3
As	mg/kg	0.4 ± 0.14	0.03	0.02	0.08 – 0.31	0.17 ± 0.10	0.09– 0.32	0.18 ± 0.10	0.09 – 0.13	0.11 ± 0.03
Sr	mg/kg	4.0 ± 2.8	130.3	104.8	45.5 – 67.9	54 ± 10.5	54 – 109.4	81.6 ± 22.8	50 – 56.6	53.3 ± 4.6
Mo	mg/kg	118.2 ± 61.4	3.1	1.6	0.2 – 2.4	1.1 ± 0.9	1.3 – 3	1.9 ± 0.7	1 – 1.1	1 ± 0
Cd	mg/kg	0.00 ± 0.00	0.04	0.01	0.09 – 0.17	0.11 ± 0.04	0.00 – 0.00	0.02 ± 0.01	0.05 – 0.06	0.05 ± 0.01
Sn	mg/kg	0.3 ± 0.1	1.5	1.6	0.8 – 1.5	1.2 ± 0.2	1.1 – 1.3	1.2 ± 0	1.4 – 1.5	1.4 ± 0.1
Ba	mg/kg	21.3 ± 12.6	42.9	46.7	15.9 – 66.7	45.8 ± 22.1	46 – 72.4	60.2 ± 11	91.9 – 126.2	109.1 ± 24.2
Hg	mg/kg	<0.01	0.00	0.01	0.00 – 0.00	0.01 ± 0.01	0.00 – 0.00	0.01 ± 0.00	0.01 – 0.00	0.01 ± 0.00
Pb	mg/kg	2.2 ± 0.9	0.1	0.1	0.3 – 1.3	0.7 ± 0.4	0.3 – 0.6	0.4 ± 0.1	0.3 – 0.4	0.3 ± 0

Trace metals (Na, Mg, P, K, Ca); Heavy metals (B, Al, Si, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Sn, Ba, Hg, Pb)

Table 4.2: Analysis of variance results for metal concentrations in leafy edible vegetables. Values in bold indicate significant differences at $p < 0.05$. Abbreviations: df – degrees of freedom

Variable	**Df**	**F**	***P***
Trace metals			
Na	**4**	**45.548**	**<0.001**
Mg	**4**	**4.745**	**0.036**
P	**4**	**7.045**	**0.013**
K	**4**	**4.840**	**0.034**
Ca	**4**	**29.207**	**<0.001**
B	4	2.910	0.103
Si	4	0.299	0.870
V	4	2.866	0.106
Heavy metals			
Al	**4**	**6.515**	**0.016**
Cr	4	2.395	0.148
Mn	4	4.018	0.053
Fe	**4**	**5.954**	**0.021**
Co	4	1.594	0.277
Ni	4	2.406	0.147
Cu	**4**	**9.410**	**0.006**
Zn	**4**	**6.273**	**0.018**
As	**4**	**4.433**	**0.042**
Sr	**4**	**4.983**	**0.032**
Mo	4	1.566	0.283
Cd	**4**	**20.965**	**0.001**
Sn	4	1.250	0.373
Ba	4	1.786	0.236
Hg	4	0.304	0.867
Pb	4	2.962	0.100

4.4.2 Variation and relationships of metals in vegetables

Our data revealed considerable variation and relationships across different species, reflecting differences in their uptake and accumulation capacities (Figure 4.2). Principal component analysis (PCA) results (Table 4.3) revealed that axis 1 accounted for 42.5 % of the total variance. This axis showed high and moderate positive relations for Sr, Ca, Mg, and Sn and high negative relations for V, Al, Cr, Mn, Fe, Co, Ni, Cu, As, Hg, and Pb (Figure 4.2; Table 4.3). Axis 2 explained 18.1 % of the total variance with positive and significant loadings for P, K, B, and Zn and high negative relations for Na and Sn (Table 4.3). The PCA biplot indicated two distinct metal components, showing the clustering of heavy and trace metals. Metals including As, Cu, Ni, Co, Al, Pb, Ca, Fe, Pb, and Fe clustered with Lettc3. Further, the plot indicates that V had a strong relationship with Lettc1, Mn with Lettc2, and Cd with Spinc2 and Spinc1. A negative relationship between Ba and other metals was observed, where increasing concentrations of other metals corresponded to a decrease in Ba. In terms of P, it clustered with all four pumpkin leaves. The results suggest that concentrations of B and Mo in pumpkins could be lowered with increasing P, and K and Zn could lower Si in pumpkins. The Ca had a strong association with rape and Sn with cabbage. It is clear that Sn in cabbage and lettuce will be lowered with an increase in Na.

Table 4.3: Principal component analysis (PCA) of trace and heavy metals for various vegetables

Variance extracted				
Axis	**Eigenvalue**	**% of variance**	**Cumulative % of variance**	**Broken-stick eigenvalue**
1	10.62	42.46	42.46	3.82
2	4.51	18.05	60.51	2.82
3	3.02	12.08	72.59	2.32
4	2.67	10.68	83.28	1.98
Eigen vectors				
Metals	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
Trace metals				
Na	0.14	**-0.70**	0.56	-0.25
Mg	**0.54**	-0.14	0.31	0.11
P	0.14	**0.86**	0.18	0.09
K	-0.20	**0.90**	0.06	-0.18
Ca	**0.59**	-0.19	-0.26	0.59
Si	-0.40	0.14	**-0.49**	-0.11
V	**-0.95**	-0.19	-0.04	0.1
B	0.38	**0.57**	0.55	0
Heavy metals				
Al	**-0.98**	-0.01	0.08	0.13
Cr	**-0.94**	-0.15	0.02	0.28
Mn	**-0.73**	-0.43	0.4	-0.21
Fe	**-0.97**	-0.13	0.02	0.1
Co	**-0.94**	-0.12	-0.08	0.3
Ni	**-0.95**	0.00	0.06	0.23
Cu	**-0.78**	0.34	0.41	-0.22
Zn	-0.27	**0.83**	-0.13	-0.37
As	**-0.76**	0.40	0.15	0.2

Sr	**0.60**	0.22	-0.01	0.65
Mo	0.04	0.15	0.08	**0.88**
Cd	-0.41	-0.26	-0.49	-0.55
Sn	0.47	**-0.48**	-0.2	0.11
Sb	0.07	0.61	0.13	0.04
Ba	0.07	-0.26	**0.93**	-0.08
Hg	**-0.78**	-0.12	0.05	0.36
Pb	**-0.99**	0.06	-0.01	0.09

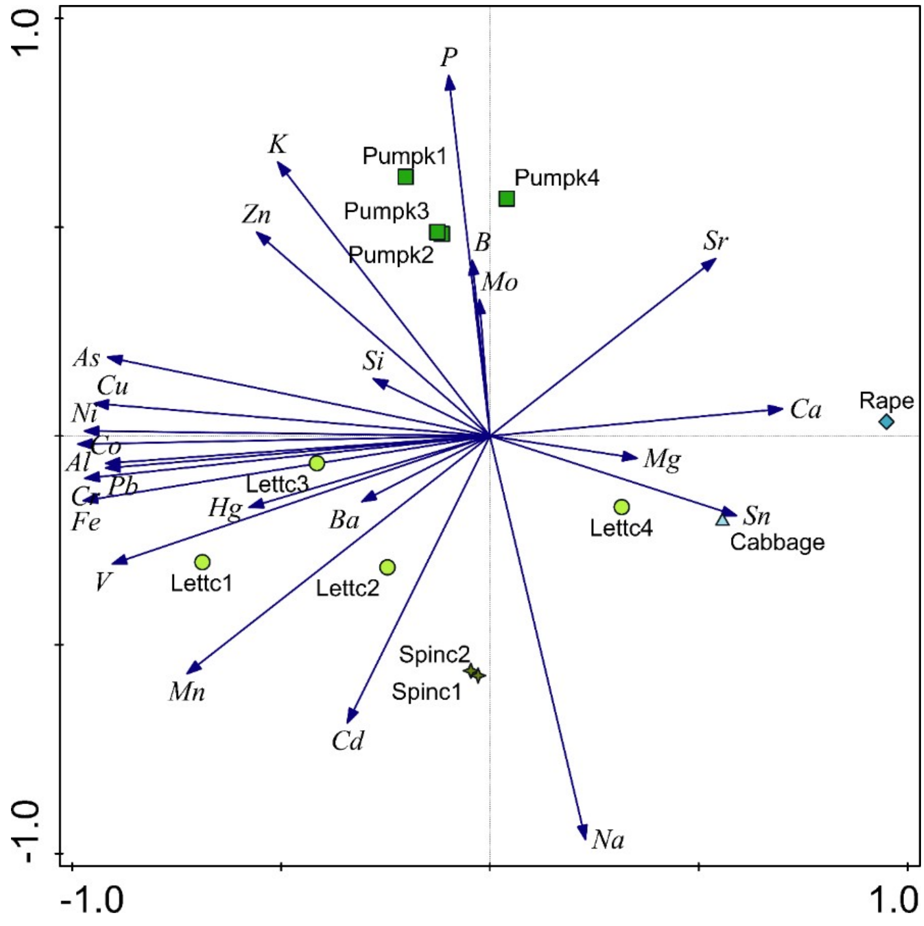

Figure 4.2: The principal component plot in rotated space for trace and heavy metals in the study area. Abbreviations for edible leafy vegetables: Spinc – spinach, Lettc – lettuce, Pumpk – pumpkin leaves.

4.4.3 Health indices

4.4.3.1 Bio-concentration factors

The BCF of trace metals exhibited the following order: Ca > Sr > Mg > B > Si > V for trace metals (Figure 4.3a). Generally, the study indicated that all vegetables showed accumulation of trace metals, except Si and V, which showed low accumulation. However, the BCF values for V were accumulated in lettuce (Figure 4.3a). The BCF of heavy metals showed maximum translocation of Cr, Co, Mn, Ni and Ba, in lettuce, spinach and pumpkin leaves. There was a maximum translocation of B in spinach and Cu in cabbage. Minimum translocation was observed in As, Mo, Cd, Pb and Al from the soil to all leafy vegetables (Figure 4.3b).

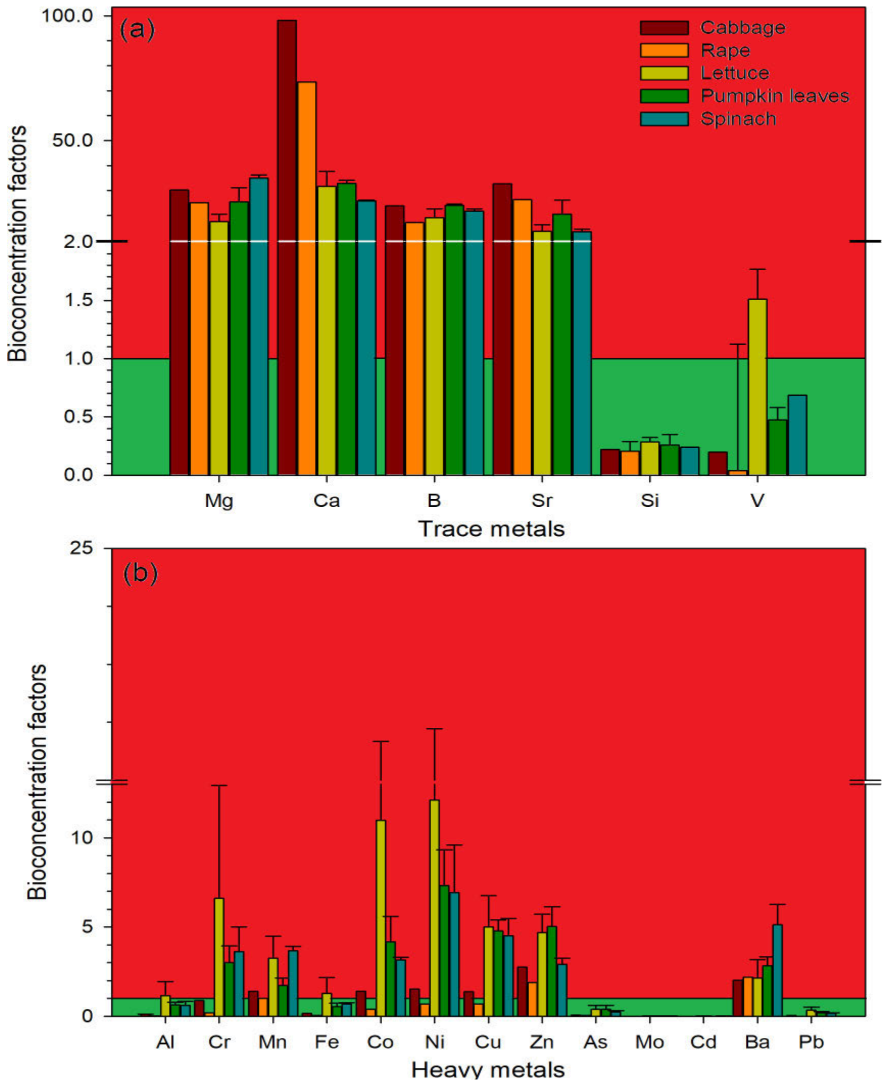

Figure 4.3: The bio-concentration factors (BCF) of leafy edible vegetables from Tonga, South Africa. Red shade – BCF value greater than 1 suggesting a high metal translocation from soil to the vegetables and green shade – BCF values of less than 1 means that there is minimal translocation of metals from the soils to vegetables

4.4.3.2 Daily intake of metals (DIM)

The daily intake of metals by humans when consuming leafy edible vegetables within the study area was compared to the RfD limits set by USEPA. A high intake of K was observed from the consumption of pumpkin leaves and lettuce and Na from the consumption of spinach. In the case of heavy metals, the highest intake of Fe was from lettuce, pumpkin leaves, and spinach (Figure 4.4). Our study showed that Fe exceeded the oral dose described by USEPA guidelines.

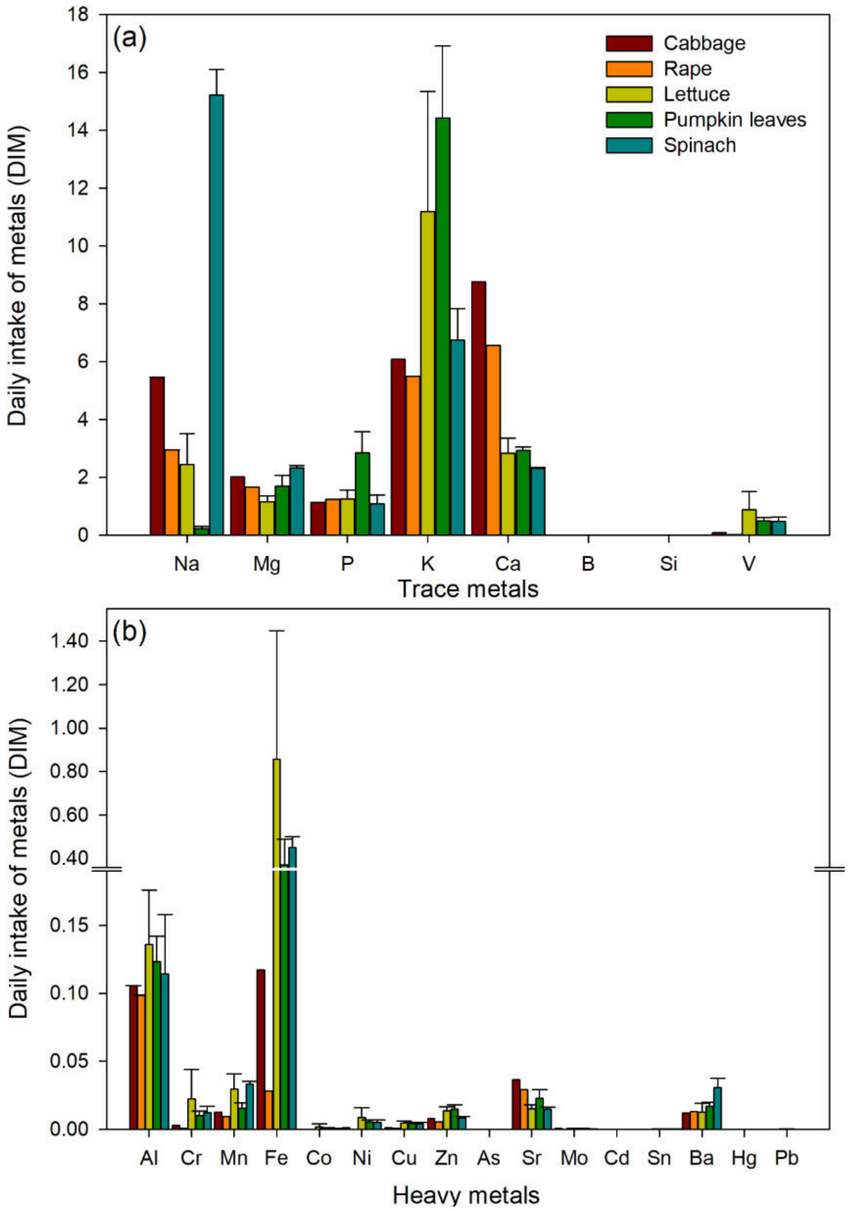

Figure 4.4: The daily intake of metals (DIM) values from consuming leafy edible vegetables.

4.4.3.3 Human health risk index (HRI)

The HRI values for all vegetables were very high and in the following pattern lettuce > spinach > cabbage > pumpkin leaves > rape (Figure 4.5). The study indicated that all HRIs for all edible leafy vegetables were greater than 1, which indicated all vegetables accumulated metals that pose significant harm to humans.

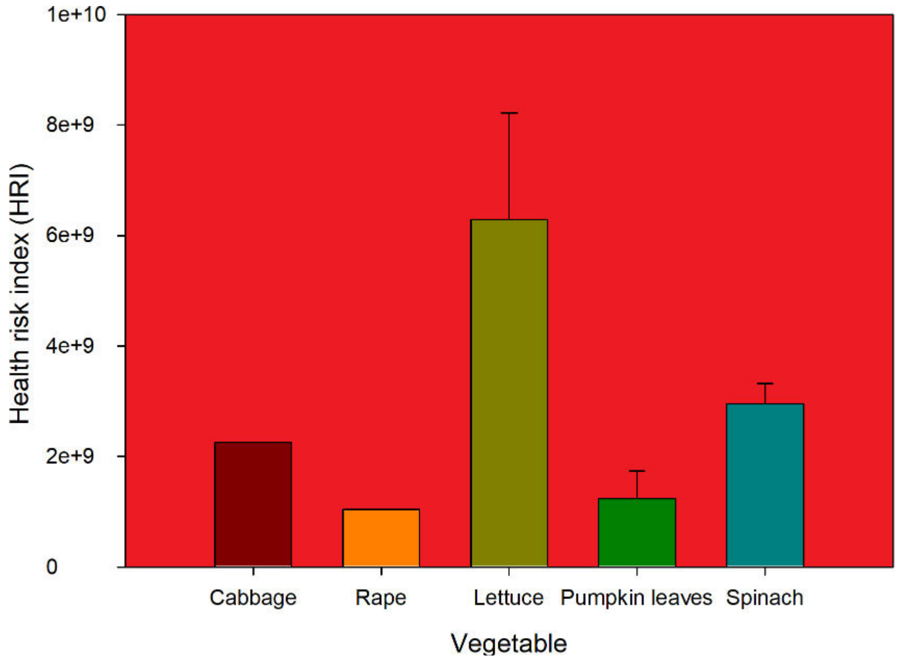

Figure 4.5: The risk to human health (HRI) by intake of metal through the consumption of vegetables. Red shade – HRI value is equal to or higher than 1, it is not safe for humans as a potential health risk may occur Green shade – HRI ratio is lower than 1, the vegetables are not likely to induce any health hazard to humans

4.4.3.4 Target hazard quotient (THQ)

The THQ values for all trace metals (B, Si and V) were greater than 1 for all vegetables. For heavy metals (Al, Cr, Mn, Fe, Ni, Zn, Cd and Pb) THQ values were greater than 1 for all vegetables, whereas for Cu, As, Mo, Sn, and Ba in all edible leafy vegetables, the THQ values were less than 1; however, Ba was greater than 1 in spinach, with Co being less than 1 in rape and high in other vegetables (Table4. 4.4).

Table 4.4: The target hazard quotient (THQ) for leafy edible vegetables. The red and green colour indicate potential of substantial non-carcinogenic risks and no non-carcinogenic risks, respectively

Metals	Cabbage	Rape	Lettuce	Pumpkin leaves	Spinach
Trace metals					
B	975.1	702.7	782.3 ± 143	984.1 ± 21.6	890.6 ± c3.3
S	4476.3	4173.5	5762.2 ± 1690.6	5214.4 ± 806.8	4846.7 ± 1847.3
i	340.7	64.8	2576 ± 1853.2	809 ± 439	1167.7 ± 179.1
V					
Heavy metals					
Al	670.2	190.2	6454.4 ± 4554.5	3618.4 ± 836.9	3437.1 ± 1219.6
Cr	118.1	27.2	891.2 ± 846.9	403.7 ± 126.1	487 ± 185.4
Mn	308.2	224.2	713.9 ± 271.4	380.3 ± 91.8	809.1 ± 52.2
Fe	2334.2	559.3	17110.3 ± 11768.7	7379.4 ± 2388	9015.7 ± 974
Co	1.1	0.3	8.8 ± 8.5	3.3 ± 1.1	2.5 ± 0.1
Ni	>10000	>10000	>10000	>10000	>10000
Cu	0.1	0.03	0.2 ± 0.2	0.2 ± 0.01	0.2 ± 0.01
Zn	>10000	>10000	>10000	>10000	>10000
As	0.01	0.01	0.05 ± 0.03	0.05 ± 0.03	0.03 ± 0.01
Sr	>10000	>10000	>10000	>10000	>10000
Mo	0.0001	0.0001	<0.0001	<0.0001	<0.0001
Cd	>10000	>10000	>10000	>10000	>10000
Sn	0.0001	0.0001	<0.0001	<0.0001	<0.0001
Ba	0.5	0.5	0.5 ± 0.2	0.6 ± 0.1	1.2 ± 0.2
Pb	659.6	338.4	4392.4 ± 2393.2	2660.4 ± 710.2	2300.8 ± 228.2

4.5 Discussion

The present study revealed varying concentrations of metals with high concentrations of Na, Mg, K, and Ca recorded in soils. Contrariwise, we found lower concentrations in P, V, and B. Variation in metal concentrations might be due to the natural processes and anthropogenic activities within the soils (Yahaya *et al.*, 2012) and environmental changes affect the soil concentrations. These results align with a previous study that reported high concentrations of Na, K, Ca, and Mg in soil from fields where cabbage was cultivated (Mohamed *et al.*, 2023).

Among the identified metals, Si had a high concentration in the soil, which might be a reason that Si has a limited uptake ability by plants and remains in the soil (Camargo and Keeping, 2021). Silicon is found naturally in the soil (Tayade *et al.*, 2022) but is not beneficial for plant growth. However, it plays a major role in diseases and pest defence in the soil (Vashi *et al.*, 2020). Potassium had a high mean concentration in the study area's soils. Soils in the study area indicated that K uptake was higher than that of other metals, such as Na, Ca, and Mg. This has been confirmed by Arienzo *et al.* (2009) that absorption of K can be 10 times greater than that of Na. Potassium availability in the soil is dependent on pH of the soil and wastewaters (Arienzo *et al.*, 2009).

For heavy metals, the high mean concentrations were found in Al and Fe only, and other metals such as Cr, Cu, Co, Ni, As, and Zn had low concentrations. Similar results were observed in agricultural fields where Fe (Affum et al., 2020) and Al had high concentrations (Ene et al., 2024). On the contrary, high concentrations of Cr, Zn, Ni and Cu were observed in agricultural soils where different vegetables were grown (Ahmed et al., 2022). These differences might be due to activities, such as mining, emissions from vehicles and the littering of plastics and household waste near agricultural land. Our study reported higher concentrations of trace metals than the heavy metals in the soil, thus suggesting that the accumulation of these trace metals in the soil is an indicationof an impact of fertilisers from agricultural activities, human activities and climate change by affecting their mobility (Swain, 2024).

Cabbage and rape leaves in the study area had high concentrations of Ca, K, Na, Mg, and P. These concentrations were consistent with the results reported by Golubkina et al. (2023) where high concentrations of Ca, K and P were observed and a high concentration ofNa was also observed in cabbage (Mohamed et al., 2023). Moreover, according to Golubkina et al. (2023) cabbage had Si (2.1 mg kg^{-1}) which was reported not to be Si accumulator, however, our study reported that cabbage had accumulated about 377.2 mg kg^{-1} of Si. Potassium levels were higher in lettuce and pumpkin, as well as in all leafy vegetables compared to Ca (Punchay et al., 2020). The current study found high Na accumulation in spinach, which was low in the leafy vegetables tested by Punchay et al. (2020) in Thailand. Overall, Mg was higher than B, Si, and Vi in all leafy vegetables, which is consistent with a study by Punchay et al. (2020).

Even if Mg is important for bone structure, metabolism, and as a powerful antioxidant, however a high level of Mg can be harmful and affect nervous system, which, in turn, may cause insomnia, depression, or illusions and, eventually, progressive alterations in gait and balance and tremor and Parkinson-like symptoms (Buchman, 2014). This study indicates that mean concentrations of Al and Fe were high in all heavy metals in all the vegetables. Similar results were reported by Gebeyehu and Bayissa (2020) and Guadie et al. (2021) where they observed leafy vegetables having high concentration of Fe than Cr, Cu and Pb. Furthermore, lettuce accumulated the highest Fe as compared to other vegetables. Contrarily, it was observed that cabbage was the highest accumulator of Fe (Guadie *et al.*, 2021). Aluminium had higher mean concentrations in lettuce and spinach. Ghasemidehkordi *et al.* (2018) observed that spinach accumulated a high concentration of Al as compared to other investigated leafy vegetables.

The PCA axis 1 and 2 contributed 42.5 % and 18.1 %, total variance, respectively, and showed high and moderate positive relations for Sr, Ca, Mg, Sn and Zn. The current study reported that Cu, Cd, Pb, and Zn have a strong relationship with lettuce. This trend was also reported by Manzoor et al. (2018), who found that Cu, Cd, Pb, and Zn are strongly associated with lettuce.

The current study indicated that trace metals (Mg, Ca, B, Sr, V in lettuce) had maximum translocation from the soil into all leafy vegetables, with their BCF values greater than 1. For Si and V had a minimal translocation from the soil to the leafy vegetables. Contrary to the study of Polechońska et al. (2022), trace metals had a lower BCF, meaning that these metals had a passive translocation from the soil into the leafy vegetables. Maximum translocation of metals suggests that the tested vegetables may be useful in phytoextraction of Mg,Ca, B, Sr and V. The results of this study show that BCF values vary, they have shown species dependent. The BCF values indicate if the plant is an accumulator or an excluder (Eid et al., 2012).

Regarding heavy metals, the BCF showed maximum translocation of Cr, Co, Mn, Ni and Ba, in lettuce, spinach and pumpkin leaves. There was a maximum translocation of B in spinach and Cu in cabbage. Minimum translocation was observed in As, Mo, Cd, Pb and Al from the soil to all leafy vegetables. Similarly, Latif et al. (2018) reported that Cd was passively translocated from the soil into pumpkin leaves and contrarily high BCF for Cr was observed in spinach, however, Chang et al. (2014) observed Cd was highly translocated from the soil into lettuce. Translocation of metals from soils into the plant tissues depends on the soil properties, vegetable's ability to absorb metals and nutrient management (Qazi *et al.*, 2004). Therefore, it is important when choosing vegetables for consumption, the risk of human exposure to metal contamination can be considerably decreased. This study indicates that the tested leafy vegetables are accumulators of metals.

Daily intake of metal (DIM) is used to determine the exposure of metals being consumed by human beings (Ahmed *et al.*, 2022). There are possible human exposure routes such as food-chain, skin-contact, and inhalation, but oral intake is recommended to be the main route for exposure in the food chain (Begum *et al.*, 2021). Our study indicated that consuming pumpkin leaves and spinach there is a chance of consuming high amounts of K and Na, respectively.The study indicated that HRI for all edible leafy vegetables were greater than 1, which indicated all vegetables accumulated metals which pose significant harm to humans (Andleeb *et al.*, 2023).The high HRI values show the possibility of high BCF values of heavy metals being transferred from the soil to the vegetables (Fonge *et al.*, 2021). The results suggest that the population near the study area is highly exposed to high health risks from the metals.

The target hazard quotient method was used to measure the potential human health risk posed by metals through consumption of vegetables (Sridhara Chary *et al.*, 2008). The THQ values for all trace metals (B, Si and V) were greater than 1 for all vegetables. For heavy metals (Al, Cr, Mn, Fe, Ni, Zn, Cd, and Pb). THQ values were greater than 1 for all vegetables, whereas for Cu, As, Mo, Sn, and Ba in all edible leafy vegetables, the THQ values were less than 1; however, Ba wasgreater than 1 in spinach, with Co being less than 1 in rape and high in other vegetables. The current study agrees with results reported by Sharma et al. (2016), where spinach was found to bemore hazardous due to high THQ values greater than 1 of Cd, Co, Fe and Pb. Irrespective of the hazardous effects of these heavy metals, they have some important roles in human bodies; Co is found in Vitamin B12 with various physiological functions (González-Montaña et al., 2020). Sharma et al. (2016) further reported that these heavy metals have health hazards. They target some specific organs: Cd targets kidneys, Co targets the endocrine gland, and Cu and Fe target thegastrointestinal tract due to their high THQ values. If the THQ values of less than 1 mean no non-carcinogenic risks and greater than 1 value, there is a considered potential of substantial health riskhazard (Gupta et al., 2022). In the current study, we observed that these leafy vegetables pose a significant hazard to human beings due to high THQ values.

4.5 Conclusion

The study concludes that different vegetables accumulated different metals at different concentration levels. Furthermore, the study indicates potential health risks associated with consuming vegetables from the current study area due to the high accumulation of metals. There is a high translocation of metals from the soil into the vegetables. In addition, the results of the health risk assessment revealed that the HRI and THQ values through consumption of vegetables were >1, revealing that the people consuming these vegetables might suffer a higher non-carcinogenic risk. Thus, the study recommends an extensive investigation into heavy metals in this area should be a priority since metal concentrations exceeded the limits guided by WHO/FAO. The community members from the study area need to be made aware of the dangers of continuous consumption of vegetables from nearby wetlands, since they pose serious health implications.

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CHAPTER 5.

METAL CONCENTRATIONS, ACCUMULATION AND TRANSLOCATION WITHIN SELECTED AQUATIC MACROPHYTES FROM URBAN WETLANDS

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5.1 Abstract

Globally, metal contamination raises serious concerns within aquatic ecosystems. Wetlands can accumulate high concentrations of metals, acting as sinks for these contaminants. In aquatic systems, metal contamination can be removed by phytoremediation. This study assessed the ability of four macrophytes species (i.e., *Phragmites australis*, *Typha capensis, Cyperus papyrus* and *Cyperus dives*) used in the phytoremediation of metals from five differentwetlands across three seasons (i.e., cool-dry, hot-wet, hot-dry). This study assessed the bioconcentration factor (BCF) and translocation factor (TF). Generally, macrophytes accumulate different metals within the roots and leaves of all species during the cool-dry followed by hot-dry and hot-wet seasons. The study further indicated high BCF ≥ 1 in all macrophytes *C. papyrus,* P. *australis* and *T. capensis* during cool-dry and hot-dry seasons. *Phragmites australis* had the lowest accumulation of metals among the species studied. The translocation factor (TF) was generally greater than 1, indicating efficient movement of most metals from roots to leaves, particularly during the hot-dry and cool-dry seasons. Both *Cyperusdives* and *Typha capensis* exhibited strong potential as phytoremediation candidates. Further research is recommended to evaluate additional species for enhanced phytoremediation effectiveness.

Keywords: *Aquatic macrophytes, bioconcentration factor, phytoremediation, seasons, translocation factor, urban wetlands*

5.2 Introduction

Global rapid urbanisation, industrialisation and an increase in human population; aquatic environments receive significant concentrations of metals from urban wastewater discharge (Tabatabaei *et al.*, 2020; Derwis *et al.*, 2023), which result in wetland malfunctioning (Lettoof *et al.*, 2020; Asongwe *et al.*, 2022). Metals originate from both natural processes, such as volcanic activity and the weathering of rocks, as well as from anthropogenic sources, including discharges of untreated municipal waste, industrial emissions and effluents, traffic-related pollution, mining operations, and the use of fertilizers in agriculture (Muthoni and Kinyua, 2020; Heisi *et al.*, 2023). These activities may directly discharge heavy metals into wetlands, causing bioaccumulation of metals in aquatic environments and degradation of water quality (Yin *et al.*, 2020; Datta *et al.*, 2022; Chakraborty *et al.*, 2023).

The most common metals such as aluminum (Al), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), and cadmium (Cd), tend to accumulate to high toxic concentrations within wetlands (Vidu *et al.*, 2020; Yan *et al.*, 2020), since these metals do not dissolve naturally (Vidu *et al.*, 2020). Furthermore, these metals can be strongly absorbed in the sediments, where they can be transferred within the food chain, which later causes serious health risks to both animals and human beings (Munir *et al.*, 2021; Uddin *et al.*, 2021; Mitra *et al.*, 2022; Validandi *et al.*, 2025). Currently, conventional methods used to remove these metals from the environment, are often time-consuming and ineffective (Maurya *et al.*, 2024). Thus, this calls for more interest in using alternative methods to remove metals from water such as phytoremediation (Vidu *et al.*,2020).

Phytoremediation is defined as a green technology which is currently considered to be environmentally friendly and cost-effective (Bhat *et al.*, 2022; Yadav *et al.*, 2022; Lavanya *et al.*, 2024). Phytoremediation is the preferred method due to its ability to remove metals from water and sediments naturally (Nguyen *et al.*, 2021; Munyai and Dalu, 2023; Kola *et al.*, 2025). Previous studies (Rahman and Hasegawa, 2011; Ali *et al.*, 2013; Amare and Workagegn, 2022; Munyai and Dalu, 2023; Kola *et al.*, 2025) have proved that various aquatic macrophytes have the capability to carry out phytoremediation. Aquatic macrophytes have shown some phytoremediation potential, however, this is normally restricted to different plant parts such as roots, stems, and leaves before releasing them back into the aquatic environment after decomposition (Hossain *et al.*, 2021; Töre and Özkoç, 2022; Rawat and Singh, 2023).

Moreover, these macrophytes are metal-specific, which tends to affect the metal uptake ability (Das *et al.*, 2021); thus, plants absorb some metals and leave others.

The present study aims to evaluate the efficacy of aquatic macrophytes (*Typha capensis* (Rohrb.) N.E.Br., *Cyperus papyrus* L., *Cyperus dives* Delile and *Phragmites australis* Cav. Steud.) as agents for phytoremediation. The main objectives were to (i) determine different types of metal concentrations accumulated in aquatic macrophytes (*T. capensis*, *C. papyrus*, *C. dives,* and *P. australis*) found in Riverside wetlands across three seasons (i.e., cool-dry, hot-dry, and hot-wet), (ii) assess metal accumulation capacity in various macrophyte tissues (i.e., roots, leaves, and stems) to evaluate their effectiveness as bio accumulators and (iii) evaluate contaminant concentrations based on standard indices. This study hypothesises that these macrophytes will accumulate different metals in different tissues across different seasons.

5.3 Materials and methods

5.3.1 Study area

The study was carried out within Riverside's wetlands, City of Mbombela, South Africa (Figure 5.1), and these wetland systems range in size between 1 500 and 38 000 m^2. Riversideis located within the Lowveld region of Mpumalanga province, Ehlanzeni District Municipalityin the north-eastern part of the country (Nkosi and Mathivha, 2025). The elevation ranges from632 m to 688 m above sea level. The wetlands are located within the Riverside improvement District area, with the catchment dominated by industrial areas. The area has a mild subtropicalclimate, with its temperatures during summer ranging between 16–19°C and 25–27 °C, to 10–16 °C and 22–26 °C in winter. The study area receives a mean annual rainfall of 667 mm yearly(Nkosi and Mathivha, 2025). Five sites were randomly selected from the five Riverside Wetlands, each with 6 locations where sediments were collected. The sampling was conductedin the hot-wet (December 2023), the hot-dry (September 2023) and cool-dry (March 2024) seasons.

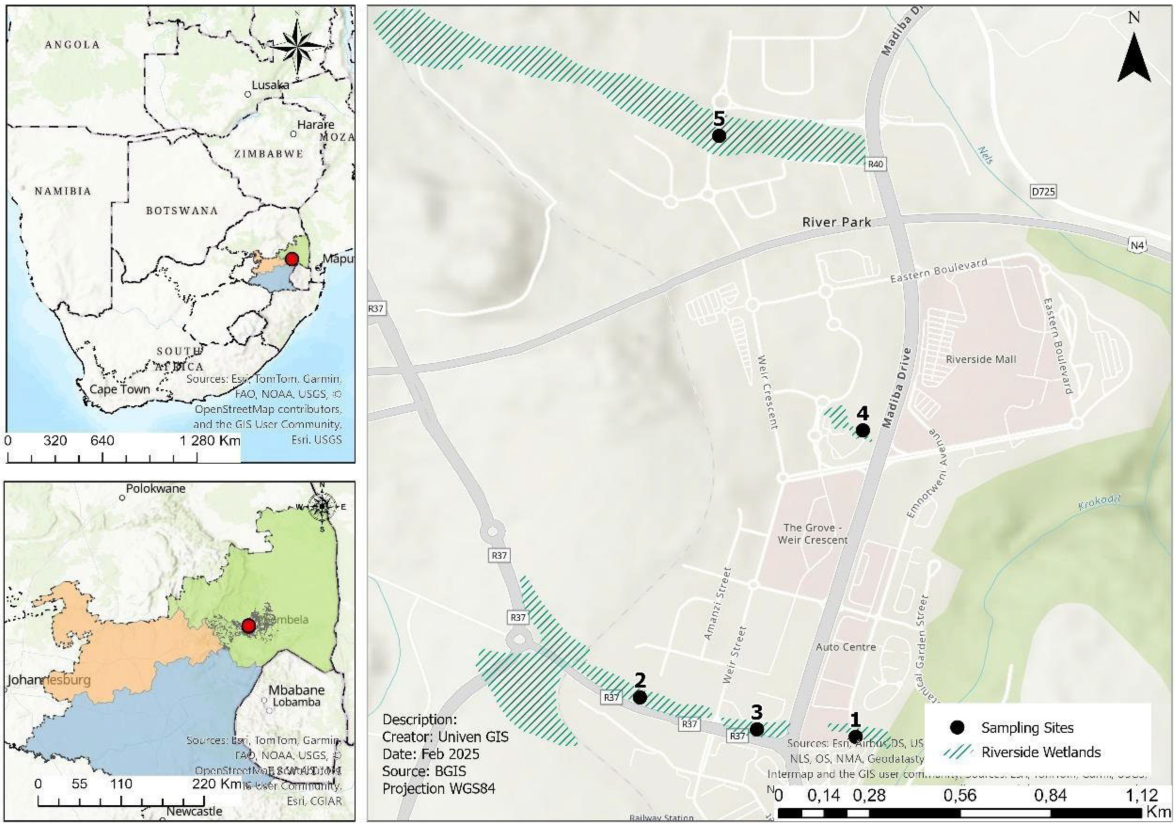

Figure 5.1. Location of the sampling sites within the Riverside Wetlands in the City of Mbombela, South Africa

5.3.2 Collection and processing of samples

Four perennial common emergent macrophytes which are dominant and naturally growing in the Riverside wetlands, namely, *C. papyrus, C. dives, P. australis* and *T. capensis,* were collected from five different sites for three seasons [hot–wet season (December 2023), hot–dry season (September 2023) and cool–dry season (March 2024)]. About four plants per species were randomly collected at every site per season. Then, macrophytes were carefully uprooted using a digging fork, ensuring that all roots were not damaged and properly preserved, then washed with wetland water thoroughly to remove any particles and sediments. Further, macrophytes were then divided into three different parts (i.e. leaves, stems, and roots) and placed into three separate polyethylene Ziplock bags.

In the laboratory, the roots, stems, and leaves were further washed with deionized water and oven dried at 70°C for 72 h. After drying, the plant parts were ground into a fine powder using a kitchen blender (Summit ® Pro Bar Blend series BBS1200, Hamilton Beach Brands Inc., Glen Allan, VA) and hard plant parts were ground using a grinding machine (Retsch, SM 300). A 20 g powder of each macrophyte part was weighed and placed into a labelled Ziplock bag for the determination of metal and nutrient concentration.

5.3.3 Metal analysis

To determine metal concentrations, 20 g of each powdered macrophyte was weighed and transferred into a Teflon digestion vessel, where 7 mL of high purity concentrated 65 % nitric acid (HNO3) and 1 mL high purity concentrated 30 % of hydrogen peroxide (H2O2) were added. Then, vessels were capped and fitted into rotor segments and inserted into the microwave digestion system, where the samples were radiated for 20 minutes. Upon cooling, the solution was transferred into a 50 mL volumetric flask before analysis using an Inductively Coupled Plasma Mass–Spectrophotometer (ICP–MS) instrument with an Elan 9000 and Agilent 7500a (Fatai *et al.*, 2024). All sample analysis was conducted at the Central Analytical Facility, Stellenbosch University, South Africa.

5.3.4 Data analysis

Bio–concentration factor (BCF) of metals translocated from the sediments to macrophytes was measured by calculating the ratio of the concentration of each metal in different parts (i.e. roots, stems, leaves) of macrophytes and the concentration of corresponding metal in the respective sediments (Ngoc Ba *et al.*, 2024). A BCF value of less than 1 means minimal accumulation of metals from the sediments to macrophytes, and a value greater than 1 means a high accumulation of metals from soils to macrophytes. Equation used by Ngoc Ba *et al.* (2024) was used to calculate BCF as follows:

$$\text{BCF} = \frac{C_{macrophyte}}{sediment}$$

Where Cmacrophyte is the metal concentration in the fern tissue (i.e., root, rhizome or fronds) andCsediment is the metal concentration in sediment where the ferns were kept. A BCF value > 1 indicates that the macrophyte can accumulate more metals than are available in the water (i.e.,hyperaccumulation). A BCF value ≤ 1 suggests that the macrophyte can only absorb metals inproportion to what’s available in the sediments.

The transportation index (*Ti*) gives the leaves/root or stem/root metal concentration, and it depicts the macrophyte ability to translocate metal species from roots to the stems or leaves at different concentrations. It is calculated based on the equation:

$$T_i = \frac{Metals\ in\ leaves\ or\ stems}{metals\ in\ roots} \times 100$$

A $Ti > 1$ means the macrophyte can efficiently move the metal from its roots to its stems or leaves, and a $Ti < 1$ is ideal for phytostabilisation.

A three-way analysis of variance (ANOVA) was used to examine the effect of seasons (i.e., cool-dry, hot-dry, and hot-wet), macrophyte species (i.e., *C. papyrus, C. dives, P. australis*, *T. capensis*) and macrophyte part/location (i.e., roots, stems, and leaves) on the measured metal concentrations and bioconcentration factors (BCF). A two-way ANOVA was used for models considering translocation factors (TF). All significant variables were further assessed using the Tukey HSD tests to determine which specific group (i.e., parts/locations, seasons, species) means were significantly different from each other.

5.4 Results

5.4.1 Metal concentrations in macrophytes parts across various seasons

The four macrophyte species differed in metal concentrations (Figure 5.2, Table 5.1). Of all the metals, Mg was high in all macrophyte species (i.e., *C. papyrus, C. dives* and *T. capensis*) across all the seasons, whereas *P. australis* had high Mg only in the roots during cool-dry and hot- wet and in stems in hot-wet. Furthermore, the study revealed high Cr concentration in the *C. dives* roots (cool-dry) and leaves (cool-dry and hot-dry), also high in the stems cool-dry and hot-wet) and in the leaves (cool-dry) in *T. capensis.* High Fe, Pb and Al were generally observed in all *C. dives, P. australis* and *T. capensis* parts during cool-dry season. The results of ANOVA showed that Mg, Cr, Fe, Pb and Al concentrations were significantly different (p < 0.05) across species (Table 5.2). Furthermore, the interaction macrophyte species × location showed significant differences (ANOVA, $p < 0.05$) in Na, Pb, B, Cd, Mo, Zn, Cu, Co, Fe, Mn, Cr, Ca, K and Mg concentrations (Table 5.2), with macrophyte location being a major driver. However, the interaction of macrophyte species × location × season only indicated significant differences for Mo (0.026) and B (0.008) concentrations, with and seasons being major drivers of metal accumulation (Table 5.2), with season × location showing significant differences for Fe (0.014), Co (0.050), Mo (0.016), and Al (0.017) (Table 5.2).

5.4.2 Transportation index (translocation factors) of metals

Transportation factors (TF) of metals shown in Figure 5.3 indicates that most metals in *C. papyrus* had leaves/roots TF of > 1 across all the seasons, except Na, K, Ca, Mn and B during cool-dry season, and Fe, Al, Pb, B, As and Cd in the hot-dry season. For stem/roots translocation, the TF values for Na, K and Ca were >1 during the cool-dry season. *Cyperus dives* metals such as Na, K, Ca and P indicated TF of > 1 across all the seasons. Additionally, *C. dives* TF > of Na, K, Ca and P were observed in stem/roots across all seasons, except for B, which was translocated only during hot-dry and cool-dry seasons. *Phragmites australis* TF in leaves/roots was > 1 for Mg, K, Ca, P and B across all seasons, whereas Mn TF value was > 1, during the hot-dry and hot-wet seasons. The higher translocation in stem/roots, Na, Ca, P and B, were observed during the hot-wet season and for K across all seasons. *Typha capensis* had TF of >1 of Na and P during the hot-dry season, K, Ca and Mn across all seasons and B during the hot-dry and hot-wet seasons. Generally, most metals in *T. capensis* were translocated from the roots to the stems across all seasons, whereas, Al, Pb, Fe and Cd were translocated during the hot-wet season. Based on the three-way ANOVA, metals such as Cr, Fe, Co, Cu, Zn, As, B, Pb, and Al were significantly different ($p < 0.05$) among macrophytes species, with seasons showing significant differences ($p < 0.05$) for Ca, P, Cr, Fe, Co, Cu, Zn, Mo, As, B, Pb and Al (Table 5.3).

Table 5.1: Mean ± standard deviation of metal concentration (mg kg^{-1}) in different macrophyte species across various macrophyte parts (i.e., roots, stems, leaves) amongst three seasons (i.e., cool–dry, hot-dry, and hot–wet).

Metals	**Leaves**				**Roots**				**Stems**			
	Cool-dry											
	Cyperus papyrus	*Cyperus dives*	*Phragmites Australis*	*Typha capensis*	*Cyperus papyrus*	*Cyperus dives*	*Phragmites Australis*	*Typha capensis*	*Cyperus papyrus*	*Cyperus dives*	*Phragmites australis*	*Typha capensis*
Na	8.30 ± 0	6.30 ± 2.05	0.09± 0.70	4.03 ± 1.21	9.76 ± 0	3.67 ± 1.72	3.32 ± 1.81	3.11 ± 1.74	18.29 ± 0	6.78 ± 2.01	0.15 ± 0.20	9.72 ± 2.99
Mg	1.96 ± 0	0.00 ± 0.00	1.56 ± 0.10	2.48 ± 0.37	1.94 ± 0	2.42 ± 0.58	2.23 ± 0.61	2244.6 ± 0.60	1.36 ± 0	2.14 ± 0.50	301.1 ± 94.6	4.02 ± 0.70
K	17.92 ± 0	0.03 ± 0.00	14.08 ± 1.78	39.01 ± 11.03	33.80 ± 0	2.61 ± 20.13	18.12 ± 18.48	10.76 ± 4914	41.55 ± 0	27.82 ± 310.41	20.02 ± 3.61	58.28 ± 22.46
Ca	5.40 ± 0	0.01 ± 0.00	5.11 ± 2.72	13.01 ± 2.07	3.05 ± 0	8.25 ± 5.29	6.44 ± 4.49	4.41 ± 0.165	4.63 ± 0	6.07± 1.71	0.48 ± 0.43	25.71 ± 7.53
P	2.87 ± 0	0.00 ± 0.00	1.65 ± 0.38	2.54 ± 0.79	2.08 ± 0	2.46 ± 0.8	2.23 ± 1.11	1.80 ± 0.69	1.11 ± 0	2.02 ± 0.58	0.97 ± 0.41	2.80 ± 1.49
Cr	1.57 ± 0	0.00 ± 0.00	5.49 ± 0.99	2.48 ± 2.21	28.17 ± 0	19240.9 ± 19.06	23.34± 16.21	26.07 ± 12.18	4.10 ± 0	14.45 ± 7.48	3.00 ± 1.92	11.66 ± 12.43
Mn	126.49 ± 0	0.35 ± 0.01	197.88 ± 168.012	1015.78 ± 438.34	86.99 ± 0	635.52 ± 356.15	601.24 ± 400.40	429.57 ± 282.62	80.25 ± 0	468.20 ± 229.39	28.65 ± 7.74	1039.31 ± 526.73

Fe	284.85 ± 0	0.26 ± 0.02	351.13 ± 324.13	319.67 ± 173.53	1593.82 ± 0	5769.1 ± 6 7062.2 0	6749.4 ± 3 6396.0 11	7472.8 ± 0 5635.5 9	14.22 ± 0	530.5 ± 7 164.3 9	128.3 ± 5 65.54	975.4 ± 0 1137. 89
Co	0.31 ± 0	0.27 ± 0.12	0.12 ± 0.07	0.26 ± 0.17	1.74 ± 0	3.08 ± 3.37	3.68 ± 2.94	4337.9 ± 2241.7	0.56 ± 0	0.61 ± 0.44	0.12 ± 0.47	0.70 ± 0.35
Cu	11.52 ± 0	3.93 ± 1.08	4.01 ± 0.62	5.01 ± 1.51	82.12 ± 0	11.88 ± 6.23	14.13 ± 4.95	17.43 ± 4.45	8.90 ± 0	4.84 ± 1.84	3.26 ± 2.73	5.19 ± 5.25
Zn	19.55 ± 0	18.61 ± 1.44	38.38 ± 7.02	38.62 ± 14.82	433.00 ± 0	97.30± 51.02	139.61 ± 87.80	166.30 ± 61.74	176.36 ± 0	27.20 ± 10.54	19.41 ± 14.25	66.09± 42.80
Mo	0.21 ± 0	0.91 ± 0.36	1.03 ± 0.14	0.78 ± 0.40	0.51 ± 0	0.65 ± 0.15	0.73± 0.14	0.81 ± 0.14	0.21 ± 0	0.67 ± 0.16	0.53 ± 0.53	0.68 ± 0.12
Cd	0.14 ± 0	0.02 ± 0.09	0.00 ± 0.00	0.02 ± 0.00	0.27 ± 0	0.04 ± 0.02	0.05 ± 0.02	62.4 ± 14.7	0.26 ± 0	0.034 ± 0.02	0.01 ± 0.00	0.02 ± 0.01
As	0.05 ± 0	0.12 ± 0.05	0.16 ± 0.05	0.122 ± 0.00	0.34 ± 0	2.9 ± 3.63	3.15 ± 3.47	3.31 ± 3.32	0.06 ± 0	243.2 ± 157.7	0.19 ± 0.19	0.25 ± 0.19
B	17.23 ± 0	7.73 ± 1.28	5.30 ± 1.62	15.74 ± 4.28	8.84 ± 0	12.08 ± 6.98	10.42 ± 7.23	7.91 ± 1.86	8.41 ± 0	11.59 ± 1.69	1.98 0.00 ±	13.04 ± 3.43
Pb	0.32 ± 0	0.53 ± 0.07	0.34 ± 0.09	0.51± 0.28	6.05 ± 0	0.00 ± 5.35	7.11 ± 4.75	8.27 ± 3.10	0.38 ± 0	1.05 ± 3.35	0.25 ± 0. 18	1.47 ± 1.11
Al	116.15 ± 0	273.11 ± 10	89.30 ± 54.19	309.30 ± 238.52	1785.12 ± 0	4103.0 ± 6 5700.0 5	5066.2 ± 7 5251.2 9	6091.0 ± 8 4523.8 9	117.27 ± 0	568.8 ± 40 272.6 9	82.35 ± 68.201	1029.98. ± 0
						Hot- dry						
Na	6.57 ± 0	5.67 ± 0.54	0.13 ± 0.05	4.51 ± 2.29	3.15 ± 0	1.64 ± 0.47	0.00 ± 0.00	6.99 ± 3.81	9.41 ± 0	5.21 ± 0.66	0.22 ± 0.25	6.61 ± 3.69

Mg	1.69 ± 0	1.73 ± 0.34	1.55 ± 0.99	2.51 ± 0.66	1.79 ± 0	1.55 ± 0.07	0.00 ± 0.00	2.85 ± 0.75	1.18 ± 0	2.54 ± 0.29	0.35 ± 0.04	3.80 ± 1.23
K	28.40 ± 0	24.01 ± 5.54	16.63 ± 6.04	28.84 ± 14.15	22.58 ± 0	5.75 ± 0.33	0.0 ± 0.00	20.72 ± 6.13	47.60 ± 0	28.33 ± 7.34	16.30 ± 3.09	45.38± 15.18
Ca	2.87 ± 0	4.75 ± 0.94	3.91 ± 0.84	10.50 ± 4.75	1.64 ± 0	2.62 ± 0.59	0.00 ± 0.00	6.43 ± 1.06	1.90 ± 0	4.49 ± 0.78	362.3 ± 9.8	16.11 ± 3.63
P	2.54 ± 0	2.88 ± 0.87	1.37 ± 0.07	2.12 ± 0.57	2.34 ± 0	1.52 ± 0.36	0.00 ± 0.00	3.05 ± 1.11	1.95 ± 0	4.20± 1.44	622.5 ± 285.1	2.96 ± 0.90
Cr	44.21 ± 0	21.83 ± 12.64	55.25 ± 0.36	174.4 ± 67.56	143.71 ± 0	685.76 ± 177.09	0.11 ± 0.04	245.29 ± 245.86	11.08 ± 0	56.09 ± 34.88	13.02± 4.65	24.01 ± 22.36
Mn	122.37 ± 0	330.18 ± 104.91	183.3 ± 2 136.4 2	59.58 ± 305.22	68.11 ± 0	412.54± 11.87	0.09± 0.01	53.45 ± 419.17	46.23 ± 0	435.9 ± 4 143.5 0	23.49 ± 1.98	557.1 ± 6 243.9 7
Fe	848.08 ± 0	273.64 ± 130.60	431.8 ± 4 145.1 3	2020.5 ± 0 4167.1 1	1359.30 ± 0	10507. ± 93 3176.7 2	1.85 ± 0.84	5923.1 ± 8 5087.5 5	111.14 ± 0	576.7 ± 6 213.7 3	102.51 ± 8.93	369.45± 184.30
Co	3.65 ± 0	1.49 ± 1.04	2.34 ± 0.13	18.83 ± 40.09	11.47 ± 0	70.07 ± 19.61	0.00 ± 0.00	19.98 ± 17.13	0.76 ± 0	4.55 ± 4.62	0.78± 0.02	2.39 ± 2.10
Cu	13.29 ± 0	3.88 ± 1.39	5.10 ± 0.28	9.18 ± 13.24	56.49 ± 0	3.16 ± 5.01	0.00 ± 0.00	16.23 ± 9.54	11.82 ± 0	6.05 ± 1.79	0.00 ± 0.00	3.83 ± 1.53
Zn	283.01 ± 0	16.69 ± 1.22	22.73 ± 2.43	54.18 ± 56.42	412.12 ± 0	175.53 ± 6.00	26.70 ± 3.38	218.06 ± 136.79	185.31 ± 0	26.37 ± 3.91	14.83 ± 7.40	105.0 ± 4 86.61
Mo	0.85 ± 0	0.68 ± 0.31	1.37 ± 0.22	3.12 ± 5.80	1.97 ± 0	10.44 ± 3.02	1.73 ± 0.57	3.90 ± 29.78	0.28 ± 0	1.19 ± 0.64	461.1 ± 285.9	0.74 ± 0.38

Cd	0.09 ± 0	0.01 ± 0.00	0.07 ± 0.00	0.03 ± 0.05	0.17 ± 0	0.14 ± 0.06	0.00 ± .00	0.11 ± 0.068	0.01 ± 0	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
As	0.38 ± 0	0.11 ± 0.02	0.22 ± 0.08	0.55 ± 1.14	0.27 ± 0	4.22 ± 4.83	1.76 ± 1.03	1.43 ± 0.99	0.01 ± 0	0.17 ± 0.09	0.10 ± 0.01	0.11 ± 0.06
B	21.32 ± 0	6.27 ± 0.24	5.62 ± 0.07	8.54 ± 2.06	6.51 ± 0	4.72 ± 0.45	1.50 ± 0.01	10.07 ± 1.87	5.01 ± 0	10.48 ± 0.64	1.14 ± 0.04	11.13 ± 2.15
Pb	1.01 ± 0	0.41 ± 0.11	0.63 ± 0.33	2.25 ± 4.57	1.41 ± 0	13.07 ± 8.80	1.39 ± 0.11	4.24 ± 3.48	0.11 ± 0	0.55 ± 0.23	0.075 ± 0.05	0.61 ± 0.68
Al	1173.36 ± 0	195.45 ± 92.30	125.98 ± 36.72	872.28 ± 1693.5 5	156.99 ± 0	455.06 ± 1955.1 7	1231.9 ± 5 20.70	2286.5 ± 9 1512.5 7	67.11 ± 0	353.1 ± 5 256.7 6	42.301 ± 4.53	0.32 ± 240.34
	Hot-wet											
Na	3.01 ± 0	5.25 ± 1.52	150.8 ± 54.2	4.01 ± 1.85	7.47 ± 0	2.29 ± 1.06	0.23 ± 0.08	4.22 ± 1.67	10.42 ± 0	5.85 ± 1.31	0.29 ± 0.14	9.02 ± 5.97
Mg	1.44 ± 0	1.71 ± 0.45	678.1 ± 83.2	2.03 ± 0.29	1.34 ± 0	1.87 ± 0.36	0.26 ± 0.14	1.99 ± 0.39	1.08 ± 0	1.86 ± 0.55	0.18 ± 0.11	3.02 ± 0.76
K	12.18 ± 0	18.48± 2.80	14.69 ± 5.43	18.66 ± 4.68	31.64 ± 0	6.8 3± 1.97	12.01 ± 1.53	11.10 ± 1.06	44.81 ± 0	25.39 ± 6.57	17.64 ± 5.31	24.27 ± 5.55
Ca	3.00 ± 0	4.96 ± 0.98	3.19 ± 0.99	9.77 ± 1.48	2054.3 ± 0	4.46 ± 1.23	0.34 ± 0.15	5.31 ± 1.37	3.02 ± 0	3.32 ± 0.91	0.32 ± 0.15	12.81 ± 3.82
P	2.64 ± 0	1.76 ± 0.22	1.41 ± 0.81	1.55 ± 0.34	1.56± 0	1.66 ± 0.36	0.61 ± 0.41	1.54 ± 0.52	2.38 ± 0	2.07 ± 0.70	0.96 ± 0.95	1.78 ± 0.53
Cr	9.51 ± 0	111.32 ± 112.25	4.62 ± 7.67	38.19 ± 32.56	49.04 ± 0	33.014 ± 202.14	99.06± 95.88	512180 ± .1 281534 .7	56.08 ± 0	168.9 ± 5 148.9 6	20.84 ± 9.55	87.43 ± 43.85
Mn	188.18 ± 0	219.00 ± 128.47	83.80 ± 40.47	645.38 ± 91.98	128.96 ± 0	352.84 ± 255.33	72.46 ± 37.39	511.55 ± 311.65	147.20 ± 0	214.1 ± 9	31.36 ± 2.26	431.1 ± 0

										106.0 2		114.4 1
Fe	17.75 ± 0	1233.60 ± 1 1477.30	342.87 ± 17.38	557.33 ± 496.51	482.10 ± 0	6447.8 ± 0 4370.1 0	2029.9 ± 4 2472.5 5	9824.7 ± 8 4695.9 1	568.23 ± 0	1415. ± 59 1402. 57	258,90 ± 155.35 5	1518. ± 48 2216. 47
Co	1.40 ± 0	4.76 ± 3.01	1.31 ± 0.77	1.10 ± 1.70	4.20 ± 0	25.22 ± 12.92	10.99± 13.42	50.87 ± 26.26	1.73± 0	6.64 ± 3.38	1.77 ± 9.86	5.86± 3.42
Cu	8.21± 0	6.21 ± 4.39	8.19± 5.57	3.59 ± 1.35	13.71 ± 0	33.93± 26.27	6.24 ± 2.92	25.29 ± 3.77	8.32 ± 0	7.45.1 ± 4.41	2.35 ± 0.34	8.14 ± 7.98
Zn	86.65 ± 0	31.88 ± 4.73	25.46 ± 10.27	26.54 ± 12.72	119.69 ± 0	257.79 ± 3 162.73	20.13 ± 5.79	0.02 ± 0.08	42.38 ± 0	31.54 ± 12.04	15.66 ± 3.98	15.83 ± 20.81
Mo	0.25 ± 0	1.79 ± 0.75	1.16 ± 0.25	0.77 ± 0.42	1.07 ± 0	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.71 ± 0	2.12 ± 0.60	0.86 ± 0.48	1.69 ± 0.64
Cd	57.4 ± 0	23.5 ± 11.6	5.6 ± 0.3	7.8 ± 3.6	0.07 ± 0	0.18 ± 0.21	10.4 ± 6.2	0.10 ± 0.03	0.05 ± 0	31.4 ± 15.5	5.9 ± 0.1	34 ± 49
As	48.4 ± 0	257.06 ± 0	264.8 ± 1.1	152.8 ± 162.8	0.18 ± 0	2.10 ± 1.04	2.29 ± 3.24	3.47 ± 3.17	0.10 ± 0	0.26 ± 0.12	0.25 ± 0.23	0.41 ± 0.69
B	6699.6 ± 0	6319.7 ± 772.9	19969. ± 9 24031. 7	8644 ± 976.7	9.82 ± 0	8.57 ± 2.60	1.16 ± 0.70	7.03 ± 2.20	10.16 ± 0	7.47 ± 1.34	1.06 ± 0.20	10.06 ± 2.78

Pb	163.2 ± 0	1463.8 ± 1637.5	265.7 ± 54.6	696.3 ± 640.9	0.64 ± 0	10.12 ± 9.40	1.18± 1.23	10.10 ± 2.72	0.58 ± 0	1.51 ± 1.30	0.25 ± 0.14	3.01 ± 5.65
Al	72943.4 ± 0	1283344.6 ± 1971246.8	138465.9 ± 19763.1	459317.5 ± 344321.6	476.35 ± 0	4.38 ± 4340.45	927.85 ± 767.13	4954.23 ± 1820.68	360.07 ± 0	984.85 ± 1167.77	161.68 ± 78.03	996.15 ± 1319.27

Table 5.2: Three-way analyses of variance (ANOVA) based on significant (p < 0.05) variables only for macrophyte metal concentration and bioconcentration and translocation factors across different species (i.e., *Cyperus papyrus, C. dives, Phragmites australis* and *Typha capensis)* location (i.e., roots, stems, and leaves), and seasons (i.e., cool-dry, hot-dry, and hot-wet).

Metal	**Species**			**Season**			**Location**			**Species × Season**			**Species × Location**			**Season × Location**			**Species × Season × Location**		
	Df	**F**	***P***	**Df**	**F**	***P***	**Df**	**F**	***P***	**df**	**F**	***p***	**Df**	**F**	***p***	**df**	**F**	***P***	**df**	**F**	***P***
Metal concentrations																					
Na	**4**	**140.578**	**<0.001**	1	0.303	0.583	4	1.603	0.182	3	0.571	0.636	**12**	**3.355**	**0.001**	2	0.342	0.711	5	0.825	0.536
Mg	**4**	**119.330**	**<0.001**	**1**	**17.828**	**<0.001**	**4**	**3.640**	**0.009**	**3**	**5.220**	**0.002**	**12**	**10.502**	**<0.001**	2	0.064	0.938	5	0.843	0.523
K	**4**	**21.922**	**<0.001**	1	3.594	0.061	**4**	**20.843**	**<0.001**	**3**	**4.549**	**0.005**	**12**	**4.367**	**<0.001**	2	0.415	0.661	5	0.346	0.883
Ca	**4**	**225.756**	**<0.001**	1	0.356	0.553	**4**	**25.889**	**<0.001**	**3**	**3.150**	**0.029**	**12**	**20.068**	**<0.001**	2	0.115	0.892	5	1.819	0.118
P	**4**	**17.361**	**<0.001**	**1**	**7.098**	**0.009**	4	2.113	0.087	3	0.993	0.400	12	1.856	0.052	2	0.312	0.733	5	1.911	0.101
Cr	**4**	**5.837**	**<0.001**	**1**	**4.488**	**0.037**	**4**	**22.902**	**<0.001**	**3**	**2.837**	**0.043**	**12**	**2.366**	**0.011**	**2**	**4.893**	**0.010**	5	1.265	0.287
Mn	**4**	**42.602**	**<0.001**	1	0.001	0.978	**4**	**2.850**	**0.029**	3	1.997	0.121	**12**	**3.041**	**0.001**	2	0.233	0.792	5	0.639	0.670
Fe	**4**	**6.354**	**<0.001**	1	3.865	0.053	**4**	**43.531**	**<0.001**	3	1.719	0.169	**12**	**2.580**	**0.006**	**2**	**4.509**	**0.014**	5	0.951	0.453
Co	**4**	**6.086**	**<0.001**	1	1.865	0.176	**4**	**41.780**	**<0.001**	3	2.559	0.061	**12**	**2.051**	**0.030**	**2**	**3.101**	**0.050**	5	2.262	0.056
Cu	**4**	**10.824**	**<0.001**	1	0.012	0.912	**4**	**32.645**	**<0.001**	3	2.447	0.070	**12**	**2.503**	**0.007**	2	0.724	0.488	5	0.779	0.568

Zn	**4**	**21.539**	**<0.001**	1	1.684	0.198	**4**	**18.993**	**<0.001**	**3**	**2.758**	**0.047**	**12**	**3.531**	**0.000**	2	0.126	0.881	5	0.271	0.928
Mo	**4**	**10.627**	**<0.001**	**1**	**4.766**	**0.032**	**4**	**20.336**	**<0.001**	3	1.350	0.264	**12**	**2.437**	**0.009**	**2**	**4.340**	**0.016**	**5**	**2.707**	**0.026**
Cd	**4**	**35.073**	**<0.001**	1	0.646	0.424	**4**	**23.832**	**<0.001**	3	1.452	0.234	**12**	**2.505**	**0.007**	2	0.920	0.403	5	0.455	0.808
As	**4**	**4.225**	**0.004**	1	1.797	0.184	**4**	**32.906**	**<0.001**	3	1.285	0.285	12	1.760	0.069	2	1.364	0.261	5	0.264	0.931
B	**4**	**75.954**	**<0.001**	1	1.854	0.177	**4**	**17.088**	**<0.001**	**3**	**3.189**	**0.028**	**12**	**11.377**	**<0.001**	2	1.813	0.170	**5**	**3.409**	**0.008**
Pb	**4**	**9.915**	**<0.001**	**1**	**5.028**	**0.028**	**4**	**39.627**	**<0.001**	3	2.614	0.057	**12**	**2.239**	**0.017**	**2**	**4.338**	**0.016**	5	1.321	0.263
Al	**4**	**8.737**	**<0.001**	**1**	**5.097**	**0.027**	**4**	**38.879**	**<0.001**	3	1.342	0.266	12	1.612	0.104	**2**	**4.314**	**0.017**	5	0.906	0.481

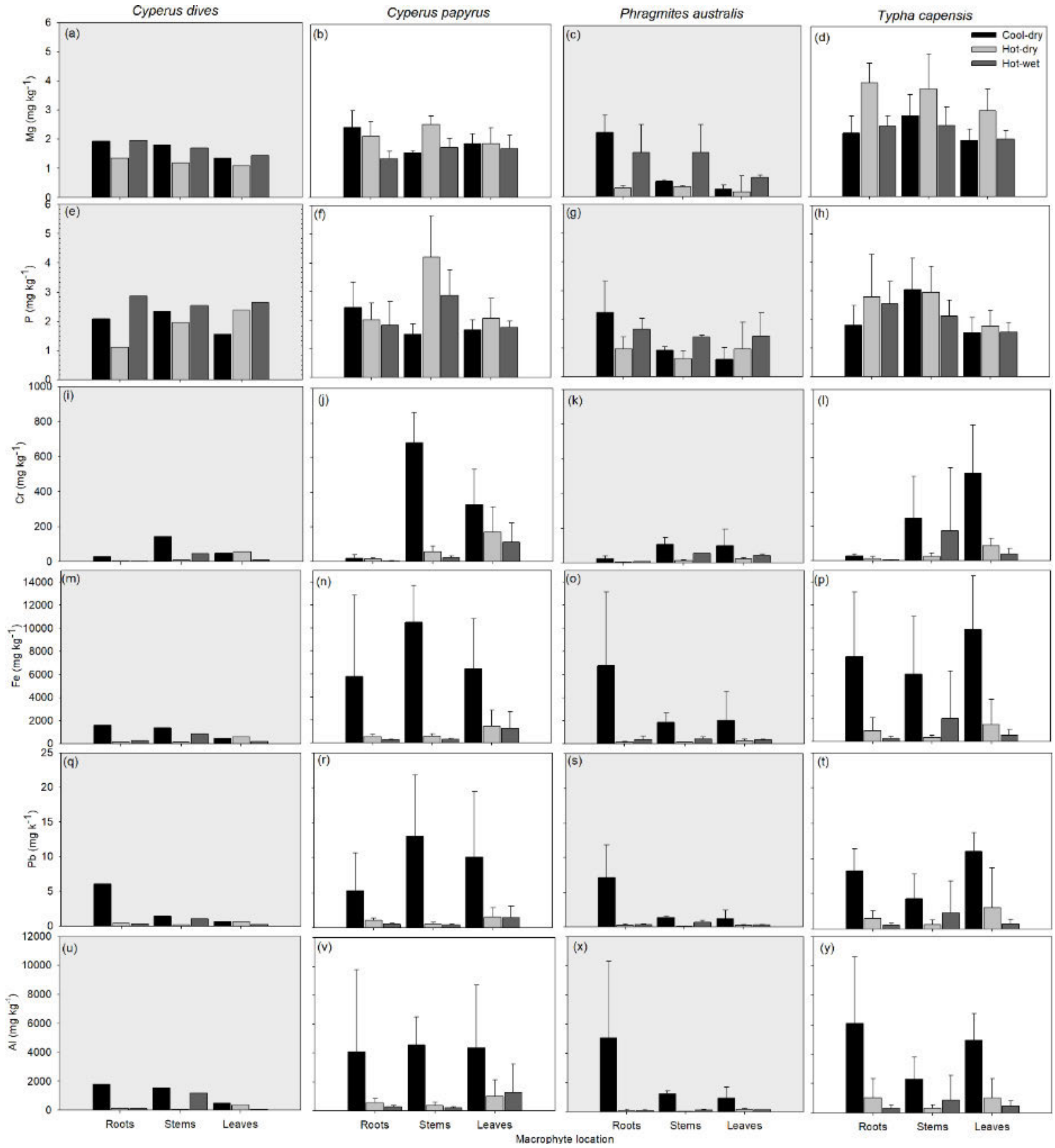

Figure 5.2: Mean (±standard deviation) of significant metal concentration variation in plant parts (i.e., roots, stems, and leaves) of different macrophytes (i.e., *C. papyrus, C. dives, P. australis* and *T. capensis*) across various seasons (i.e., cool–dry, hot-dry, and hot–wet) sampled within Riverside Wetlands, Mbombela, South Africa; (a–d) magnesium (Mg), (e-h) phosphorus (P), (i-l) chromium (Cr), (m–p) iron (Fe), (q-t) lead (Pb) and (u-y) aluminum (Al) for the respective macrophyte species at the top.

Table 5.3: Three-way analyses of variance (ANOVA) based on significant ($p < 0.05$) variables only for macrophyte metal concentration and bioconcentration and translocation factors across different species (i.e., *Cyperus papyrus, C. dives, Phragmites australis* and Typha *capensis)* location (i.e., roots, stems, and leaves), and seasons (i.e., cool-dry, hot-dry, and hot-wet).

Metals	***Species***			***Season***			***Species* × *Season***		
	df	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
Translocation factors									
Na	**3**	**17.49**	**<0.001**	2	1.531	0.238	6	1.821	0.139
Mg	**3**	**24.134**	**<0.001**	**2**	**3.881**	**0.035**	6	2.364	0.063
K	**3**	**12.255**	**<0.001**	2	0.998	0.384	6	0.888	0.519
Ca	**3**	**18.794**	**<0.001**	2	0.444	0.647	**6**	**4.309**	**0.005**
P	**3**	**4.634**	**0.011**	2	0.682	0.515	**6**	**3.347**	**0.016**
Cr	**3**	**8.962**	**<0.001**	**2**	**9.255**	**0.001**	**6**	**6.488**	**<0.001**
Mn	**3**	**3.164**	**0.044**	2	0.067	0.935	6	0.87	0.532
Fe	**3**	**30.704**	**<0.001**	**2**	**19.639**	**<0.001**	**6**	**13.297**	**<0.001**
Co	**3**	**15.684**	**<0.001**	**2**	**19.983**	**<0.001**	**6**	**6.038**	**0.001**
Cu	**3**	**23.604**	**<0.001**	**2**	**15.886**	**<0.001**	**6**	**7.605**	**<0.001**
Zn	**3**	**83.07**	**<0.001**	**2**	**23.826**	**<0.001**	**6**	**9.407**	**<0.001**
Mo	**3**	**6.604**	**0.002**	2	3.12	0.063	**6**	**4.027**	**0.007**
Cd	**3**	**20.287**	**<0.001**	2	1.719	0.201	6	2.239	0.076
As	**3**	**26.393**	**<0.001**	**2**	**15.473**	**<0.001**	**6**	**10.601**	**<0.001**
B	**3**	**56.039**	**<0.001**	**2**	**6.168**	**0.007**	**6**	**28.819**	**<0.001**
Pb	**3**	**25.201**	**<0.001**	**2**	**20.573**	**<0.001**	**6**	**10.69**	**<0.001**
Al	**3**	**10.061**	**<0.001**	**2**	**12.989**	**<0.001**	**6**	**9.491**	**<0.001**
Bioconcentration factors									
Na	**3**	**76.972**	**<0.001**	2	1.695	0.189	6	0.692	0.657
Mg	**3**	**66.289**	**<0.001**	**2**	**6.182**	**0.003**	6	1.893	0.09
K	**3**	**7.834**	**<0.001**	2	1.04	0.357	6	0.836	0.545
Ca	**3**	**66.942**	**<0.001**	**2**	**4.27**	**0.017**	6	0.569	0.754
P	**3**	**12.152**	**<0.001**	2	2.093	0.129	6	1.334	0.25
Cr	3	2.295	0.083	**2**	**11.035**	**<0.001**	6	0.767	0.598
Mn	**3**	**37.962**	**<0.001**	2	1.658	0.196	6	1.375	0.233
Fe	3	1.961	0.125	2	0.117	0.889	6	0.396	0.88
Co	3	1.539	0.21	**2**	**6.663**	**0.002**	6	0.729	0.627
Cu	**3**	**2.712**	**0.049**	2	0.152	0.859	6	0.602	0.728
Zn	**3**	**5.84**	**0.001**	2	0.967	0.384	6	0.698	0.652
Mo	**3**	**4.141**	**0.008**	2	2.787	0.067	6	0.856	0.53
Cd	**3**	**9.108**	**<0.001**	2	1.839	0.165	6	0.477	0.824

As	3	1.969	0.124	2	0.029	0.971	6	0.412	0.87
B	**3**	**26.467**	**<0.001**	2	1.701	0.188	6	0.481	0.821
Pb	**3**	**3.278**	**0.024**	2	0.817	0.445	6	0.538	0.778
Al	3	2.381	0.074	2	0.465	0.629	6	0.39	0.884

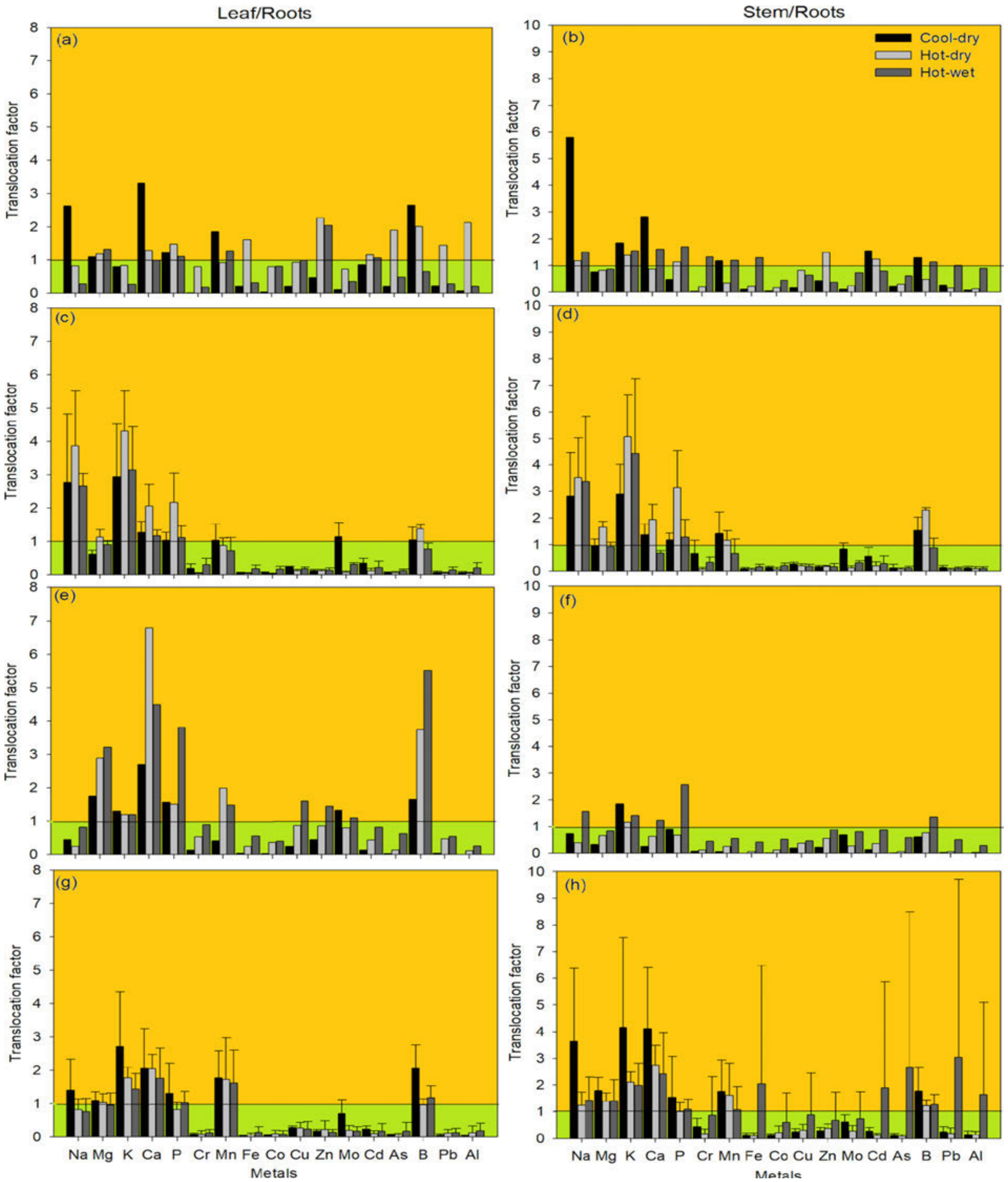

Figure 5.3: Mean (±standard deviation) of translocation factors in plant parts (i.e., leaves/roots and stems/roots) of different macrophytes (i.e., *C. papyrus, Cyperus dives, P. australis*, *T.capensis*) across various seasons (i.e., cool–dry, hot-dry, and hot–wet) sampled within Riverside Wetlands, Mbombela, South Africa; (a–b) *C. papyrus*, (c-d) *C. dives,* (e-f) *P. australis* and (g-h) *T. capensis.*

5.4.3Bioconcentration factor (BCF)

The bioconcentration factor (BCF) values were ≥1 for all macrophyte species parts for most metals across all seasons, suggesting that these macrophytes are capable of accumulating metals within their different plant parts. *Cyperus papyrus* (all parts) indicates high BCF values for Fe were between the range of 16.61- 201.71 mg kg^{-1} and Al (230.89 mg kg^{-1}) in the roots during the cool-dry season (Table 5.4). High BCF values for Fe (967.29 – 1 108.92 mg kg^{-1}) across all seasons, Pb of 2 155.9 mg $kg^{-1}$and 33 330 mg kg^{-1}were observed in the roots of *C. dives* across all seasons (Table 5.5). Apart from BCF values being ≥ 1, *Phragmites australis* recorded low BCF values than other macrophytes species, where Fe had high BCF values in the roots during cool-dry and hot-dry seasons. However, all macrophyte species had BCF > 1, generally, the study indicated that P. *australis* was the least accumulator as compared to other species (Table 5.6). High BCF for Fe was observed in stems (cool-dry) and roots across all seasons, roots (all seasons) of *Typha capensis* Al was high in the leaves (cool-dry), stems (hot-wet) and roots (all seasons) (Table 5.7). Our study generally indicated that macrophytes are best phytoremediation candidates. Using two-way ANOVA, significant BCF differences ($p < 0.05$) were recorded for Na, Mg, K, Co, Ca, Mn, Mo, Cd, Cr, Zn, Cu, Cd, B and Pb for macrophyte species (Table 3).

Table 5.4: Mean (± standard deviation) variation of bioconcentration factor (BCF) of *Cyperus papyrus* sampled from Riverside wetlands

Metal	**Leaves**			**Stems**			**Roots**		
	Cool -dry	**Hot - Dry**	**Hot-wet**	**Cool -dry**	**Hot - Dry**	**Hot-wet**	**Cool -dry**	**Hot - Dry**	**Hot-wet**
Na	11.67	10.00	6.18	25.82	14.31	21.35	4.44	12.13	14.28
Mg	0.73	0.65	0.56	0.51	0.45	0.42	0.67	0.55	0.49
K	6.07	9.59	4.18	14.07	16.08	15.37	7.65	11.57	9.96
Ca	1.86	0.99	1.05	1.59	0.66	1.06	0.56	0.76	0.66
P	1.37	1.24	1.31	0.53	0.95	1.18	1.11	0.84	0.70
Cr	0.35	10.09	2.21	0.92	2.53	13.04	32.08	12.69	9.87
Mn	24.58	23.81	36.74	15.59	9.00	28.74	13.23	26.13	24.14
Fe	42.27	126.71	26.63	21.10	16.61	85.24	201.71	78.54	65.79
Co	0.09	1.10	0.42	0.16	0.23	0.53	3.38	1.37	1.16
Cu	2.88	3.34	2.07	2.22	2.97	2.10	14.11	3.62	3.28
Zn	43.45	61.08	19.27	39.20	40.00	9.43	91.60	26.92	25.50
Mo	0.08	0.34	0.10	0.08	0.11	0.29	0.79	0.46	0.40
Cd	0.11	0.06	0.04	0.19	0.06	0.04	0.12	0.05	0.05
As	0.02	0.12	0.02	0.02	0.02	0.03	0.08	0.07	0.06
B	5.13	6.31	1.98	2.51	1.50	3.01	1.94	3.15	2.67
Pb	0.08	0.26	0.04	0.10	0.03	0.15	0.37	0.18	0.15
Al	17.08	171.25	10.75	17.25	9.80	53.05	230.89	80.50	59.10

Table 5.5: Mean (± standard deviation) variation of bioconcentration factor (BCF) of *Cyperus dives* sampled from Riverside wetlands

Metal	Leaves			Stems			Roots		
	Cool-dry	Hot-dry	Hot-wet	Cool-dry	Hot-dry	Hot-wet	Cool-dry	Hot-dry	Hot-wet
Na	8.88 ± 2.90	7.47 ± 2.65	11.17 ± 3.28	9.58 ± 2.84	7.45 ± 2.96	11.44 ± 2.60	4.39 ± 2.46	2.50 ± 0.71	4.70 ± 2.17
Mg	0.50 ± 0.10	0.55 ± 0.27	0.69.8 ± 0.17	0.80 ± 0.19	0.82 ± 0.36	0.64 ± 0.08	0.84.1 ± 0.23	0.59 ± 0.25	0.73 ± 0.14
K	8.87 ± 1.66	6.84 ± 3.32	6.20 ± 0.81	9.42 ± 3.52	8.40 ± 3.52	8.07 ± 1.80	3.6 ± 1.66	1.94 ± 0.11	2.34 ± 0.67
Ca	1.91 ± 0.47	1.41 ± 0.59	1.70 ± 0.37	2.08 ± 0.59	1.36 ± 0.49	1.05 ± 0.190	1.51 ± 0.06	0.90 ± 0.20	1.56 ± 0.43
P	0.87 ± 0.39	1.20 ± 0.58	0.87 ± 0.11	0.96 ± 0.28	1.70 ± 1.01	0.93 ± 0.26	0.86 ± 0.33	0.74 ± 0.18	0.83 ± 0.18
Cr	0.84 ± 0.32	4.05 ± 3.25	23.26 ± 27.62	3.23 ± 1.67	10.60 ± 8.49	29.78 ± 28.67	5.82 ± 2.72	15.64 ± 40.40	76.78 ± 47.01
Mn	0.07 ± 0.03	56.8 ± 24.34	47.68 ± 20.41	90.97 ± 44.6	76.9 ± 30.30	36.724 ± 18.46	83.46 ± 54.91	80.28 ± 23.10	68.89 ± 49.85
Fe	38.37 ± 2.46	33.2 ± 24.01	175.5 ± 227.21	78.7 ± 24.40	73.9 ± 39.06	152.2 ± 167.77	1108.9 ± 836.29	1569.9 ± 474.6	967.3 ± 655.60
Co	0.08 ± 0.03	0.36 ± 0.33	1.25± 0.98	0.18 ± 0.13	1.12 ± 1.32	1.82 ± 1.01	1.28 ± 0.66	21.06 ± 5.89	7.66 ± 3.93
Cu	0.98 ± 0.20	0.83 ± 0.44	1.48 ± 1.15	1.21 ± 0.46	1.31 ± 0.61	1.44 ± 0.40	4.35 ± 1.112	7.95 ± 1.26	8.55 ± 6.62
Zn	4.14 ± 0.32	2.95 ± 1.49	7.26 ± 0.87	6.05 ± 2.34	5.05 ± 1.66	6.68 ± 2.85	36.96 ± 13.72	37.88 ± 12.958	57.34 ± 36.20
Mo	0.37 ± 0.15	0.24 ± 0.12	0.65 ± 0.36	0.27 ± 0.06	0.39 ± 0.28	0.81 ± 0.23	322.5 ± 55.4	4.1 ± 1.19	2.22 ± 1.18
Cd	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.05 ± 0.010	0.09 ± 0.04	0.13 ± 0.15
As	35.8 ± 17	0.03 ± 0.01	0.08± 0.67	0.75 ± 0.48	0.56 ± 0.03	0.08 ± 0.33	1.02 ± 1.03	1.39 ± 1.60	0.71 ± 0.354
B	2.30 ± 0.38	1.56 ± 0.66	1.88 ± 0.23	3.45 ± 0.50	2.58 ± 1.17	2.07 ± 0.20	2.35 ± 0.55	1.40 ± 0.13	0.00 ± 0.00
Pb	0.14 ± 0.02	88.2 ± 45.1	347 ± 427.2	273.9 ± 87.3	128.8 ± 56.8	297.8 ± 283	2155.9 ± 809.1	3330.2 ± 2242.2	2.58 ± 2.39
Al	40.2 ± 15.2	25.9 ± 13.1	183.5 ± 293.6	83.7 ± 40.1	49.3 ± 32.8	85.7 ± 102.8	895.9 ± 665.4	664.2 ± 285.4	645.1 ± 639.5

Table 5.6: Mean (± standard deviation) variation of bioconcentration factor (BCF) of *Phragmites australis* sampled from Riverside wetlands

Metals	Leaves			Stems			Roots		
	Cool-dry	**Hot-dry**	**Hot-wet**	**Cool-dry**	**Hot-dry**	**Hot-wet**	**Cool-dry**	**Hot-dry**	**Hot-wet**
Na	0.13	0.20	0.31	0.21	0.33	0.59	0.29	0.85	0.38
Mg	0.58	0.59	0.26	0.11	0.14	0.07	0.34	0.20	0.08
K	4.77	5.62	5.04	6.78	5.51	6.05	3.67	4.71	4.26
Ca	1.75	1.35	1.12	0.16	0.12	0.11	0.65	0.20	0.89
P	0.78	0.67	0.70	0.46	0.30	0.48	0.50	0.45	0.18
Cr	1.23	12.60	9.68	0.67	2.98	4.85	9.39	24.04	1.08
Mn	38.45	35.67	16.36	5.57	4.57	6.12	95.40	17.95	11.14
Fe	52.11	64.52	51.44	19.05	15.32	38.84	1615.72	274.93	94.11
Co	0.36	0.70	0.40	0.03	0.23	0.54	1.80	1.99	1.02
Cu	1.00	1.29	2.06	0.81	0.56	0.59	4.23	1.47	1.30
Zn	8.53	4.90	5.66	4.31	3.20	3.48	19.33	5.76	3.94
Mo	0.41	0.54	0.47	0.21	0.18	0.35	0.31	0.68	0.43
Cd	0.00	0.00	0.00	0.01	0.00	0.00	0.04	0.01	0.00
As	0.49	0.07	0.09	0.06	0.03	0.08	3.06	0.58	0.14
B	1.58	1.66	5.91	0.60	0.34	0.31	0.96	0.44	0.23
Pb	0.00	0.16	0.68	0.66	0.02	0.06	2.78	0.35	0.13
Al	13.13	18.39	20.40	12.11	6.17	23.82	1230.02	179.80	83.93

Table 5.7: Mean (± standard deviation) variation of bioconcentration factor (BCF) of *Typha capensis* sampled from Riverside wetlands

Metal	Cool-dry	Hot-dry	Hot-wet	Cool-dry	Hot-dry	Hot-wet	Cool-dry	Hot-dry	Hot-wet
	Leaves			Stems			Roots		
Na	0.00 ± 0.00	7.61 ± 2.61	8.21 ± 3.80	13.72 ± 4.21	12.851 ± 6.67	13.82 ± 4.95	5.04 ± 2.61	10.63 ± 5.80	15.28 ± 15.12
Mg	0.93 ± 0.14	1.09 ± 0.17	0.79 ± 0.11	1.51 ± 0.26	1.52 ± 0.46	1.12 ± 0.28	0.88 ± 0.22	1.09 ± 0.29	0.92 ± 0.35
K	13.21 ± 3.73	12.02 ± 2.10	6.40 ± 1.60	19.73 ± 7.60	14.77 ± 5.53	8.15 ± 2.07	6.29 ± 3.85	6.99 ± 2.07	4.88 ± 2.43
Ca	4.46 ± 0.71	4.46 ± 0.63	3.42 ± 0.52	8.81 ± 2.58	5.99 ± 1.40	4.42 ± 1.5	2.58 ± 1.05	2.22 ± 0.37	2.45 ± 1.38
P	1.21 ± 0.38	1.15 ± 0.25	0.77 ± 0.169	1.34 ± 0.71	1.43 ± 0.44	0.86 ± 0.29	1.11 ± 0.45	1.48 ± 0.54	0.81 ± 0.25
Cr	0.55 ± 0.49	2.31 ± 1.44	8.88 ± 7.57	2.60 ± 2.77	5.30 ± 5.24	22.23 ± 10.14	6.73 ± 4.03	55.955 ± 56.08	97.462 ± 74.56
Mn	0.20 ± .09	0.12 ± 0.054	0.01 ± 0.02	20.19 ± 0.10	117.8 ± 51.58	81.02 ± 23.43	147.65 ± 101.95	104.01 ± 81.57	99.90 ± 52.
Fe	47.44 ± 0.75	31.12 ± 0.60	83.61 ± 4.49	144.74 ± 68.86	48.13 ± 9.91	255.21 ± 64.10	1329.03 ± 703.34	884.95 ± 60.10	1197.27 ± 68.81
Co	0.08 ± 0.05	0.28 ± 0.16	0.61 ± 0.52	0.21 ± 0.10	0.68 ± 0.66	1.88 ± 1.13	1.58 ± 0.57	6.00 ± 5.15	12.62 ± 9.37
Cu	1.25 ± 0.38	0.81 ± 0.28	0.90 ± 0.34	1.30 ± 1.31	0.90 ± 0.44	2.13 ± 2.24	4.60 ± 2.16	4.08 ± 2.40	5.43 ± 2.26
Zn	8.58 ± 3.30	7.65 ± 3.90	5.90 ± 2.83	14.69 ± 0.951	21,45 ± 9.68	32.63 ± 51.27	51.83 ± 26.92	47.06 ± 29.52	43.56 ± 15.43
Mo	0.31 ± 0.16	0.20 ± 0.08	0.31 ± 0.17	0.27 ± 0.50	0.27 ± 0.162	0.74 ± 0.26	0.49 ± 0.19	1.54 ± 1.17	2.61 ± 1.87
Cd	0.01 ± 0.00	6.8 ± 3.5	5.4 ± 2.5	14.4 ± 8.1	6.7 ± 2.5	26.2 ± 37.2	60.9 ± 34.3	73.9 ± 44.5	59.4 ± 33
As	37.9 ± 27.3	0.02 ± 0.00	0.05 ± 0.05	0.08 ± 0.06	0.027 ± 0.02	0.16 ± 0.26	1.01 ± 1.06	0.47 ± 0.33	0.95 ± 1.06
B	4.69 ± 1.28	2.80 ± 0.199	2.56 ± 0.29	3.88 ± 1.02	3.61 ± 0.75	2.87 ± 0.87	2.41 ± 0.71	2.98 ± 0.55	2.37 ± 0.86
Pb	0.13 ± 0.74	0.07 ± 42.5	0.18 ± 0.16	0.38 ± 0.29	0.13 ± 0.18	0.87 ± 1.58	2.29 ± 1.62	1.01 ± 0.8	2.29 ± 1.29
Al	45.5 ±	24.5 ±	67.7 ±	151.5 ±	38.35 ±	162.3 ±	1281.8 ± 1043.4	333.7 ±	597.8 ± 375.85

	35.08	20.31	50.73	192.49	37.69	213.14		220.75	

5.5 Discussion

The results of the present study revealed variations in metal concentrations amongst macrophyte species within the Riverside Wetlands. *Cyperus dives* and *T. capensis* had high phytoremediation ability as compared to *P. australis* and *C. papyrus*, suggesting that potentially not the best phytoremediation candidate compared to the other studied species. The findings are similar to Munyai and Dalu (2023), who also found *P. australis* as not conducive for phytoremediation. In addition, the results of the present study indicated that high metal concentrations were accumulated during the cool-dry and hot-dry seasons compared to the hot-wet season. Emenike *et al.* (2020) indicated that during cool-dry and hot-dry seasons, metal accumulation and concentrations in wetlands diminish as a result of water withdrawal and reduced evaporation. However, once metals are introduced into the system, their concentrations tend to remain relatively stable, suggesting limited remobilization or removal under these conditions. Such patterns underscore the importance of seasonal variability in influencing metal retention and potential ecological risks in wetland environments. In contrast Edokpayi et al. (2016), observed elevated metal concentrations during the hot-wet season which were likely influenced by increased rainfall in the study area, our findings indicate that metal concentrations were generally high during the hot-dry season where concentrations was expected to occur to reduced inflows and high evaporation rates, whereas the dilution effects of rainfall during the hot-wet season, could be attributed to low metal concentrations in the wetlands. However, de Lima *et al.* (2022) found macrophytes to be effective phytoremediation agents throughout the year.

The current study revealed that metals were accumulated in different macrophyte parts (i.e. leaves, stems, and roots) across different seasons. Iron and Aluminium were found to be highly accumulated by all macrophytes within roots of all macrophytes during the cool-dry season. Furthermore, Mg was accumulated only in the roots (cool-dry and hot-wet) and stems (hot-wet) of *P. australis,* which was in contrast with Munyai and Dalu (2023), who found that Mg was high in *P. australis* leaves across all seasons in the Mvudi river systems of Limpopo province of South Africa. Our study results were similar to Netshiongolwe et al. (2020) who reported high Fe and Mg in the *P. australis* roots and stems across the seasons. Moreover, *P. australis* accumulated high Pb concentrations in the roots compared to other parts during the cool-dry season. In contrast, Eid et al. (2021) reported that Pb concentrations were high in the leaves and stems of *P. australis* during the hot-wet season. The findings of the present study were similar to those of Kayirangwa et al. (2024), who reported that *C. papyrus* was not a good metal accumulator compared to other macrophyte species studied such as *C. dives, T. capensis* and *P. australis.*

This study recorded bioconcentration factors (BCF) values of >1, which indicates that the studied macrophyte species have phytoremediation potential. The current study indicates that macrophyte species accumulated higher metal concentrations in the roots and leaves than in the stems. Various studies (Nguyen et al., 2021; Basallote et al., 2023) reported that most macrophytes often accumulate metals in their roots, compared to their above-ground parts. Generally, roots accumulate more of the heavy metals than stems and leaves. Apart from that, roots are the first contact of a plant that absorbs heavy metals from sediments or a waterbody before their transfer to the other parts (Hanafiah et al., 2020). In addition, compared to stems and leaves, roots are the only covered part, and they are in contact with the growth medium. Some factors, such as plant physiology, can alter the accumulation of metals within the plant, such as *P. australis* having the ability to release some phytocompounds from the roots into the rhizosphere, which can reduce their toxicity and mobility (Kalu et al., 2021). The study indicated that the macrophytes were hyperaccumulators of Fe and Al, and this suggests that the presence of Al and Fe in the natural environment likely facilitated its uptake and accumulation by the macrophytes (Hanafiah *et al.*, 2020; Coimbra and Borges, 2023). Our study indicated that *C. papyrus* had a high BCF value for Fe in the roots and leaves, which supports findings by Al-Hamad (2023), who reported a high BCF value for Fe in the roots and stems. Generally, *T. .capensis* had high Fe in leaves and roots during the cool-dry and hot-wet seasons, which agreed with the findings reported by Mthombeni (2022). All four macrophyte species translocated metals differently among seasons within the Riverside Wetlands, having high translocation potential for Na, K, Ca and P. Generally, most metals were translocated from the roots to the leaves during hot-dry and cool-dry seasons rather than hot-wet season, with variation among macrophyte species being observed. The findings of this study were supported by Netshiongolwe et al. (2020), Munyai and Dalu (2023) and Kola et al. (2025), who reported the presence and seasonal translocation of metals such as K, Ca, Mg, B, and Mn. In the current study, *P australis* exhibited pronounced translocation of Ca, P and B from the roots to the leaves across all seasons, whereas Mn showed limited translocation during the hot-dry and hot-wet periods. These patterns are in line with those observed by Munyai and Dalu (2023), who reported high translocation of Mn during the hot-dry season. Overall, the observed translocation trends highlight the species potential role in the uptake and redistribution of specific metals within the macrophytes. The high translocation factors in *P.australis* indicated in this study highlighted that metals (i.e., Ca, P, Ba and Mn) had high mobility (Wdowczyk and Szymańska-Pulikowska, 2023). Furthermore, the current study indicated high Mn translocation in all macrophyte species. These results were similar to Samadi et al. (2024), who reported high translocation of Mn and low translocation of Fe in *P. australis.* Similarly, *P. australis* highlighted high Mn translocation from the roots to the leaves during the hot-wet season (Netshiongolwe et al. (2020). Limited mobilisation of metals was because metals were rather accumulated within the roots and the amount of rainfall during the study (Netshiongolwe et al., 2020).

Our results are in support of the previous study (Munyai and Dalu, 2023) who reported that *T. capensis* was better in translocating Mn as compared to other macrophyte species across all seasons (Wdowczyk and Szymańska-Pulikowska, 2023). Furthermore, B in *T. capensis* did increase from the roots to the aboveground parts, which agreed with the results of Sasmaz et al. (2021) who reported the increase of B from roots to the above ground plant parts. Based on seasonal variation, the study indicated that during the hot-wet season, most metals were not transferred, thus indicating that metals might have been diluted in the wetlands.

Translocation factor explains the transfer of metals from the roots to their aboveground organs (Samadi et al., 2024). The translocation of these metals is directly influenced by several factors, such as different plant species, environmental factors, metal composition, and sediment parameters. Therefore, based on the current study findings, the good macrophyte candidate for phytoremediation is dependent on the specific metals which will need to be removed and the environmental conditions at the site and season. Where the TF is high it may also be due to other ions, high organic matter content, and nutrient levels (Subramanian et al., 2022). The current findings indicate that *P. australis* and *C. papyrus* were least effective in translocating the metals as compared to *C. dives* and *T. capensis.* Our results agree with some studies (Abdelaal et al., 2021; Munyai and Dalu, 2023). The TFs value below one showed that the studied aquatic macrophytes exhibited a lower rate of metal translocation from the roots to their above-ground parts. The current study indicates that aquatic macrophytes accumulated heavymetals in their roots and did not efficiently translocate metals from the roots to the stems.

Hence, another study suggests that studied plants might be considered as metal excluders and for phytostabilisation of polluted wetlands (Bonanno, 2013). Our results indicate that B, Na, Mg, K, P, Ca were translocated from roots to the stems by different species. Contrarily, Abdelaal et al. (2021) found that metals such as Fe, Cu, Zn and Mn, which are responsible for metabolic functions of the plants, were translocated to the shoots. Therefore, the translocation of these metals varied significantly among the studied aquatic macrophytes and parts.

5.6 Conclusion

The present study found that macrophyte species accumulated metal concentrations in the different parts across seasons. When considered holistically high metal concentrations are observed during the cool-dry compared to hot-dry and hot-wet seasons, irrespective of macrophyte species. In turn, the macrophyte *C. dives,* C. *papyrus* and *T. capensis* can be major accumulators of metals compared to *P. australis.* Plant metal concentrations were generally highest in the roots and leaves as compared to stems. These results provide baseline information for general management of the Riverside Wetlands and advocate for further investigations into the long-term wetlands and plant metal variability to better understand the metal and nutrient dynamics involving several plant species across different seasons.

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CHAPTER 6.

METAL TOLERANCE AND PHYTOREMEDIATION POTENTIAL OF *DRYOPTERIS PENTHERI* (KRASSER) C. CHR

The chapter is currently submitted: ***Kola, E***., *Munyai, C., Mpopetsi, P., Munyai, L.F., Dondofema, F. and Dalu, T. Metal tolerance and phytoremediation potential of Dryopteris pentheri (Krasser) C. Chr. Applied Ecology and Environmental Research. Major revision*

6.1 Abstract

Globally, heavy metals from natural and anthropogenic activities cause serious health and environmental risks, especially in water systems. Phytoremediation is a promising approach to remediating metal contaminants in the environment. The present study aimed to evaluate the ability of a fern, *Dryopteris pentheri* (Krasser) C. Chr. to uptake, accumulate, and tolerate heavy metals. The study was conducted in a controlled environment, where plants were cultivated in 20 L dishes filled with wetland water for 7 days in control, low and high metal concentrations. Overall, *D. pentheri* contained high concentrations of selected metals, however, composition and concentrations differed across plant parts, with roots containing exceptionally high concentrations of Na, Mg and K. Both bioaccumulation and translocation factors were more significant than 1 for some selected metals in the roots, indicating that *D. pentheri* is hyperaccumulator and has shown an ability to tolerate metals by increasing the chlorophyll content in the high concentration after a long metal exposure. Therefore, *D. panther* stands as a good candidate for removing metals from aquatic environments.

Keywords: *Bioaccumulation factor, ferns, translocation factor, phytostabilisation, hyperaccumulator*

6.2 Introduction

Heavy metals pose a significant threat to ecosystems and human health (Dalu and Tavengwa, 2022; López-Botella et al., 2021; Parui et al., 2024). Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are among the most pervasive and hazardous environmental pollutants (Balali-Mood et al., 2021; Dalu and Tavengwa, 2022; Sharma et al., 2021). These metals are introduced into the environment through various anthropogenic activities, including mining, industrial processes, agricultural practices, and urban discharges (Akhtar et al., 2021; Pandey and Kumari, 2023; Parui et al., 2024; Rashid et al., 2023). In contrast to organic pollutants, heavy metals cannot be degraded and tend to accumulate in soils and water bodies, leading to long-term environmental and health risks (Yaashikaa et al., 2022). Prolonged exposure to heavy metals can have severe health implications in humans, including neurological disorders, kidney damage, and cancer (Shetty et al., 2023). Moreover, heavy metals can disrupt ecosystems by affecting soil fertility, plant growth, terrestrial (Gall et al., 2015) and aquatic life (Priyadarshanee et al., 2022; Xu et al., 2022). Given the widespread contamination of heavy metals and their potential detrimental effects, there is an urgent need for effective remediation strategies.

Phytoremediation is the use of plants to remove, stabilise, or degrade contaminants from soil, water, and air, which has emerged as a sustainable and cost-effective approach to environmental clean-up (Kafle et al., 2022; Shen et al., 2022). Various methods have been used to clean up metal contaminants from the environment. Among these include, traditional metal remediation methods, such as excavation and chemical treatment, which are often expensive, energy-intensive, and disruptive to the environment (Azhar et al., 2022; Rajendran et al., 2022). In contrast, phytoremediation offers an eco-friendly alternative that leverages the natural capabilities of plants to uptake and accumulate metals, thereby restoring contaminated sites (Shen et al., 2022).

Phytoremediation encompasses several mechanisms by which plants can mitigate metal contamination, including phytoextraction, phytostabilisation, phytovolatilisation, and rhizofiltration (Kafle et al., 2022; Kristanti et al., 2023). Phytoextraction involves the uptake of metals from the soil or water and their translocation to above-ground plant tissues, which can then be harvested and removed (Gul et al., 2021; Mudgal et al., 2023). Phytostabilisation focuses on immobilising metals in the soil or water, reducing their bioavailability and preventing their spread (Bhat et al., 2022; Gavrilescu, 2022; Moreira et al., 2021). Phytovolatilisation involves the uptake and transpiration of volatile metals, such as mercury and selenium, into the atmosphere (Somagattu et al., 2024). Rhizofiltration refers to the absorption or adsorption of metals by plant roots in aquatic environments (Green, 2024; Kristanti et al., 2021; Woraharn et al., 2021). Among these, phytoextraction is particularly promising for the remediation of heavy metal-contaminated soil, because it enables the plant to uptake metals from the soil to aboveground parts, which also detoxifies the metals inside the plant organs (Gavrilescu, 2022; Kafle et al., 2022). The success of phytoremediation depends on the selection of appropriate plant species that exhibit high metal uptake capacity, tolerance to metal toxicity, and the ability to thrive in contaminated environments (Kafle et al., 2022; Liang et al., 2024; Yadav et al., 2023). Hyperaccumulator plants, those that can accumulate exceptionally high concentrations of metals in their tissues, are particularly valuable for phytoextraction (Yaashikaa et al., 2022). However, they are often slow-growing and have limited biomass production, which can limit their practical application (Li and Liu, 2022; van der Ent et al., 2024). Therefore, there is a growing interest in identifying non-hyperaccumulator plants with moderate-high metal accumulation capacity but higher biomass production, as these may offer a more feasible solution for large-scale remediation efforts.

Fern plants have been recognised for their ability to tolerate and accumulate heavy metals, making them promising candidates for phytoremediation (Sharma and Sharma, 2022). Ferns are perennial species belonging to the family Dryopteridaceae (Maroyi, 2024). Specifically, plants of the genus *Dryopteris* are a promising group for phytoremediation due to their ability to uptake and accumulate heavy metals, as well as their tolerance to metal toxicity (Grosjean et al., 2019; Senila et al., 2013). *Dryopteris pentheri* (Krasser) C. Chr., commonly known as Penther's wood fern, has the potential as a phytoremediator of heavy metals, owing largely to its adaptability to harsh environmental conditions, including low nutrient availability and high metal concentrations (Gao et al., 2018; Hietz, 2010). The ability of *D. pentheri* to uptake and accumulate heavy metals can also be attributed to other factors such as root morphology, metal transporters, and chelation mechanisms (Sharma and Sharma, 2022). Despite this, research on *D. pentheri* and its metal uptake capabilities is relatively limited.

Preliminary studies on *Dryopteris* have shown that it can accumulate moderate levels of metals such as cadmium, lead, and zinc in its fronds and roots (Grosjean et al., 2019; Sajeev et al., 2022; Senila et al., 2013). However, the extent of metal accumulation and the plant's tolerance thresholds vary depending on the metal species, soil properties, and environmental conditions (Sharma and Sharma, 2022). For example, *Dryopteris* may exhibit a higher accumulation of certain metals in acidic soils, where metal bioavailability is increased (Khan et al., 2024), while the presence of other nutrients and organic matter in the soil or water can influence metal uptake and translocation patterns (Yan et al., 2021). However, research on *D. pentheri* and its metal uptake capabilities is relatively limited.
One advantage of *Dryopteris* is its adaptability to harsh environmental conditions, including low nutrient availability and high metal concentrations (Gao et al., 2018; Hietz, 2010). Thus, understanding these mechanisms is crucial for assessing the fern plant's phytoremediation potential and optimising its use in contaminated environments. The ability of *D. pentheri* to uptake and accumulate heavy metals can be attributed to several factors, including root morphology, metal transporters, and chelation mechanisms (Sharma and Sharma, 2022). Ferns typically have extensive root systems that enhance their capacity to absorb metals from the soil

or water (Sharma and Sharma, 2022). Once absorbed, metals are transported to various plant tissues, where they may be sequestered in vacuoles or bound to metal-chelating compounds such as phytochelatins (Cobbett, 2000a, b). The phytochelatins play a critical role in detoxifying metals and preventing their interference with essential cellular processes.

Metal tolerance in plants is a complex trait that involves multiple physiological and biochemical adaptations (Chen et al., 2022; Sajeev et al., 2022; Wang et al., 2023). In *D. pentheri,* similar to other fern species, tolerance mechanisms may include the production of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), which mitigate oxidative stress induced by metal toxicity (Alam et al., 2021; Zhang et al., 2019). The plant may also upregulate the synthesis of osmoprotectants, such as proline and glycine betaine, to maintain cellular homeostasis under metal stress (Drăghiceanu et al., 2016). Furthermore, *D. pentheri* may employ exclusion strategies, such as binding metals to cell walls or the secretion of organic acids, to limit metal uptake and translocation. Thus, understanding how *D. pentheri* moves metals is essential for enhancing the potential phytoremediation of the taxa.
Dryopteris genus is a promising group for phytoremediation due to its ability to uptake and accumulate heavy metals, as well as its tolerance to metal toxicity (Grosjean et al., 2019; Senila et al., 2013). Thus, further research is needed to fully characterise its metal accumulation capacity, tolerance mechanisms, and potential for large-scale application particularly in subtropical regions. By leveraging the natural capabilities of *D. pentheri*, it may be possible to develop sustainable and effective strategies for mitigating heavy metal contamination and restoring polluted ecosystems.
This study aims to contribute to the growing body of knowledge by assessing the phytoremediation potential of *D. pentheri*, thereby paving the way for its practical application in environmental clean-up efforts. Specifically, the study aims to evaluate the ability of a fern *D. pentheri* to uptake, accumulate, and tolerate heavy metals (e.g., lead, cadmium, arsenic, iron, chromium, and magnesium) in a controlled environment. It was hypothesised that *D. pentheri*

will uptake and accumulate heavy metals from contaminated water. Furthermore, they will exhibit differential tolerance and accumulation capacities for different metals among different tissues (i.e., roots, rhizomes, fronds).

6.3 Materials and methods

6.3.1 Reagents and chemicals

All chemicals (i.e., potassium nitrate AR (cas no. 7757-79-1), calcium nitrate tetrahydrate AR (cas no. C1843), potassium dihydrogen phosphate AR (cas no. 7778-77-0), magnesium sulphate AR (cas no. 10034-99-8), zinc sulphate heptahydrate AR (cas no. 7446-20-0), cupric sulphate pentahydrate crystals AR (cas no. 10099-74-8) used in the experiment were of analytical grade. Most of the chemicals were obtained from RadChem, South Africa, except for calcium nitrate tetrahydrate AR which was obtained from Minema Chemicals, South Africa.

6.3.2 Experimental design

Indigenous potted fern plants *Dryopteris pentheri* (n = 18) were sourced from a local warehouse store in Nelspruit, South Africa. The fern plants *D. pentheri* were taken to the University of Mpumalanga, where they were placed in dishes (diameter 80 cm, depth 45 cm) filled with 20 L of wetland water. The wetland water was collected from a nearby wetland system. The fern plants *D. pentheri* (n = 3 per dish) were randomly placed in the dishes, and the plants were left to acclimatise for 3 days before the experiment was initiated. After three days, the fern plants *D. pentheri* were briefly taken out of the water level was topped to the initial level and the chemicals were added before being thoroughly mixed while ensuring all chemicals dissolved in the water after which the fern plants *D. pentheri* were placed back in the dishes (see Table 6.1). The experimental design consisted of two dishes for each of the treatments: control (n = 3 plants per dish), low (n = 3 plants per dish) and high (n = 3 plants per dish) metal concentrations (Figure 6.1).

Table 6.1: The concentrations of chemicals (g) added in water tanks (20 L) for the phytoremediation experiments

Chemical	Control	High concentration	Low concentration
Pb	0	80	40
Cu	0	196.5	98.25
Zn	0	220	110
Mg ($MgSO4 \cdot 7H_2O$)	0	51.5	25.75
K (KH_2PO_4)	0	60	30
Ca (CaNO3)	0	45	22.5
NO_4(KNO_3)	0	45	22.5

Physico-chemical water parameters (i.e., temperature, total dissolved solids, conductivity, pH, salinity, oxygen reduction potential, resistivity) were measured using a handheld HANNA multiparameter. The water measurements were recorded on days 1, 3, 5 and 7. The leaf chlorophyll-*a* concentration was measured from 20 randomly selected leaves per treatment dish (n = 40 per treatment) using a handheld chlorophyll SPAD 520 meter on days 1, 4 and 7. Furthermore, water and fern plant *D. pentheri* samples were collected at days 1 and 7 for metal analysis (i.e., sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), aluminium (Al), arsenic (As), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), nickel (Ni), phosphorous (P) and zinc (Zn). The fern plant *D. pentheri* sampled on day 1 was from the 3 extra pots to know the background values on metals in the plants and were placed in labelled Ziplock bags after dividing them into roots, stems and leaves. Water samples were collected in labelled 500 mL polyethylene water bottles.

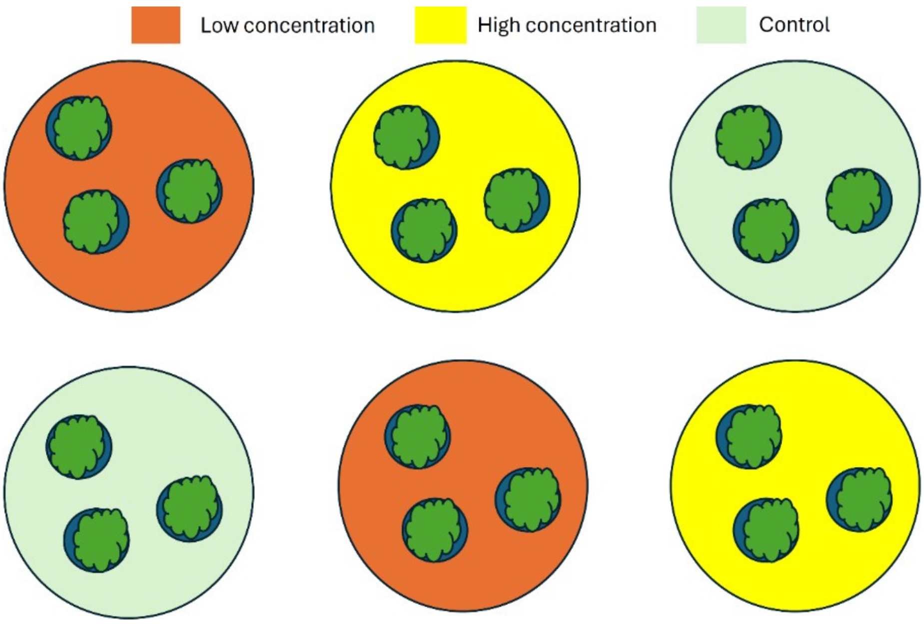

Figure 6.1: Experimental design for the fern *Dryopteris pentheri* phytoremediation study

6.3.3 Metal analysis

6.3.3.1 Water

At the beginning and end of the experiment, collected water samples were sent to Water Lab, Pretoria, South Africa for metal analysis, where major anion samples were filtered through a 0.45 µm filter paper before analysis. The Na, Ca, K and Mg were analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Varian, Mulgrave). The reliability of the analysis results was determined using the ionic balance error of samples; the samples showed an ionic balance of ≤ ±10 % error, with four samples showing an ionic balance error of ±10 to ±15 %.

6.3.3.2 Plants

The collected fern plant *D. pentheri* parts (i.e., roots, stem and leaves) were oven-dried at 60 °C for 72 days before being ground into powder. Approximately 10 g of the sample for each plant part were analysed at the Stellenbosch University Central Analytical Facility after microwave digestion (MARS®-5) at 280 PSI pressure and 180 °C for 20 min using 6 mL (65 %) nitric acid: 2 mL (30 %) hydrochloric acid and 6.5 mL (65 %) nitric acid: 0.5 mL (30 %)

hydrochloric acid, respectively (EPA, 2007). Post-digestion, de-ionised water was added to make 50 mL final volume of each sample. Metal analyses were performed on 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). For quality control, each set of matrix digestions included dual quality controls, with a blank acid mixture of 1 573a tomato leaf (~0.3 g) Certified Reference Material (National Institute for Science and Technology, Gaithersburg, USA) were used as controls for plants. The accuracy range was 81.8–113.4 %), with a recoveryrange of 83.6–113.2 % was recorded.

6.3.4 Data analysis

Bioconcentration factor (BCF) is indicative of the degree of enrichment of a heavy metal in a biota (i.e., fern plant *D. pentheri*) relative to that in its habitat (i.e., water). The BCF is calculated based on the equation:

$$BCF = \frac{C_{fern}}{C_{water}}$$

where C_{fern} is the metal concentration in the fern tissue (i.e., root, rhizome or fronds) and C_{water} is the metal concentration in water where the ferns were kept. A BCF value > 1 indicates that the fern plant *D. pentheri* can accumulate more metals than are available in the water (i.e., hyperaccumulation). A BCF value ≤ 1 indicates that the fern plant *D. pentheri* can only absorb metals in proportion to what's available in the water.

The transportation index (Ti) gives the fronds/root or rhizome/root metal concentration, and it depicts the fern plant *D. pentheri* ability to translocate metal species from roots to the rhizome/fronds at different concentrations. It is calculated based on the equation:

A $Ti > 1$ means the fern plant *D. pentheri* can efficiently move the metal from its roots to its rhizome or fronds, and a $Ti < 1$ is ideal for phytostabilisation.

6.3.5 Statistical analysis

A repeated measures analysis of variance (ANOVA) was used to assess the differences in basic water variables (i.e., pH, conductivity, salinity, total dissolved solids, ORP, resistivity, temperature), metals (i.e., Na, K, Ca, Mg, Al, As, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Ni, P, Zn) in water and plants across days, after testing for homogeneity of variances (Levene's test) and normality of distribution (Shapiro-Wilk test). For significant results, Tukey post-hoc tests were performed via estimated marginal means when the effects were significant. All statistical analyses were performed using IBM SPSS version 28.0.0.0 based on log(x + 1) transformed data.

6.4 Results

6.4.1 Water

Conductivity, TDS and salinity were generally high in the high-concentration treatment and ow in the control treatment (Table 6.2). Resistivity was high in the control treatment. Based on repeated measures ANOVA analysis, temperature, salinity, resistivity and pH were found to be significantly ($p < 0.001$) different across days, conductivity, TDS, salinity and resistivity were significantly ($p < 0.001$) different among treatments (Table 6.2).

Metals (i.e., Al (range 0.100–0.375 mg L^{-1}), As (range 0.002–0.005 mg L^{-1}), B (range 0.025–0.025 mg L^{-1}), Cd (range 0.001–0.001 mg L^{-1}), Cr (range 0.025–0.025 mg L^{-1}), Co (range 0.025–0.025 mg L^{-1}), Fe (range 0.339–0.534 mg L^{-1}), Li (range 0.001–0.001 mg L^{-1}), Mn (range 0.025–0.139 mg L^{-1}), Ni (range 0.025–0.025 mg L^{-1}) were below 1 mg L^{-1} across all treatments, before experiment. The control treatment had lower metal concentrations than the control and high metal concentration treatments (Figure 6.2). For example, the Mg was 6.5 mg L^{-1}, 109 mg L^{-1} and 218.5 mg L^{-1} in control, low and high concentration treatments, respectively, with similar patterns being observed for Na, K, Ca, Cu, P and Zn before the experiment (Figure 5.2). At the end of the experiment, an increase in Al (range 0.531–1.111 mg L^{-1}), Fe (range 0.231–1.350 mg L^{-1}) and Mn (range 0.736–3.920 mg L^{-1}) concentrations was observed across all treatments. There was decrease in concentrations for Na, K, Mg, Cu, Pb, P and Zn, except for Ca which increased among treatments (Figure 2). The repeated measures ANOVA test indicated significant ($p < 0.05$) differences for Ca, Al, AS, B, Cr, Co, Cu, Fe, Li, Mn, Ni, P and Zn before and after the experiment, with K, Ca, Mg, Cr, Co, Cu, Fe, Mn, Ni, P and Zn being significantly ($p < 0.05$) different among treatments (Table 6.3).

Table 6.2: Mean (±standard deviation) of basic water quality variables measured over the experimental period

Time	Treatment	Conductivity (µS cm^{-1})	Temperature (°C)	TDS (ppt)	Salinity (ppt)	Resistivity (Ω)	pH	ORP (mV)
Day 1	Control	0.2 ± 0.01	20.2 ± 1.0	0.1 ± 0.01	0.1 ± 0.004	5717 ± 492.1	7.5 ± 0.3	-45.6 ± 0.3
	Low	8.9 ± 0.1	19.6 ± 0.4	6.1 ± 3.7	5.0 ± 0.09	136.5 ± 6.0	7.4 ± 0.09	-46.0 ± 0.07
	High	15.3 ± 0.1	20.4 ± 1.2	6.2 ± 0.3	8.9 ± 0.01	79.9 ± 4.9	7.3 ± 0.01	-45.8 ± 0.2
Day 3	Control	0.2 ± 0.03	17.9 ± 0.1	0.1 ± 0.003	0.1 ± 0.0	5485.5 ± 613	7.8 ± 0.04	-46.1 ± 1.3
	Low	12.4 ± 4.5	17.4 ± 0.5	3.3 ± 0.08	5.1 ± 0.08	148.9 ± 2.4	7.8 ± 0.04	-46.9 ± 0.8
	High	15.7 ± 0.02	17.5 ± 0.2	5.8 ± 0.05	9.1 ± 0.002	85.8 ± 1.0	7.8 ± 0.001	-46.8 ± 0.5
Day 5	Control	0.3 ± 0.01	16.0 ± 0.2	0.1 ± 0.01	0.1 ± 0.01	4840 ± 257.3	7.7 ± 0.02	-39.2 ± 1.2
	Low	10.0 ± 0.07	14.9 ± 0.6	3.2 ± 0.1	5.6 ± 0.1	160.5 ± 0.1	7.7 ± 0.01	-38.6 ± 0.4
	High	17.2 ± 0.05	15.7 ± 1.1	5.6 ± 0.2	10.0 ± 0.1	88.7 ± 4.7	7.7 ± 0.01	-39.4 ± 0.1
Day 7	Control	0.3 ± 0.002	22.5 ± 0.4	0.1 ± 0.004	0.1 ± 0.003	3278 ± 59.3	7.6 ± 0.01	-36.2 ± 1.9
	Low	10.7 ± 0.01	22.8 ± 1.6	4.9 ± 0.3	6.1 ± 0.02	102 ± 8.5	7.6 ± 0.03	-35.8 ± 0.4
	High	18.3 ± 0.2	23.1 ± 0.5	8.4 ± 0.3	10.9 ± 0.1	59.1 ± 2.0	7.6 ± 0.02	-37.1 ± 0.3

Table 6.3: Repeated measures analysis of variance of water quality variables and fern plant *Dryopteris pentheri* parts over the entire experimental period. Values in bold indicate significant differences at $p < 0.05$

Variable	**Days**			**Treatment**			**Days × Treatment**		
	Df	**F**	***P***	***df***	**F**	***P***	***df***	**F**	***P***
Basic water parameters									
Conductivity	3	2.248	0.135	**2**	**159.000**	**<0.001**	6	0.944	0.500
Temperature	**3**	**105.611**	**<0.001**	2	1.426	0.278	6	0.440	0.838
TDS	3	0.704	0.568	**2**	**45.986**	**<0.001**	6	0.776	0.604
Salinity	**3**	**26.665**	**<0.001**	**2**	**16.797**	**<0.001**	**6**	**26.278**	**<0.001**
Resistivity	**3**	**73.741**	**<0.001**	**2**	**118.681**	**<0.001**	**6**	**3.830**	**0.023**
pH	**3**	**17.741**	**<0.001**	2	0.475	0.633	6	0.844	0.560
ORP (mV)	3	0.754	0.541	2	0.914	0.427	6	1.019	0.458
Water metals									
Na	1	1.667	0.244	2	2.317	0.180	2	0.817	0.486
K	1	3.522	0.110	**2**	**38.544**	**<0.001**	2	0.452	0.656
Ca	**1**	**32.139**	**0.001**	**2**	**17.736**	**<0.001**	**2**	**8.732**	**0.017**
Mg	1	0.791	0.408	**2**	**16.046**	**<0.001**	2	0.056	0.946
Al	**1**	**17.415**	**0.006**	2	3.168	0.115	**2**	**13.048**	**0.007**
As	**1**	**3.769**	**0.100**	2	2.385	0.173	2	0.538	0.609
B	**1**	**7.361**	**0.035**	2	1.994	0.217	2	1.994	0.217
Cd	1	1.000	0.356	2	1.000	0.422	2	1.000	0.422
Cr	**1**	**2.601**	**<0.001**	**2**	**19.000**	**0.003**	**2**	**8.103**	**<0.001**
Co	**1**	**12.462**	**0.012**	**2**	**15.346**	**0.004**	**2**	**20.192**	**0.002**
Cu	**1**	**35.306**	**0.001**	**2**	**15.755**	**<0.001**	**2**	**8.869**	**0.016**
Fe	**1**	**11.306**	**0.015**	**2**	**17.447**	**0.003**	**2**	**5.854**	**0.039**
Pb	1	1.775	0.231	2	1.503	0.296	2	1.234	0.356
Li	**1**	**9.000**	**0.024**	2	3.000	0.125	2	3.000	0.125
Mn	**1**	**57.629**	**<0.001**	**2**	**10.467**	**0.011**	**2**	**22.983**	**0.002**
Ni	**1**	**16.427**	**0.007**	**2**	**8.515**	**0.018**	2	0.444	0.661
P	**1**	**21.921**	**0.003**	**2**	**134.268**	**<0.001**	**2**	**9.508**	**0.014**
Zn	**1**	**45.496**	**0.001**	**2**	**255.096**	**<0.001**	**2**	**11.858**	**0.008**
Plant metals									

	Plant part			Treatment			Plant part × Treatment		
Na	2	0.290	0.750	2	3.032	0.062	4	1.497	0.226
Mg	**2**	**183.390**	**<0.001**	2	0.951	0.397	4	2.145	0.098
P	**2**	**30.481**	**<0.001**	**2**	**13.104**	**<0.001**	4	1.785	0.156
K	**2**	**27.678**	**<0.001**	**2**	**4.750**	**0.016**	4	1.707	0.173
Ca	**2**	**70.443**	**<0.001**	2	0.314	0.732	4	2.596	0.055
B	**2**	**78.823**	**<0.001**	2	1.840	0.175	4	1.407	0.254
Al	**2**	**130.488**	**<0.001**	2	1.930	0.162	4	2.580	0.056
Cr	**2**	**71.737**	**<0.001**	2	0.760	0.476	4	0.699	0.599
Mn	**2**	**79.409**	**<0.001**	2	0.124	0.884	**4**	**3.403**	**0.020**
Fe	**2**	**125.520**	**<0.001**	2	0.140	0.870	4	0.172	0.951
Co	**2**	**42.100**	**<0.001**	2	0.329	0.722	4	0.928	0.460
Ni	**2**	**29.418**	**<0.001**	2	0.495	0.614	4	0.780	0.547
Cu	2	1.971	0.156	**2**	**9.218**	**0.001**	4	1.452	0.240
Zn	**2**	**4.524**	**0.019**	**2**	**12.356**	**<0.001**	4	2.541	0.059
As	**2**	**103.024**	**<0.001**	2	0.346	0.710	4	0.663	0.622
Cd	**2**	**15.243**	**<0.001**	2	0.758	0.477	4	2.095	0.105
Pb	2	1.744	0.191	2	2.668	0.085	4	0.941	0.453

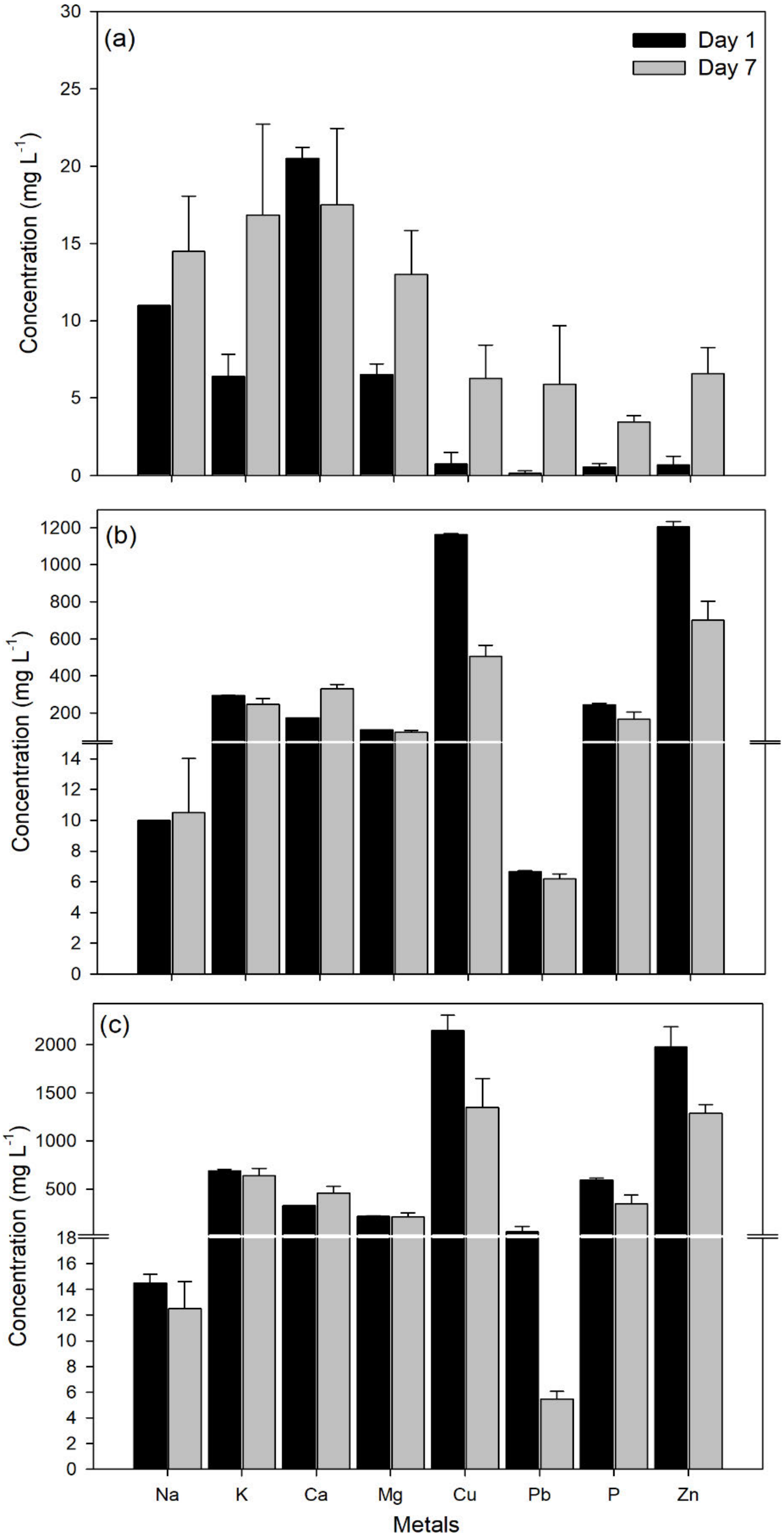

Figure 6.2: Water metal variation (mean ± standard deviation) measured at the beginning and end of experiment: (a) control, (b) low and (c) high concentration treatment.

6.4.2 Fern plant Dryopteris pentheri metal concentrations

In the plant roots, Ca, Mg and K were found in high concentrations exceeding 2 000 mg kg^{-1}, with relatively high concentrations in the low (i.e., Ca, Mg) and high (i.e., K) concentration treatments (Figure 6.3a). Potassium, Na, Ca, Mg, Cu, Pb, P, and Zn were high in plant stems with the high concentration treatment (Figure 5.3b). In the plant leaves, Na, K, Mg, Cu, Pb, P and Zn were high in the high-concentration treatment (Figure 6.3c). Repeated measures ANOVA indicated significant ($p < 0.05$) differences in Mg, P, K, Ca, B, Al, Cr, Mn, Fe, Co, Ni, Zn, As and Cd among the different plant parts, with P, K, Cu and Zn being significantly ($p < 0.05$) different among treatments (Table 6.3). Based on Tukey's posthoc analysis, most of the metals were found to be significantly ($p < 0.05$) different among the different plant parts except Mg (roots *vs* stems, $p = 0.816$), P (leaves *vs* stems, $p = 0.222$), Ca (leaves *vs* roots, $p = 0.990$), B (roots *vs* stems, $p = 0.055$), Cr (leaves *vs* stems, $p = 0.917$), Ni (leaves *vs* stems, $p = 0.875$), As (leaves *vs* stems, $p = 0.801$), Cd (leaves *vs* stems, $p = 0.222$), Fe (leaves *vs* stems, $p = 0.961$), Co (leaves *vs* stems, $p = 0.873$), and Zn (leaves *vs* roots, $p = 0.086$; leaves *vs* stems, $p = 0.262$; roots *vs* stems, $p = 0.785$).

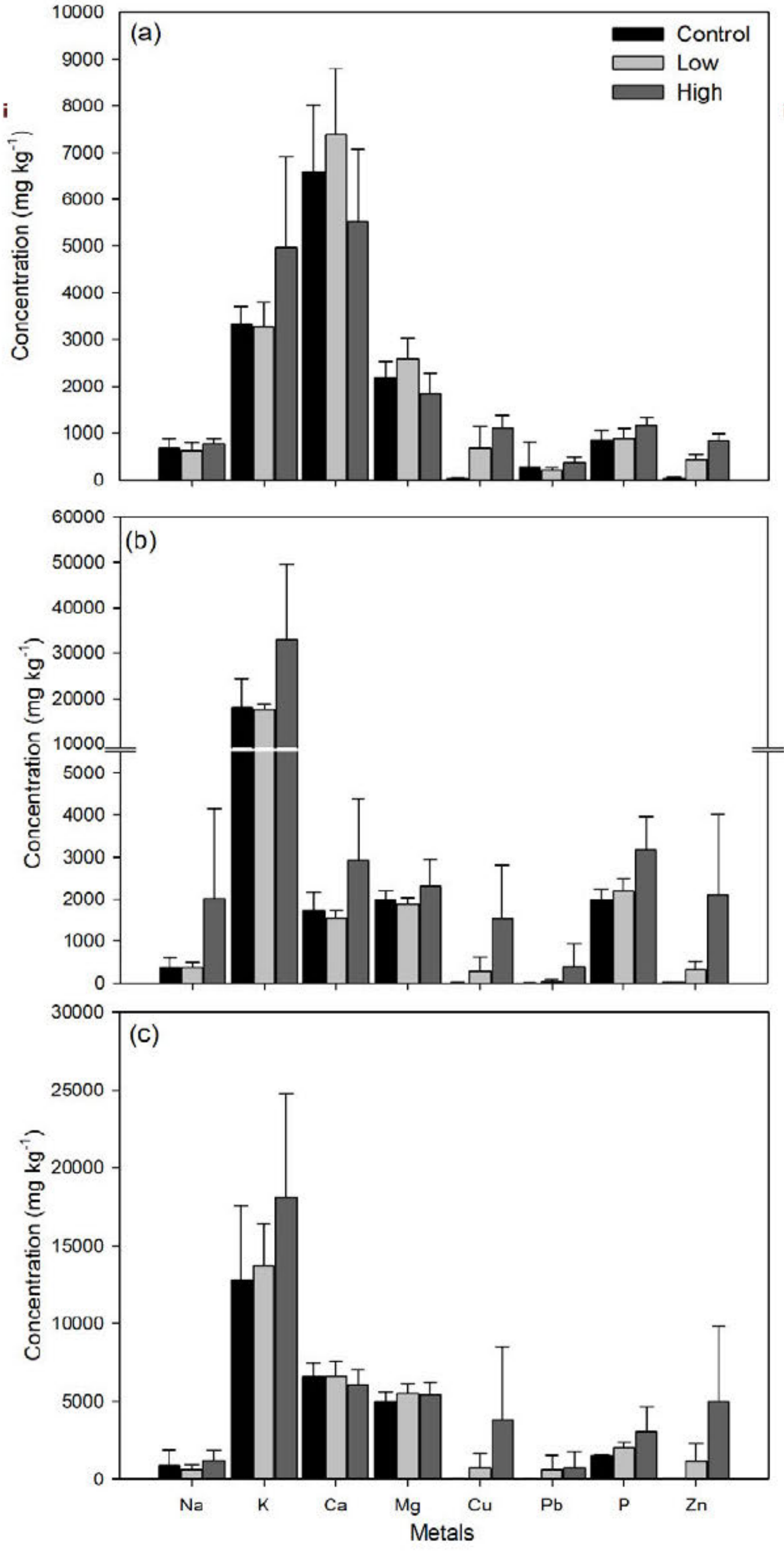

Figure 6.3: Fern plant *Dryopteris pentheri* metal variation (mean ± standard deviation) measured at the end of experiment among different plant parts: (a) roots, (b) steams and (c) leaves.

6.4.3 Dryopteris pentheri leaf chlorophyll concentrations

The plant leaf chlorophyll concentrations in the control were 4.52 ± 3.39 SPAD units at day 1 before decreasing at day 3 (2.54 ± 1.49 SPAD units) and then increasing to day 7 (4.32 ± 2.83 SPAD units). In the low concentration treatment, chlorophyll concentrations decreased from day 1 (5.02 ± 3.85 SPAD units) to 7 (2.22 ± 0.95 SPAD units), with similar pattern being observed in the high concentration treatment, where concentration decreased from day 1 (2.88 ± 2.03 SPAD units) to day 3 (1.80 ± 0.76 SPAD units) and there was a slight increase at day 7 (1.96 ± 0.77 SPAD units). Repeated measures ANOVA indicated significant differencesamong days ($F = 13.813$, $df = 2$, $p < 0.001$) and treatments ($F = 16.063$, $df = 2$, $p < 0.001$). The Tukey's posthoc analysis indicated significant differences for day 1 *vs* 2 ($p < 0.001$) and day 1*vs* 3 ($p < 0.001$), and furthermore for control *vs* high ($p < 0.001$) and low *vs* high ($p < 0.001$).

6.4.4 Transportation index (translocation factors) of metals

The stem/root metal concentration index (*Ti*) indicated less ideal for phytostabilisation as most metal concentration values were less than 1, with the exception of K and B which were greater than 1, indicating that the fern plant *D. pentheri* could efficiently move these two metals from its roots to its shoots among all treatments (Table 6.4). Based on one-way ANOVA analysis, no significant ($p > 0.05$) differences were observed for the stem/root metal concentration index.The leaf/root metal concentration index (*Ti*) indicated that Na, Mg, B, Cu, Zn and Pb metals could be efficiently moved from the roots to the leaves as the value was greater than 1 across all treatments, with Ca being > 1 in the control treatment only. The high-concentration606 treatment generally had high values (Table 6.4). Based on one-way ANOVA, only Co ($F =$ 607 11.111, $df = 2$, $p = 0.004$) was significantly different for the leaf/root metal concentration index among treatments, with the Tukey's posthoc indicating significant 609 differences for control *vs* high ($p = 0.009$) and control *vs* low ($p = 0.008$).

Table 6.4: The stem/root and leaf/root concentration ratios (transportation index) of metals in fern plant *Dryopteris pentheri* grown for 7 days. Red – values < 1 = less ideal for phytoremediation, and green – values > 1 = plant could efficiently move these two metals from its roots to its shoots

Metals	**Stem/Root**			**Leaf/Root**		
	Control	**Low**	**High**	**Control**	**Low**	**High**
Na	0.51 ± 0.18	0.55 ± 0.14	0.64 ± 0.18	1.17 ± 0.23	1.06 ± 0.33	1.51 ± 0.06
Mg	0.9 ± 0.14	0.73 ± 0.11	0.89 ± 0.16	2.31 ± 0.2	2.16 ± 0.2	2.56 ± 0.09
K	5.76 ± 1.71	5.44 ± 0.68	5.89 ± 0.89	3.96 ± 0.57	4.2 ± 0.26	5.05 ± 0.23
Ca	0.27 ± 0.07	0.21 ± 0.04	0.28 ± 0.03	1.02 ± 0.06	0.91 ± 0.08	0.92 ± 0.03
B	1.13 ± 0.2	1.33 ± 0.25	1.39 ± 0.14	3.66 ± 0.1	3.51 ± 0.3	3.72 ± 0.13
Al	0.07 ± 0.06	0.02 ± 0.01	0.01 ± 0	0.09 ± 0.02	0.07 ± 0.03	0.04 ± 0.03
Cr	0.06 ± 0.04	0.03 ± 0.02	0.02 ± 0	0.1 ± 0.02	0.1 ± 0.06	0.06 ± 0.02
Mn	0.15 ± 0.04	0.17 ± 0.05	0.21 ± 0.04	0.54 ± 0.14	0.75 ± 0.24	0.82 ± 0.02
Fe	0.06 ± 0.06	0.01 ± 0	0.01 ± 0	0.07 ± 0.01	0.06 ± 0.03	0.03 ± 0.02
Co	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.07	0.18 ± 0.05	0.19 ± 0
Ni	0.07 ± 0.04	0.05 ± 0.02	0.09 ± 0.05	0.12 ± 0.02	0.15 ± 0.05	0.17 ± 0.01
Cu	0.29 ± 0.37	0.46 ± 0.66	0.53 ± 0.05	1.07 ± 0.82	1.28 ± 1.62	2.6 ± 0.12
Zn	0.55 ± 0.45	0.81 ± 0.79	0.87 ± 0.15	1.54 ± 1.94	3.23 ± 4	5.42 ± 0.17
As	0.06 ± 0.04	0.03 ± 0.01	0.02 ± 0	0.13 ± 0.02	0.10 ± 0.04	0.08 ± 0.02
Cd	0.22 ± 0.06	0.19 ± 0.1	0.21 ± 0.04	0.12 ± 0.06	0.18 ± 0.1	0.24 ± 0.01
Pb	0.17 ± 0.14	0.2 ± 0.22	0.13 ± 0.08	1.04 ± 1.3	3.61 ± 4.28	2.77 ± 0.03

6.4.5 Bioaccumulation factors

Al, Cr, As, Cd, Mn, Fe, Co, and Ni were hyperaccumulating in the roots > fronds > rhizomes in all treatments (Table 6.5). Lead in control treatment was hyperaccumulating the roots, whereas in the low and high concentrations, it was hyperaccumulating in the fronds. Copper and Zn did not show accumulation in roots and rhizomes for low and high-concentration treatments. Trace metals (i.e., Na, Mg, K, Ca) were below 1, meaning that the fern plant *D. pentheri* was only able to absorb what was available in the water except for K in rhizomes and fronds of the control treatment that showed hyperaccumulation (Table 6.5). The low-concentration treatment had high BCF values compared to other treatments. Based on the two-way ANOVA, all metals except Na (p = 0.057), Cu (p = 0.407) and Pb (p = 0.379) were significantly different among fern plant *D. pentheri* parts. Among treatments Mg, K, Ca, Al, Mn, Cu, As and Zn were significantly ($p < 0.01$) different among the treatments (Table 6.6).

Table 6.5: Comparison of metal accumulation in *Dryopteris pentheri* leaves, stems and roots across different treatments after 7 days. Red shade – values ≤ 1 = plant can only absorb metals in proportion to what's available in the water, and green shade – values > 1 = plant can accumulate more metals than are available in the water (i.e., hyperaccumulation)

Variable	Control			Low			High		
	Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves
Na	0.06 ± 0.01	0.03 ± 0.02	0.08 ± 0.08	0.06 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.08 ± 0.04
Mg	0.33 ± 0.05	0.30 ± 0.03	0.77 ± 0.08	0.02 ± 0.004	0.02 ± 0.001	0.05 ± 0.01	0.01 ± 0	0.01 ± 0.001	0.02 ± 0.003
K	0.52 ± 0.05	2.91 ± 1.02	2.00 ± 0.74	0.01 ± 0.001	0.06 ± 0.004	0.05 ± 0.01	0.01 ± 0	0.03 ± 0.01	0.03 ± 0.01
Ca	0.37 ± 0.08	0.09 ± 0.02	0.37 ± 0.04	0.04 ± 0.01	0.01 ± 0.001	0.04 ± 0.01	0.02 ± 0.001	0.01 ± 0	0.02 ± 0.003
B	260.4 ± 46.0	291.5 ± 46.3	944.6 ± 269.3	260.8 ± 42	340.1 ± 26.2	906.8 ± 72.5	264.7 ± 32.9	369.2 ± 66.1	975.5 ± 186.6
Al	18947 ± 6720	1323 ± 1611	1760.3 ± 826.4	87836 ± 34636	1643 ± 720.5	6097 ± 1333	84573 ± 7332	1256 ± 286.9	3475 ± 1845
Cr	1200 ± 368.0	85.4 ± 88.9	111.4 ± 28.8	1496.5 ± 782.2	39.6 ± 22.1	111.3 ± 28.4	1172.3 ± 100.9	25.9 ± 6.1	75.1 ± 27.6
Mn	4328 ± 752.5	678.0 ± 147.1	2281 ± 468.3	22431 ± 7412	3661 ± 256.2	15646 ± 1931	16365 ± 1762	3478 ± 417.4	13487 ± 936.8
Fe	16488 ± 4518	977.6 ± 1422	1073 ± 413.5	28094 ± 12107	437.7 ± 180.3	1532 ± 355.5	23833 ± 945.2	333.3 ± 64.8	950 ± 445.4
Co	241.8 ± 157.7	10.9 ± 8.2	11.6 ± 1.4	187.9 ± 57.4	9.1 ± 2.5	31.9 ± 4.5	179 ± 11.2	10.4 ± 2.4	34 ± 9.8
Ni	506.1 ± 379.8	29.8 ± 16.5	53.6 ± 24.9	445.6 ± 172.6	22.8 ± 4.6	59.9 ± 4.4	382.1 ± 26.5	34.9 ± 19.7	65.5 ± 9.1
Cu	43.34 ± 18.2	12.7 ± 9.6	50.8 ± 60.6	0.5 ± 0.3	0.2 ± 0.3	0.6 ± 0.7	0.5 ± 0.1	0.3 ± 0.1	1.7 ± 2.2
Zn	60.6 ± 21.6	31.5 ± 6.6	89.7 ± 27.4	0.3 ± 0	0.2 ± 0.1	0.9 ± 0.9	0.4 ± 0	0.3 ± 0.1	2.5 ± 2.4
As	1298 ± 366.2	75.7 ± 65.7	162.5 ± 41.7	1594 ± 610.1	44.2 ± 12.3	147.4 ± 211	525.7 ± 17.8	11.7 ± 1.2	44.7 ± 11

Cd	81.2 ± 36.0	17.2 ± 6.1	9.5 ± 1.9	87.6 ± 32.3	15.0 ± 3.6	14.1 ± 4.1	76.4 ± 22	16 ± 2.3	17.8 ± 5.2
Pb	1755 ± 3717	20.6 ± 10.8	109.2 ± 116.2	1331 ± 382.4	256.3 ± 313.4	4433 ± 5943	5.8 ± 2.3	0.6 ± 0.3	14 ± 18.5

Table 6.6: Two-way analysis of variance of bioaccumulation factors for the different fern plant *Dryopteris pentheri* parts. Bold values indicate significance at $p < 0.05$

Variable	Plant parts			Treatment			Plant parts × Treatment		
	df	F	*P*	*Df*	F	*P*	*Df*	F	*P*
Na	2	3.197	0.057	2	0.049	0.953	4	0.128	0.971
Mg	**2**	**61.347**	**<0.001**	**2**	**503.125**	**<0.001**	**4**	**51.775**	**<0.001**
K	**2**	**8.299**	**0.002**	**2**	**58.198**	**<0.001**	**4**	**8.614**	**<0.001**
Ca	**2**	**30.454**	**<0.001**	**2**	**201.677**	**<0.001**	**4**	**21.602**	**<0.001**
B	**2**	**105.493**	**<0.001**	2	0.291	0.750	4	0.194	0.939
Al	**2**	**99.039**	**<0.001**	**2**	**16.914**	**<0.001**	**4**	**14.559**	**<0.001**
Cr	**2**	**62.656**	**<0.001**	2	0.486	0.620	4	0.487	0.745
Mn	**2**	**59.375**	**<0.001**	**2**	**69.423**	**<0.001**	**4**	**10.158**	**<0.001**
Fe	**2**	**93.310**	**<0.001**	2	2.533	0.098	4	2.684	0.053
Co	**2**	**32.236**	**<0.001**	2	0.172	0.843	4	0.599	0.667
Ni	**2**	**24.727**	**<0.001**	2	0.151	0.860	4	0.224	0.923
Cu	2	0.930	0.407	**2**	**8.797**	**0.001**	4	0.945	0.453
Zn	**2**	**6.360**	**0.005**	**2**	**83.386**	**<0.001**	**4**	**6.299**	**0.001**
As	**2**	**69.216**	**<0.001**	**2**	**7.210**	**0.003**	**4**	**4.714**	**0.005**
Cd	**2**	**47.871**	**<0.001**	2	0.085	0.918	4	0.217	0.926
Pb	2	1.006	0.379	2	1.912	0.167	4	1.407	0.258

6.5 Discussion

The present study reported that *Dryopteris pentheri* accumulated different metal concentrations in various plant parts. Moreover, bioaccumulation and translocation factors were greater than 1 for some selected metals in the roots, showing that *D. pentheri* is a hyperaccumulator and showed metal tolerance ability due to the increased chlorophyll content under the high metal concentration over time. Plants can accumulate metal levels differently in their different parts. The present study indicates that more metals were accumulated in the roots than in the fronds and rhizomes. The increased chlorophyll content shows that *D. pentheri* is tolerant to the metal concentrations in the water. The current study demonstrated that most metals accumulated increased remarkably inthe roots, then in the aboveground plant parts. Numerous studies observed similar results (Eid et al., 2021; Galal et al., 2018; Kumar et al., 2020; Kumar et al., 2019). The bioaccumulation factors (BCF) are used to determine plant's ability to transport metals from soil or water to the roots (Eid

et al., 2021). If BCF is greater than 1, then it means the plant can accumulate metals in the roots (Ang et al., 2023). The fronds/roots translocation factor (TF) showed that the plants can translocate the metals from the roots to the leaves in high concentrations. The TF value of rhizome/roots was less of phytostabilisation, with TF values being less than 1. Laptiev et al. (2024) stated that if the TFvalues are less than one, then the plant plays a lesser role in phytostabilisation processes.
The current study results indicated an increase in water parameters (conductivity, total dissolved solids and salinity) in high concentration and resistivity had increased in control. Similar results by Helard et al. (2021) reported that high metal concentrations had increased water parameters. Our study reported that metal accumulation by *D. pentheri* differed among the treatments. This suggests that it might be due to the solubility and mobility of the metals in the water (Munyai and Dalu, 2023). Trace metals such as Ca, Mg, Na and K were accumulated in roots and transferred to the shoots, irrespective of the treatments. Ferns typically have extensive root systems that enhance their capacity to absorb metals from the soil or water (Sharma and Sharma, 2022). Once absorbed, metals are transported to various plant tissues, where they may be sequestered in vacuoles or bound to metal-chelating compounds such as phytochelatins (Cobbett, 2000a, b). These compounds play a critical role in detoxifying metals and preventing their interference with essential cellular processes.

Our current findings show that the chlorophyll content increased in the control group after a period of 7 days, confirmed by Drăghiceanu et al. (2021) who reported an increase in the chlorophyll content of *Dyropteris* sp. in control. The results of our study indicate that chlorophyll content decreased at low concentrations over time, while it increased slightly in both the control and high concentration groups after 7 days. In contrast, a study by Drăghiceanu et al. (2021) reported that the chlorophyll content of *Dryopteris affinis* and *Dryopteris filix-mas* has been reduced by high metal concentrations after long exposure to metals. In addition, various other studies including Doganlar et al. (2012), Shah et al. (2017) and Singh and Pandey (2011), reported contradicting findings to the current study, where low metal concentrations stimulated the chlorophyll over time.

The results of the current study indicated that translocation factor (TF) values of rhizome/roots were less than 1, which suggests that *Dryopteris pentheri* is a metal excluder (Ang et al., 2023). A TF value of less than 1 might be due to the exclusion strategy and restriction of metal movement from the roots to the shoots (Pang et al., 2023). Additionally, plant physiology also plays an important role in excluding certain metals that are not required by the plant itself, thereby protecting the shoots (Ang et al., 2023; Netshiongolwe et al., 2020). *Dryopteri*s *pentheri*, a metal excluder, has been shown to have translocated only potassium (K) and boron (B) from roots to the rhizomes, which were explained by their TF values being greater than 1. Thus, it indicates that *D. pentheri* has a potential to remove K and B from the environment. The fronds/roots TF values of Na, Mg, B, Cu, Zn, Pb and Ca were more significant than 1, and our current study confirms that *D. pentheri* is a hyperaccumulator of these metals. *Dryopteris pentheri* showed the phytoaccumulation ability. The findings in the current study had however confirms observation by Munyai and Dalu (2023) who reported that trace metals (Na, B, Cu and Zn) have increased from the roots to the fronds. The present study had high bioaccumulation factors (BCF), indicating the ability of the plant to hyperaccumulate metals such as Al, Cr, As, Cd, Mn, Fe, Co and Ni in the roots followed by fronds and then rhizomes among all concentrations. Our results reflect those reported by Cornara et al. (2007); Senila et al. (2013) and Singh et al. (2010) who reported that most ferns, such as *D. fillix-mas, Pteris cretica* and *P. vittata* are hyperaccumulators of As, Mn, Fe, Al and Cr than any other non-fern plants, and further mentioned that control stimulated the hyperaccumulation of lead (Pb) in the roots. Our present study indicates that K had BF >1; this might suggest that K hyperaccumulation in the control concentration might have been influenced by As in the roots. Arsenic is known to induce growth stimulators such as K at low concentrations (Singh et al., 2010).

The metal uptake mechanism in plants involves complex interactions between the soil environment and highly selective transport systems within the plant. These systems regulate the absorption, transport, and detoxification of metals to support plant health while avoiding toxicity. Understanding these processes is vital for agriculture, environmental protection, and biotechnological applications like phytoremediation.

6.6 Conclusion

The study concludes that *Dryopteris pentheri* accumulated different metals in different parts (root, rhizome and fronds) at different concentration levels. Furthermore, the study indicates potential of removing and up taking metals from the aquatic environment. There is a high translocation of metals of some metals from the roots into the fronds. In addition, the results revealed that both BF and TF values were >1, revealing that *D. pentheri* hyperaccumulate metals, especially, As, Cr, As, Cd, Mn, Fe, Co and Ni among all treatments. Generally, *D. pentheri* is tolerant to the metals, as shown by the increase in chlorophyll content among the treatments. After assessing *D. pentheri* for phytoremediation, the results indicate that this species is a good candidate for hyperaccumulating metals from the environment. This suggests that these plants deserve to be conserved especially in the wetlands as they are good potentials for phytoremediation.

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CHAPTER 7.
CONCLUSION AND SYNTHESIS

7.1 General Introduction

Wetlands face serious contamination from metals, which happens every season. These metals might be coming from either human settlements, industrialisation, agricultural activities, or any other activities around wetlands, such as littering plastics and domestic waste (Rathod et al., 2024). Furthermore, these metals get absorbed or settle in the sediments and accumulate in the macrophytes. On the other hand, leafy edible vegetables can accumulate metals translocated from the soil and later have a huge impact on human health. Due to the availability of water, these vegetables are irrigated by water from wetlands. Therefore, every water resource available must be conserved to alleviate water scarcity (Adeeyo et al., 2022). Interestingly, Riverside wetlands in the City of Mbombela have sediments and plant species that are considered to purify and treat water by accumulating the metals.

Nevertheless, there is still a different community attitude towards the ecological and economic importance of wetlands (Sinthumule, 2021; Bhowik, 2022). Hence, this study explored sediments, aquatic macrophytes and edible leafy vegetables on their capability to accumulate heavy metals and nutrients. This study focused on investigating heavy metals and nutrient accumulation by sediments, aquatic macrophytes across different seasons and sites. Additionally, to determine the metal accumulation by vegetables with implications for human health. The current findings demonstrated that sediments were able to accumulate different metal concentrations, similarly aquatic macrophytes were able to accumulate metals, hence they are regarded as the best candidates for phytoremediation (Xiao et al., 2021; Rasool et al., 2023; Marques et al., 2024).

Moreover, leafy edible vegetables proved to be accumulators of metals at different concentrations. The study validated field data using *Dryopteris pentheri* to confirm the phytoremediation of the aquatic macrophytes. This study added some significant value towards the importance of wetlands.

7.2 Summary of results

The Riverside wetlands in the City of Mbombela are promising productive aquatic ecosystems that contribute to environmental, cultural, and economic values. Like many other systems within the same geographic region, however, the Riverside wetlands are at risk of being polluted by different activities around them, which result in affecting life within the wetlands (Junk et al., 2013; Bhowmik, 2022; Aslam et al., 2024). An informative assessment of spatio-temporal variation and accumulation of metals by plant species and sediments is presented. The importance of the sediments and different plant species on metal accumulation is discussed in Chapters 3 to 5. One of the key findings of this study is that seasons, plant parts and locations played a major role in the translocation of the metals within the Riverside wetlands. The current study provided better knowledge and contributed significantly to the available literature on the accumulation and translocation of metals by sediments and plant species during different seasons and sites. Validation of this study and how other plants (ferns) can tolerate metals and contribute towards phytoremediation is provided in Chapter 6.

From the researcher's perspective, this is the first study of metal accumulation conducted at Riverside wetlands. However, similar studies were conducted (Netshiongolwe et al., 2020; Munyai and Dalu, 2023) but measured few metals such as P, K, Ca, Mg, B, Fe, Zn and Cu, whereas the current study measured more metals. The current study assessed every possible metal that can be found within these wetlands across different seasons, sites and plant parts in Mpumalanga Province. This study demonstrated that metal translocation depends on the season, generally the dry seasons (i.e. cool-dry and hot-dry). Alternatively, the location of the wetlands also plays a major role in the accumulation of the metals within sediments and macrophytes. Studies suggest sediments and aquatic macrophytes can accumulate the metals within the wetlands (Fan et al. 2021; Nabuyanda et al. 2022). Since metals can cause harmful effects to aquatic organisms, it was also observed that leafy edible vegetables contaminated with metals can result in human health implications.

In this current study, the spatiotemporal distribution and sediment pollution indices of trace and heavy metals in surface sediments and potential sources of contamination in urbanised Austral wetlands were assessed (chapter 3). Sediments studies on metal accumulation are, however, often limited to their distribution in wetlands (Nabuyanda et al., 2022; Wang et al., 2023). The findings of this study, therefore, highlight the potentially broad impact of season and location on sediment chemistry in wetlands (Zarei et al., 2023). Moreover, this study demonstrated that the location of the wetlands plays an important role, since there were different levels of metals from different sites. This study (chapter 3) further identified that Riverside wetland sediments have accumulated high concentrations of metals such as Mn, Co, Cu, Pb, Zn, Al and Fe during the cool-dry season, which agreed with results by Curtis et al. (2024) who found similar metals from wetlands in the Mpumalanga Lake District near coal-fire. Since high metal concentrations were observed during hot-dry season from different sites, indicating that metals were not diluted or being washed off by rainfall during hot-dry and cool-dry seasons. The present study therefore supports observations by Chakraborty (2022), who reported that high metal concentrations were observed during the dry season from Mongla port area, Bangladesh. The study sites of the current study is surrounded by automotive industries, the study was hyopothesised that those metals are coming from those industries. However, the empirical assessments of sources of the metal dynamics are often difficult to determine and have not been observed in all areas (Wood and Shelley, 1999).

The study (chapter 4) focused on investigating the bioaccumulation of heavy metals in five different leafy green vegetables (cabbage, rape, lettuce, pumpkin leaves and spinach) and soils and determining the human health risks that may arise from consuming these vegetables. The study further explored the Mpumalanga Province, Tonga which was previously not investigated and documented a wealth of knowledge that could contribute to the awareness of consuming metal contaminated edible leafy vegetables. The study also confirmed the similar metals reported in chapters 3 and 5 which can cause health hazards as previously reported (Meftaul et al., 2023). In addition, these metals have exceeded the acceptable levels. Furthermore, this study indicates potential health risks associated with consuming edible leafy vegetables which was confirmed by Sultana et al. (2022). This study confirmed that metals will be translocated from the soil into the vegetables (Ba et al., 2024). In addition, his study revealed HRI and THQ values of >1, indicating that the people consuming these vegetables might suffer a higher non-carcinogenic risk (Meftaul et al., 2023). This study found that Si, K, Na, Ca, Mg, Al and Fe were high in the soils confirmingthe study by Mahmud et al. (2020). Indices such as bio-concentration factors, daily intake of metals, health risk and target hazard quotient were used to determine the bioaccumulation of metalsin the vegetables (Fatai et al., 2024). This study shows that vegetables indicated elevated Ca, Fe, Si, Al and Sr levels. However, spinach had high Na concentrations, which echoes the study by

Meftaul et al. (2023), where spinach was reported to be the vegetable with high metal concentration. The current study hypothesised that different vegetables would accumulate various metals at different concentration levels.

In chapter 5, it was found that all four macrophyte species accumulated metal concentrations (El-Amier et al., 2020) in the different parts across seasons (Pandey et al., 2019). When considered holistically high metal concentrations during the cool-dry compared to hot-dry and hot-wet seasons, irrespective of macrophytes species (Netshiongolwe et al., 2020). In turn, the macrophyte, *C. dives,* C. *papyrus* and *T. capensis* can be major accumulators of metals compared to *P. australis.* Plant metal concentrations were generally highest in the roots and leaves as compared to stems. Aquatic ecosystems waste treatment relies largely on macrophytes (Khan et al., 2024). An important question in determining the phytoremediation potential of aquatic macrophytes from different locations across different seasons is whether aquatic macrophytes are the best phytoremediation candidates (Singh et al., 2024; Sompura et al., 2024; Kola et al., 2025). Despite the phytoremediation potential of macrophytes, they do not have the same ability to accumulate metals from the aquatic ecosystems (Khan et al., 2024). Morphology of macrophytes influences phytoremediation (Lavanya et al., 2024), where it is reported that plants with high biomass stand a good chance of having a phytoremediation potential (Maranho and Gomes, 2024).

Chapter 6 presents the validation of Chapter 5, demonstrating that *Dryopteris pentheri* can tolerate high concentrations of heavy metals. It was established that this plant species increased chlorophyll content under high metal concentration and showed that it can also be recommended to be used for phytoremediation.

7.3 Future studies

These findings can be used as a reference to inform evidence-based policy interventions aimed at promoting and evaluating aquatic macrophyte plants used for phytoremediation, as well as to demonstrate the local status quo of consuming contaminated vegetables, particularly in peri urban areas of Mpumalanga Province. The present study highlighted the importance of the sediments within different Riverside wetlands as they can accumulate metals across seasons. These results agreed with some results from the heavy metal polluted soil profile in seasonal-flooding riparian Bangladesh wetlands (Bai et al., 2016) and were confirmed by Wang et al. (2019) and Hossain et al. (2022) from Poyang Lake, China and Dunuria Upazila in Khulna district, Bangladesh, respectively. The analysis using pollution indices indicated that wetland sediments were highly contaminated, mainly with Mn, Co, Cu, Pb, Zn, Al and Fe, especially during the cool–dry season. These results have highlighted some valuable information on possible sources of these metals around the wetlands. The current study highlights an urgent need to develop immediate measures to control severe heavy metal pollution from industrial emissions into the wetlands. These immediate measures should be carried out to minimise the rate of contamination and the extent of future metal contamination. Future studies should attempt to develop methods to determine the exact source of each metal and the control measures around the wetlands.

The accumulation of metals by different aquatic macrophytes varied across different seasons. The study observed high metal (Fe and Mn) concentrations during the cool–dry compared to hot–dry and hot–wet seasons, irrespective of macrophytes species which agree with results from Oliveira et al. (2024) In turn, the macrophytes, *C. dives,* C. *papyrus* and *T. capensis* can be a major accumulator of metals than *P. australis* and these results agreed Munyai and Dalu (2023).

Plant metal concentrations were generally most accumulated in the roots and leaves as compared to stems. These results provide baseline information for general management of the Riverside Wetlands and advocate for further investigations into the long–term wetlands and plant metal variability, so as to better understand the metal and nutrient dynamics involving several plant species across different seasons. Future studies should focus on enhancing phytoremediation efficiency through molecular research and exploring the role of rhizospheric and endophytic microbes in facilitating metal accumulation.

Overall, addressing these challenges through standardized guidelines is essential for advancing the large-scale application of macrophyte–based phytoremediation. Chapter 6 has outlined the results of the experiment carried out hydroponically, where water was amended with different metal concentrations. This study showed that *Drypteris pentheri* can tolerate high metal concentration, which was indicated by the increase in chlorophyll content. Further studies should be taken to examine heavy metal uptake by plants under flow-through conditions in both the laboratory and field. This would model heavy metal uptake in urban aquatic ecosystems and their mechanisms under more realistic and mobile conditions.

Monitoring wetlands is very important, as they serve as habitats and food for aquatic organisms. Wetlands get polluted by heavy metals from activities around them. These heavy metals pollute, change the quality, and negatively impact the various ecosystem services and functioning of wetlands. As a result, further studies should focus on protecting the wetland and the plant species.

7.4 Limitation of the study

The Riverside wetlands did not have a high diversity of aquatic macrophytes; only four common species were present. As a result this might have limited the current study to have a range of metal accumulation by various aquatic macrophytes. Additionally, some of the study sites were surrounded by forests. As a result, this might suggest that some metals might be absorbed by other plants other than aquatic macrophytes. The small sample size of edible leafy vegetables during data collection, especially for cabbage and rape, resulted in a limited range of data. This study did not assess the pH levels of the soils, which might have an effect on the metals accumulated by edible leafy vegetables. Additionally, another limitation of the study is because water from the wetland could have been used to grow those vegetables to actually show the uptake of these contaminants by them.

7.5. Conclusion

The study confirmed that Riverside Wetlands are polluted by various metals, which might be coming from activities occurring around the wetlands. This study also showed that metal accumulation varies with season, with higher concentrations observed in macrophytes and sediments during the cool- dry and hot-dry seasons. Moreover, metals were accumulated in the roots, followed by the leaves. The study found that some metal concentrations exceeded WHO/FAO recommended limits, highlighting the need for the development of appropriate regulatory measures. Policymakers play a crucial role in safeguarding wetlands by enacting and enforcing strong environmental regulations, investing in restoration efforts, and promoting sustainable land-use practices. For long-term protection, it is essential that wetland conservation becomes a central component of national and local policy agendas, guided by scientific research and inclusive stakeholder engagement. The future health of our wetlands and the benefits they provide to people and the Earth depends on informed, decisive, and collaborative policymaking.

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