Assessment of metals, carbon dynamics and macroinvertebrates diversity in pan wetlands across geological areas of central Kruger National Park, South Africa

by

Elsie Nomcebo Leshaba

201836025

A research dissertation submitted in fulfilment of the requirements for the Master of Science degree, School of Biology and Environmental Sciences, University of Mpumalanga

Supervisor: Dr Tatenda Dalu

Co–Supervisor(s): Prof Timothy Dube (UWC)

November 2023

UNIVERSITY OF MPUMALANGA

Creating Opportunities

ABSTRACT

Wetlands are ecosystems that rely on both rainfall and groundwater supply for their composition, structure and functionality. They are highly dependent on water levels and a change in climatic conditions affect water availability and this highly influences their structure and functionality. They play an important role in climate mitigation, sustaining of ecosystems, livelihood and societies by providing essential ecological services and societal benefits. The central parts of Kruger National Park comprise of a network of ephemeral pan wetlands that lay across different geological types (sandstone, granite, basalt and rhyolite) that provides an ecosystem service to both inland and aquatic species. When compared with marine and terrestrial biomes due to their limited extension, they are widely recognised as biodiversity hotspots. However, despite their ecological services and their carbon dynamics, there is still a poor understanding of these ecosystems functioning. In a pool of knowledge about what they are and what they provide, there is still no information about their distribution, pan sizes and the amount of carbon they sequestrate. Hence, this thesis aims to investigate the complex interactions between abiotic (carbon sequestration, metal accumulation and nutrient concentration) and biotic factors (invertebrates' diversity and abundance) in pan wetlands.

A field study was conducted in September 2022 when pan wetlands were drying out to assess soil organic carbon sequestration, metal and non–metal dynamics in floodplain pan wetland system across different geological types and soil profiles. Sediment samples were collected randomly across 12 pans with different geologies using a 130 cm hand auger. The results indicated that there were significant differences in pH, SOC (soil organic carbon), and concentrations of various metals (K, Mn, Na, Fe, Cu, Ca, Zn) with sediment depth. These differences were found to be statistically significant. Additionally, there were significant

differences in pH, SOC, SOM (soil organic matter), resistivity, carbon sequestration, non–metal/nutrients (S, P, B), and all metals. Despite the results highlighting a significant concentration of metals in the topsoil, there are no discernible adverse observed on the aquatic systems. This study provides an evaluation of sediment metal pollution to assist resource managers in effectively implementing management strategies for floodplain pan wetlands.

In addition, an assessment of spatiotemporal invertebrate diversity and abundances during high and low rainfall water period in relation to water and sediment chemistry was done. The sediment and water–chemistry variables and macroinvertebrates were sampled during high water period (December 2022) and low water period (June 2023). Sediment and water chemistry–variables were measured to assess the concentrations of metals and nutrient across geological areas and how they influence aquatic invertebrate biodiversity. The study collected a total of 5 145 macroinvertebrates from 41 species across 12 pan wetlands with different geological types during high water and low water periods. The high rainfall period had a higher number of species (n = 29) compared to the low rainfall period (n = 12). The most abundant order was Hemiptera, making up 57.0 % of the total sample, followed by Crustacea (24.6 %), Diptera (9.1 %), Mollusca (3.3 %), Trichopteran (2.0 %), Coleoptera (1.5 %), Annelida (1.4 %), Odonata (0.9 %), and Ephemeroptera (0.1 %).

Statistical analysis revealed significant differences in macroinvertebrate community structure among geological types and seasons. During the high–water period, the % ETOC and %EPT were higher compared to the low–water period. The % Chironomidae + Oligochaeta was only observed in the low–water period. The Shannon–Wiener diversity index was higher during the low–water period, while species abundance was higher during the high–water period. The

Simpson index showed a higher number of dominant species during the low–water period, and the evenness index was higher during the high–water period.

While the pan wetlands located in the central Kruger National Park play a major role in maintaining biodiversity and serving as water points for inland animals. This study provided an assessment of aquatic biodiversity in pans to better understand trophic interactions within (autochthonous) and contributions from outside (allochthonous) and provide management options for aquatic taxa in a protected area.

Keywords: Carbon sequestration, metal, nutrient, pan wetland, sediment, macroinvertebrate, water.

TABLE OF CONTENTS

DEDICATION

This work is dedicated to my late best friend Dorah Mbonwayini Mthimunye. Life never gave you a chance to flourish, hence I am doing it for the both of us. Continue resting Nomaziyane, my heavenly angel.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God for his kept promise (PALSM 46:5), and for the strength, wisdom, courage and resilience he gave me to go through this course. I would also like to thank myself for staying the course even when my depressive episodes would kick in now and then, girl you won cheers to that. This work was made possible through the assistance of various people and institutions. Special thanks to the following institutions and people: I am grateful to Water Research Commission (WRC) for their unconditional financial support; the host, University of Mpumalanga(UMP) for the provision of laboratory, instruments and support from the lab assistance, Kruger National Park, South Africa for granting me a permit to conduct the study within the park, Aquatic System Research Group (ASRG) members for their support, special thanks to Mr Farai Dondofema, Fannie Masina, Linton Munyai and Masimini Nkosi and Ronald Mashamba for their assistance in field data collection and data processing. To my family, friends and the AFM church, thank you for the love and prayers. Finally, and most importantly, my supervisors, Dr Tatenda Dalu and Prof Timothy Dube (UWC) for their guidance, unwavering support and patience throughout this study. It would be an understatement to say that Dr Tatenda Dalu offered only academic support. His constant commitment to his student's mental health is unmatched.

DECLARATION

I, Elsie Nomcebo Leshaba, with student number 201836025, officially affirm my knowledge and understanding of the University of Mpumalanga's policy regarding plagiarism. I have diligently ensured my adherence to the stipulated standards. The above document has undergone analysis using a software designed to discover similarities, and the resulting report has been evaluated by my supervisor(s). I assert that there is no instance of plagiarism included inside this study thesis.

Signature: ______________________ **Date:** **30 Novembe2023**

CHAPTER 1: INTRODUCTION

1.1 Background

Groundwater dependent ecosystems (GDEs) are an important but poorly understood component of the natural world, particularly within the tropical regions. Kløve et al. (2011) and Richardson et al. (2011) define groundwater dependent ecosystem (GDEs) as systems that rely on the supply of groundwater for its composition, structure and functionality and for the survival of plants and animal communities, ecological processes and ecosystem afloat. Even though they are essential for the survival of aquatic habitants, these ecosystems were not regarded as important habitats, they have been neglected for many years, until recently (Mabidi et al., 2017). Matlala (2010) concur that even though wetlands provide numerous ecological benefits, they have historically been undervalued and subjected to widespread destruction due to a lack of conservation and protection legislation. According to Hose et al. (2022), several terrestrial, marine, and coastal ecosystems rely on groundwater to sustain and maintain their biological integrity. Pérez et al. (2016) argues that their protection can only be possible if knowledge about their location, extent and biodiversity is available. Moreover, despite their small size, pans can serve as natural water purifiers, filtering and absorbing many pollutants in surface water, including phytoremediation (the removal of toxins using plants) and bioremediation (the degradation of toxins to less hazardous levels using plants), habitats of great diversity for flora and fauna, including plants, invertebrates, vertebrates, and tourism (Helson, 2012).

Whigham (1999) states that pan wetlands are more significant than other forms of wetlands because of the critical landscape and biodiversity tasks they serve, such as providing habitat for migrating birds, improving water quality, and maintaining high biodiversity. Dalu and

Chauke (2020) highlight the importance of pan wetlands in proving habitants that supports aquatic biodiversity and that there are many unique flora and fauna species found only in these systems. However, Fennessy et al. (2018) note that the potential of wetland soils to sequester and store substantial amounts of carbon has been increasingly acknowledged. According to Tooth and McCarthy (2007), defines a wetland as sites where plant growth and other biological activity are suited to the wet condition and are periodically or constantly swamped by shallow water or have waterlogged soil. According to Aldous and Bach (2014), they make use of groundwater to create hydro periods that control their structure and function. Despite their widespread distribution, which accounts for 4 and 6 percent of the earth's land area, and their variety of geomorphological and sedimentary properties, which defies easy categorization, characterization, or classification (Tooth and McCarthy, 2007), many of them are already destroyed or degraded due to changes in land use (Wachniew et al., 2014).

Were et al. (2019), wetlands have become a crucial component in the effort to address climate change due to their capacity to sequester carbon. In addition, these ecosystems they often retain floodwaters, and facilitating significant biogeochemical processes that involve the conversion and retention of nutrients, organic compounds, metals, and organic matter constituents (Suir et al., 2019). Furthermore, Bonada et al. (2006) allude that the size of pans and geology of an area can determine the number of invertebrate's species that can be supported or occur within these systems. For example, pan size has been shown to influence species composition (Bonada et al.,2006). However, temporary pans support low number of invertebrate communities than permanent ones and are still considered important for rare invertebrate organisms that are not found in permanent systems (Batzer et al., 2004). Furthermore, invertebrate diversity and abundance tend to increase in habitant heterogeneity in larger pans (Kouamé et al., 2011).

1.2 Justification

The significance of comprehending ecological dynamics for the development of well-informed conservation strategies has been emphasised in previous scholarly works (Smith et al., 2018; Jones & Brown, 2020). Nevertheless, within the framework of protected areas in Southern Africa, specifically pertaining to pan wetlands, there exists a noticeable scarcity of empirical research. The existing information is either old or insufficient in terms of depth, which hinders its ability to offer practical insights for conservationists and policymakers.

Temporal pan wetlands provide nesting sites for a variety of unique invertebrates and amphibians, as well as important foraging grounds for a variety of birds (O'Niell and Thorp, 2014) and watering points for many large mammals (Vanschoenwinkel et al., 2011). However, despite their ecological importance and carbon sequestration abilities, for many of these ecosystems there is a poor understanding of their ecosystem functioning. In Southern Africa's protected areas, there is a scant information on their distribution, hydro–period and sizes of the pan wetlands. The central parts of Kruger National Park are no exception to this fate, the non–Ramsar declared pan wetlands in there are understudied. Therefore, their occurrence in such habitats like the great Kruger National Park presents an opportunity to assess the habitant biodiversity tasks and carbon dynamics to provide a solution for their management.

1.3 Aims

This research aim was to assess the metals, nutrients, carbon sequestration as well as the distribution of macroinvertebrates in relation to metals and nutrients across different geological zones.

1.4 Objectives

- To assess soil organic carbon sequestration, metal and non–metal dynamics in floodplain pan wetland system across different geological types and soil profiles.

  To assess spatial distribution and variation of macroinvertebrate diversity and abundances during high and low water period in pans in relation to water and sediment chemistry variables.

1.5 Hypothesis

- The carbon sequestration capacity in pans will be positively correlated with the organic matter content and soil depth, while the metal concentrations in soil cores will vary depending on soil properties**.**
- The sediment metal and nutrient concentrations of pans will vary spatially with high concentrations expected due low hydro period times. Additionally, nutrient levels may fluctuate seasonally due to biological and environmental factors.
- Invertebrate diversity and abundance will be high during the high–water period compared to the low water period due to habitat suitability and reproductive opportunities.

1.6 Thesis outline

This thesis consists of general introduction (Chapter 1), which includes research problem, hypothesis, aims and objectives. It has 2 data chapters (Chapters 2 and 3) and a general synthesis conclusions chapter. Chapters 2 on the thesis focused on assessing carbon sequestration, metal and non–metal concentrations in pan wetland sediment cores, chapters 3 assessed spatiotemporal invertebrate diversity and abundances during high and low water period in pans in relation to water and sediment chemistry variables.

CHAPTER 2: ASSESSMENT OF SOIL ORGANIC CARBON SEQUESTRATION, METAL AND NON–METAL DYNAMICS IN FLOODPLAIN PAN WETLAND SYSTEM ACROSS GEOLOGICAL TYPES AND SOIL PROFILES

2.1. Introduction

Pan wetland systems are globally recognised as crucial and highly productive ecosystems, characterised by their unique and intricate nature allowing them to support high biodiversity (Masina et al., 2023). Mitsch and Gosselink (2015) highlights that wetlands are recognized as vital elements of the planet's natural systems and biologically crucial habitats that has gained growing prominence in the past few years. They vary significantly based on origin, geographic location, water patterns and composition, prevalent species, soil and sediment characteristics (Bassie et al., 2014). These special ecosystems are important to world biodiversity and several ecological processes due to their transitional nature between terrestrial and aquatic environments (Zedler and Kercher, 2005).

Despite wetlands covering only 6–8% of the world's freshwater surface, their sediments carbon is estimated to account for one–third of the freshwater organic soil carbon pool (Bernal and Mitsch, 2012). These systems function as carbon sinks within the terrestrial environment and their sediments have potential to sequester and store substantial amounts of carbon (Fennessy et al. 2018; Nag et al., 2023). According to Bridgham et al. (2006) and Were et al. (2019), wetlands ecosystems can reduce the impact of climate change as they have the capacity to sequester carbon. Furthermore, Were et al. (2019) and Villa and Bernal (2018) explain the process of wetland carbon sequestration as the conversion of carbon dioxide (CO_2) from the atmosphere into the soils for an extended period, minimizing the potential of its subsequent release into the environment. Most studies have shown that in wetland ecosystems are the primary reservoir for carbon sequestration and most of the carbon is stored within the soil rather than in plants (Bernal and Mitsch 2012; Nahlik and Fennessy, 2016; Moomaw et al., 2018). Moreover, Nahlik and Fennessy (2016) state that although 20 to 30 percent of the estimated 1 500 Pg of global soil carbon is contained in wetland soils, the quantity of carbon that a wetland stores and emits each year is highly dependent on the hydrogeochemical properties of its ecosystem. In addition, wetlands play a crucial role in carbon sequestration, owing to their anaerobic, acidic, and thermal conditions, which enable them to retain carbon for significantly prolonged periods compared to other ecosystems (Suir et al., 2019).

Despite of wetland's provision of ecosystem services and their significant role in climate mitigation, they are currently confronted with escalating challenges resulting from human activities (Trettin et al., 2020). These challenges primarily manifest in the form of the introduction of diverse pollutants (Daryadel and Talaei, 2014; Li et al., 2020; Adeeyo et al., 2022). Metals, non–metals, and excessive nutrients have been identified as significant contributors to the deterioration of these delicate ecosystems (Hoffman et al.,2012). Wetlands ecosystems have experienced a substantial introduction of anthropogenic contaminants, such as heavy metals, originating from industrial, agricultural, and urban sources (Esmaeilzadeh et al., 2016). These ecosystems have been found to be contaminated with common heavy metals, including cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) (Ayeni et al., 2010; Bai et al., 2012). However, wetland sediment can serve as a repository for these heavy metals, nutrients, and pesticides that are discharged into the water column prior to entering the food chain (Helson, 2012). Morales–García et al. (2020) argues that wetland sediments serve as both reservoirs and sources of metals. Highlighting how some of these heavy metals can be natural, but leach into the sediments, studies conducted by Bashir et al. (2020) and Dalu et al. (2022) have shown that heavy metals can be naturally occurring elements yet can reach wetlands ecosystems through slow leaching of soil and rocks. Consequently, the concentration of these naturally heavy metals can be elevated to levels that are detrimental to flora and fauna due to geological and anthropogenic factors (Chibuike et al., 2014).

Overall, since sediment quality is a significant indicator for heavy metals contamination and ecological risk assessment, when metals are discharged into aquatic environments, they form chemical bonds with particulate matter which pose a significant environmental threat due to their persistence, non–biodegradability, toxicity, and bioaccumulation (Cheng et al., 2015; Gargouri et al., 2018). According to Hu et al. (2015), the presence of heavy metal contamination, such as Cd, Cr, Ni, and Pb, are common metal pollution found in areas such as farmland, freshwater, marshes, wetlands, and aquatic systems. Due to processes such as adsorption, hydrolysis, and coprecipitation, only a small portion of free metal ions are taken away by water flows and massive quantities gets deposited (Hu et al., 2015). In contrast to organic pollutants, heavy metals are not eliminated from aquatic ecosystems through natural mechanisms (Phillips et al., 2015; Bashir et al., 2020) but through multiple physical and chemical techniques. These various physical processes, including ultrafiltration, coagulation, flocculation, adsorption, membrane filtration, and ion exchange and chemical methodologies encompass several techniques such as neutralization, solvent extraction, chemical precipitation, and electrochemical treatment (Yadav et al., 2022).

Although, there is plethora of information on the contamination of metal in aquatic ecosystems (Gaur et al., 2005) and in view of the global community's efforts to identify effective solutions to mitigate climate change, conserve and restore pan wetlands. There is a growing need to comprehend the carbon sequestration capacity of wetlands and the effects of metal contaminations. This study aims to assess carbon sequestration, metals and nutrients concentrations in wetland sediment cores across geological types and to further assess the spatiotemporal variation of metals and nutrients in sediments across the pans. These aims are based on the following hypothesis (i) Carbon sequestration capacity in pans will be positively correlated with the organic matter content and soil depth, while the metal concentrations in soil cores will vary depending on soil properties, (ii) Sediment metal and nutrient concentrations in pans will vary spatially and temporally, with high concentrations expected due low hydro period times. Moreover, nutrient levels may fluctuate seasonally due to biological and environmental factors.

2.2. Materials and methods

Research permits were obtained from SANPARKS for sampling in Kruger National Park under permit number SS287 and SS713.

2.2.1. Study area

The study was conducted in the Letaba section of the Kruger National Park on the hot–dry season (i.e., 5–9 September 2022) when the pans were drying out, as they would potentially have high metal accumulation. The 12 pans were selected based on the geological formations/zones found within the Letaba section: granite (n = 3), sandstone (n = 3), basalt (n = 3) and rhyolite (n = 3) (Figure 1). The vegetation of the area is classified as the open tree savanna characterised by *Colophospermum mopane – Combretum apiculatum – Digitaria eriantha* (Van Rooyen et al., 1981). However, most of pan wetland studied were surrounded by *C. mopane* trees, with the sandstone pans having all three-tree species with *C. mopane* dominating. The area receives summer rainfall of ~400 mm per annum and has mean daily temperature of 23.3 °C (range 7.8 °C (winter) – 34.1 °C (summer). The most representative underlying geology for this region is the granite and Makhutswi gneiss, which contains Swaziland rock formations such as amphibolite and migmatite (Siebert et al., 2010).

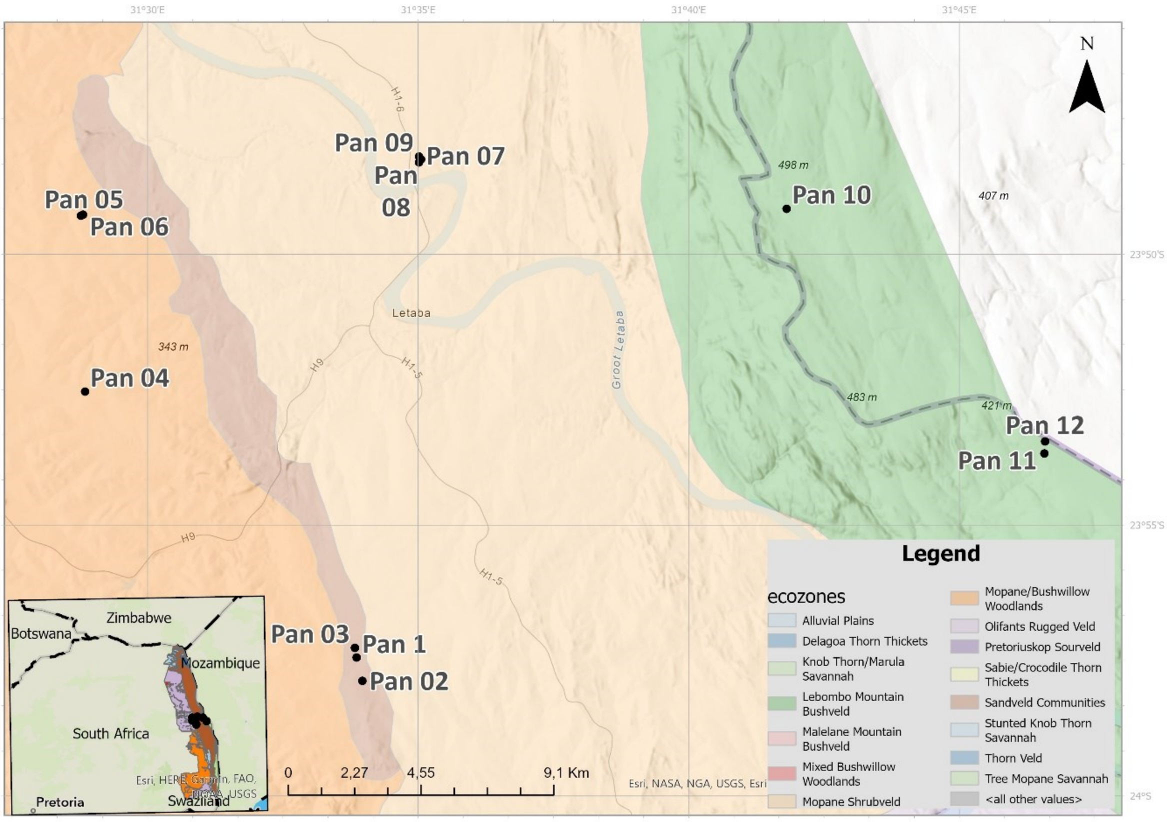

Fig. 1. Location of the study within the Letaba section of the Kruger National Park.

2.2.2. Sediment core sampling and processing

Sediments samples were collected in September 2022 from 12 pans across different geological types (i.e., granite (pans 1–3), sandstone granite (pans 4–6), basalt granite (pans 7–9), rhyolite granite (pans 10–12)) using a 130 cm hand auger. Three replicate samples were collected from each pan along varying soil depth ranges: 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm (2 kg per replicate per depth). Soil collection sites within a pan were selected randomly from within the deepest points/areas yet to dry out, as these would potentially be areas of high metal accumulation. The samples were then placed separately in clean polyethene Ziplock bags. Collected sediments were transported to the Science lab at the University of Mpumalanga, and oven dried at 70°C for 48 h, before being disaggregated in a porcelain mortar and sieved (mesh size 0.05 mm) to remove unwanted plant roots and debris.

2.2.3. Sediment chemistry variables

After the lab process from the University, all sediment samples were sent for analysis at BEMLAB, which is a South African National Accreditation System (SANAS) certified laboratory for further analysis. The following were analysed: (i) non–metal: Sulphur (S) and phosphorus (P), and Boron (B) (ii) metals: potassium (K), calcium (Ca), sodium (Na) iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu). In brief, P was analysed using a SEAL Auto Analyser, while metals were identified using the acid digestion method and an Inductively coupled plasma atomic emission spectroscope instrument (Varian, Mulgrave, Australia).

2.2.4. Bulk density

The sediment bulk density measurements for each core were calculated based on the equation 1 as it is required for the calculation of the carbon amount as we only report the carbon or organic matter concentrations. Since there were no bulk density measurements from the field, the sediment bulk density with depth was estimated based on Adams (1973) equation:

$$Bulk\ density = \frac{100}{\frac{\%OM}{0.244}+\frac{100-\%OM}{MBD}} \qquad \text{equation 1}$$

where %OM is the percentage organic matter content and MBD is mineral bulk density assumed to be the specific gravity of quartz (2.65 Mg m^{-2}). Since the actual MBD is lower than rock bulk density as soil consists of irregularly shaped mineral particles that allow large voids between them, we used a typical MBD value of 1.64 Mg m^{-3} (Mann, 1986).

2.2.5 Sediment organic carbon

The volumetric sediment organic carbon (SOC_v, units – kg m^{-3}) were obtained by multiplying the sediment organic carbon (SOC) mass concentration (g kg^{-1}) by the sediment bulk density (kg m^{-3}) using the equation 2:

$$SOC_v = BD \times \left(\frac{SOC_m}{1000}\right) \qquad \text{equation 2}$$

where SOC_v is the volumetric SOC, BD is the bulk density of the soil sample and SOC_m is the SOC mass concentration (g kg^{-1}) of each sample (Bai et al., 2016).

The SOC stock (SOC_s, kg m^{-2}) at each depth was the product of the SOC_v and interval depth (m) (Equation 3). The sediment depths used for calculations were 0–20, 20–40, 40–60, 60–80, and 80–100 cm, respectively. Thus, the SOC_c from the surface (0–20 cm) to a given depth is the sum of SOC_s in sediment layers, which was calculated based on equation 5. Thus, for a profile, the SOC_c at the depth of 0 to 100 cm is calculated by the sum of the SOC_s in the 0–20, 20–40, 40–60, 60–80, and 80–100 cm layers.

$$SOC_s = SOC_v \times H \qquad \text{equation 3}$$

$$SOC_c = \sum_{t=1}^{n} SOC_s \qquad \text{equation 4}$$

$$SOC_s i = SOC_s \qquad \text{equation 5}$$

where SOC_s is the SOC_s of a given depth interval, H is the interval depth, and SOC_c is the cumulative SOC_s at the desired depth. In our study, n is from surface to bottom depth, SOC_si is the SOC_s for the i^{th} layer, and SOC_si is equal to SOC_s (equation 5) when equation 4 is used to calculate the SOC_s in the i^{th} layer (Bai et al., 2016).

2.2.6 Measuring carbon sequestration

Carbon sequestration in the pan wetlands was determined as the product of BD, SOC concentration, and accretion rate (AR) based on Villa and Bernal (2018), see equation 6.

$$Carbon\ sequestration\ (M\ L^{-3}T^{-1}) = BD((M\ L^{-3}) \times SOC\ (M\ M^{-1}) \times AR\ (LT^{-1}) \qquad \text{equation 6}$$

The BD was determined by dividing the dry weight of undisturbed soil cores with known dimensions (Grossman and Reinsch,2002), while the SOM and SOC were analysed using the modified Walkley–Black method.

2.2.7 Data analysis

A two–way ANOVA was used to assess the significant differences in nutrients, metals, and carbon dynamics among pan wetlands (i.e., granite, sandstone, basalt, rhyolite) and sediment depths (i.e., 0–20, 20–40, 40–60, 60–80, and 80–100 cm). A one–way ANOVA was used to test the significant differences of TCFs among the pans and it was conducted using SPSS version 25. Then a multiple linear regression was applied to test if environmental variables (i.e., sediment nutrients and metals) sampled significantly predicted SOC concentrations among the different pans' geological types (i.e., sandstone, granite, basalt, rhyolite) using STATISTICA version 10.

2.3. Results

2.3.1 Vertical distribution of nutrient and metal concentration across geological types in a soil profile

2.3.1.1 Basalt

The vertical distributions of metals and non–metals along soil profiles with 4 geological types is highlighted in Figure1. The pH levels in basalt geological type increased with increasing depth (Figure 1a). The concentration of metal (K, Cu, Zn, Fe) decreased with an increase in depth. Then the concentration of elements such as (Na, Ca, Mg) increased with an increase in depth. However, more concentration of Ca was observed across the depth 60–80 cm. Moreover, an observation of Mg increasing from surface to deep layers was noted, then becoming stable across the depth 40–60 cm then slightly decreasing again in low layers.

2.3.1.2 Granite

The pH level in granite geological types was stable across all depth (Figure 1a). The concentrations of (K, Ca, Cu, Zn, Fe) decreased with an increase in depth. Moreover, the concentration of Na was persistent across all depth and Mg increased with depth to deepest layers 40–60 cm, then decreased in low layers.

2.3.1.3 Rhyolite

The pH levels in rhyolite geological type increased with an increase in depth going down 60–80 cm (Figure 1a). The concentration of Fe decreased with an increase in depth. However, metal (K, Na, Cu) shows varying distribution of metals across depth, with K having high concentration at surface level and a declining concentration with depth in low layers and Na had increased concentration with depth, then a decline across 60–80 cm. Moreover, the concentration of Ca increased with an increase in depth, while Mg was stable across depth from 0–20 cm to 60–80 cm. Then Zn had high levels of concentration across upper layer depth of 0–20 cm, while intermediate layers depth of 20–40 and 40–60 cm show a decrease in concentration and the deepest layer has a minor increase at depth 60–80 cm.

2.3.1.4 Sandstone

The concentration of metals (Fe, Zn, Mg) decreased with an increase in depth. However, the concentration of Mg decreased across the depth of 0–20 cm to 40–60 cm, then slightly increased with depth across 60–80 cm. Furthermore, Mg concentration fluctuated across all depth, while the concentration of Cu remained constant with an increased in depth across depth 0–20 cm, 20–40 cm,40–60 cm and 60–80 cm before it creased with depth across 80–100 cm. Moreover, the concentration of metals (Ca, Na) and non–metal (S) exhibited uniformity across depth (Figures 1, f, j). However, Na slightly increased with depth across depth 20–40 cm before showing uniformity (Figure 1f).

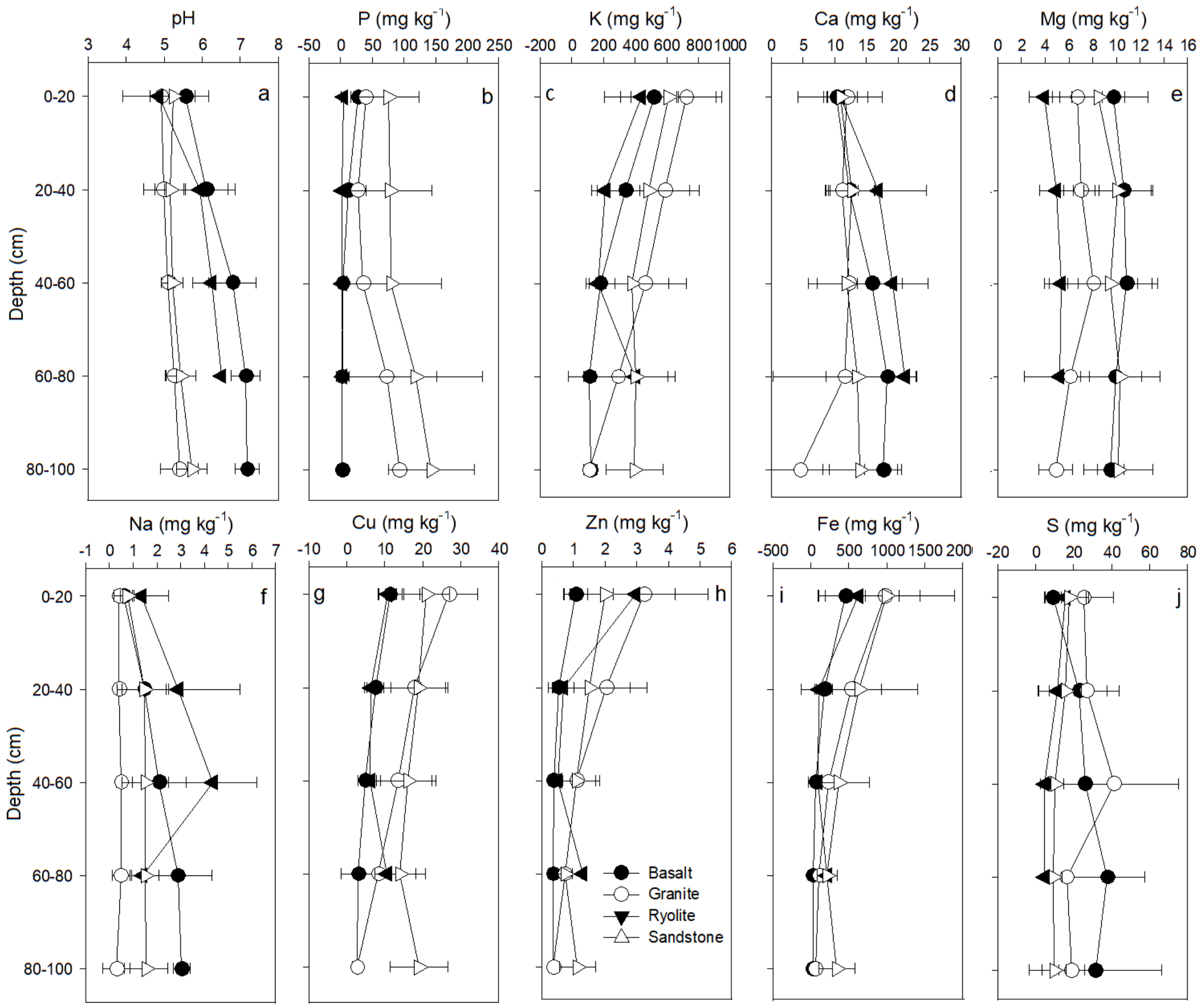

Figure 1. Distribution patterns of (a) pH, non–metals (b) P and (j) S and metal (c) K, (d) Ca, (e); Mg, (f) Na, (g) Cu, (h) Zn, and Fe concentration (mean± standard error) in vertical direction across different geological types of basalt, granite, sandstone and rhyolite. In the legend are four sampling sites.

2.3.2. Soil organic carbon dynamics

The carbon sequestration and carbon accumulation trends in various geological formations vary across different depths (Figure 2a and 2b). Basalt geological formations have a significant carbon sequestration in the upper 20 cm of the soil profile, while rhyolite geological formations show a complex pattern. The topmost layer contains moderate carbon content, while the subsequent layers show a decline. Lower layers show a rise in carbon sequestration, with a moderate level at the greatest depth. In granite formations, carbon sequestration is relatively elevated in the upper 20 cm, with a gradual decline in depth. Sandstone formations have low carbon sequestration in the topmost layer, but a rise in depths 20–40 cm. The lowermost section shows variability in carbon sequestration.

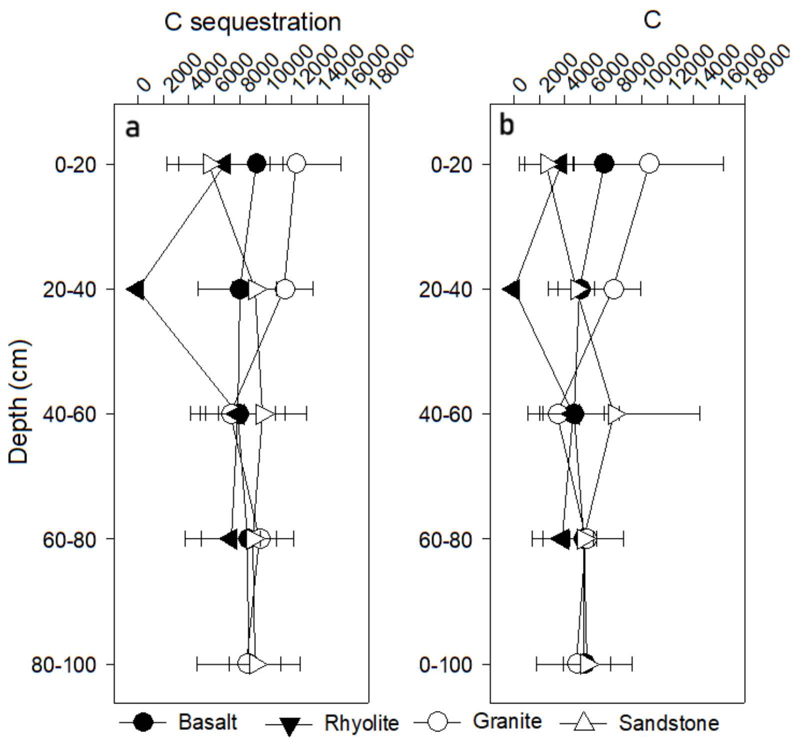

Figure 2. Distribution patterns of carbon sequestration (Figure 2a) and carbon accumulation (Figure2b) across four geological types namely the basalt, rhyolite, granite and sandstone.

Table 1. Two–way ANOVA assessing the differences in metal, carbon and non–metals concentrations across pan types with different geologies and sediment depths in the Letaba region, Kruger National Park.

Variable	**Variable**		**Pan type**		**Depth × Pan type**	
	$F_{(4,95)}$	***p***	**$F_{(3,95)}$**	***p***	**$F_{(10,95)}$**	***p***
pH	**11.288**	**<0.001**	**36.454**	**<0.001**	**2.976**	**0.003**
Resistivity	1.689	0.159	**6.299**	**0.001**	1.379	0.202
P	1.223	0.306	**32.743**	**<0.001**	1.505	0.150
K	**8.997**	**<0.001**	**9.601**	**<0.001**	0.773	0.655
Ca	**4.830**	**0.001**	**4.349**	**0.006**	1.275	0.256
Mg	1.298	0.276	**17.553**	**<0.001**	0.426	0.931
K	**9.355**	**<0.001**	**9.579**	**<0.001**	0.796	0.632
Na	**6.626**	**<0.001**	**10.784**	**<0.001**	**2.456**	**0.012**
Cu	**8.396**	**<0.001**	**35.211**	**<0.001**	1.278	0.254
Zn	**11.652**	**<0.001**	**6.964**	**<0.001**	1.454	0.169
Mn	**8.764**	**<0.001**	**18.410**	**<0.001**	**3.387**	**0.001**
B	0.456	0.768	**3.608**	**0.016**	0.912	0.526
Fe	**8.621**	**<0.001**	**4.462**	**0.006**	0.346	0.966
S	0.184	0.946	**9.284**	**<0.001**	**2.836**	**0.004**
SOC	**13.454**	**<0.001**	**15.504**	**<0.001**	1.208	0.296
SOM	0.178	0.949	**3.899**	**0.011**	0.546	0.853
Bulk density	0.387	0.818	2.330	0.079	0.503	0.884
Carbon sequestration	0.153	0.961	**5.139**	**0.002**	0.715	0.708

The results in Table 1 show significant differences in pH, SOC and in metals (K, Mn, Na, Fe, Cu, Ca, Zn) concentrations were found significant ($p < 0.05$) with sediment depth, whereas pH, SOC, SOM, resistivity, carbon sequestration, non–metal/nutrients (S, P, B) and all metals (K, Ca, Mg, Na, Cu, Zn, Mn, Fe, and non–metals were significantly different ($p < 0.05$) across pan types (Table 1). Overall, most environmental variables ($n = 12$) were found to be significant ($p < 0.05$) for rhyolite pans, with the basalt pans having the least significant environmental variables ($n = 1$) (Table 2). For example, the overall regression was statistically significant (adjusted $R^2 = 0.68$, $F_{(15,24)} = 6.48$, $p < 0.0001$) for SOC concentration in the basalt pans, and it was found that Fe concentration significantly predicted SOC concentration ($\beta = 0.001$, $p = 0.042$) (Table 2).

2.3.3. Relationship between soil organic matter and nutrient and metal concentrations

2.3.3.1 Sandstone

The regression model for sandstone R = 0.85(Table2). The results show a decrease in SOM decreases nutrient P and metal Mg concentrations and an increase in SOM increases metal and non–metal Ca and S, respectively (Table 2).

2.3.3.2 Granite

Table 2 show a regression model R = 0.88 and a significant directly proportional relationship between the SOM, pH, and metal Zn. An increase in the soil organic matter, increases pH and Zn.

2.3.3.3 Basalt

For basalt the regression model R = 0.68 and an increase in soil organic matter is associated with an increase in the concentration of Fe (Table 2).

2.3.3.4 Rhyolite

The results in Table 2 show a good regression model R = 0.99 were pH positively correlated with SOM. However, a decline in the SOM results in a decrease of metals Ca, K, Na and Mn concentration. The concentration of non–metal S, P, B and metal Cu and Mg increased with high levels of SOM (Table 2).

Table 2. Significant multi–regression results for SOC concentration, sediment non–metal/ nutrients and metal variables in pan wetlands found within the Letaba region, Kruger National Park.

Variable	***β****	**SE**	***β***	**SE**	***t***	***P***
Sandstone						
Adjusted $R^2 = 0.85$, $F_{(15,21)} = 14.215$, $p < 0.0001$, SE estimate = 0.113						
Intercept			1.25	0.78	1.605	0.123
P	–0.45	0.19	–0.002	0.001	–2.405	0.025
Ca	0.54	0.19	0.04	0.01	2.847	0.010
Mg	–1.02	0.26	–0.10	0.03	–3.947	0.001
S	0.20	0.09	0.01	0.00	2.229	0.037
Granite						
Adjusted $R^2 = 0.88$, $F_{(15,6)} = 11.184$, $p < 0.004$, SE estimate = 0.096						
Intercept			–3.07	1.51	–2.031	0.089
pH	0.80	0.29	0.63	0.23	2.776	0.032
Zn	3.49	1.05	0.71	0.21	3.322	0.016
Mn	–0.80	0.28	0.00	0.00	–2.898	0.027
Basalt						
Adjusted $R^2 = 0.68$, $F_{(15,24)} = 6.48$, $p < 0.0001$, SE estimate = 0.087						
Intercept			0.13	0.46	0.280	0.782
Fe	0.97	0.45	0.001	0.000	2.146	0.042
Rhyolite						
Adjusted $R^2 = 0.99$, $F_{(15,6)} = 70.630$, $p < 0.0001$, SE estimate = 0.028						
Intercept			–1.45	0.44	–3.307	0.016
pH	0.38	0.13	0.22	0.08	2.847	0.029
Resistivity	–0.09	0.03	–0.001	0.000	–3.667	0.010
P	0.33	0.12	0.10	0.04	2.834	0.030
K	4.03	0.88	0.01	0.00	4.600	0.004
Ca	–0.88	0.17	–0.08	0.01	–5.329	0.002
Mg	0.92	0.08	0.41	0.04	11.399	0.000
K	–4.55	0.86	–5.23	0.99	–5.298	0.002
Na	–1.00	0.08	–0.31	0.03	–12.037	0.000
Cu	0.41	0.11	0.08	0.02	3.762	0.009
Mn	–0.13	0.05	–0.001	0.000	–2.691	0.036
B	0.38	0.05	0.61	0.08	7.908	0.000
S	0.34	0.09	0.02	0.01	3.640	0.011

2.4. Discussion

This study assessed soil organic carbon sequestration, metal and non–metal dynamics in floodplain pan wetland system across different geological types and soil depths. The results show that soil carbon sequestration, metal and non–metals concentrations significantly varied across different geological types and in soil depth. According to Ruan et al. (2006), the vertical arrangement of heavy metals within soil profiles is the consequence of the accumulation and migration of these elements due to the combined effects of environmental conditions and edaphic factors. In line with the first hypothesis, it was found that, pan carbon sequestration capacity was not positively correlated with the organic matter content and soil depth. This observation suggests a progressive decline in the concentration of organic matter and microbial activity with increasing depth within the soil profile (Jansson and Hofmockel, 2020).

The results obtained in the study highlight that generally, the pH showed significant differences across depth. Considering this outcome, the pH of soil is a crucial determinant that significantly impacts the availability of nutrients and the activity of microorganisms within soil profiles (Ragot et al., 2016; Wu et al., 2017). The pH in basalt and rhyolite increased with depth, while there was a significant difference in granite and sandstone. These results correspond with Singh et al. (2015) who has indicated that the observed fluctuations in the pH among various geological types can be attributed to disparities in soil mineralogy, buffering capacity, and cation exchange capacity. Moreover, Lindsay (1979) noted that in basalts the weathered minerals release alkaline ions, increasing pH with depth. While the granite soils drain efficiently and have constant pH and mineral dissolution and leaching may raise pH deeper (Brady and Weil, 2008).

While the metal concentrations in soil cores varied across depth and geological type. The accumulation of metals (i.e., Na, Fe, K, Zn, Ca, Mg, Cu) and non–metals (i.e., P, S) were mostly concentrated on the topsoils. These results align with the findings from metal and non–metal dynamics and distribution in soil profiles across selected pans ecosystem in the Ramsar declared Makuleke Wetlands within the Makuleke Contractual National Park, in the northern Kruger National Park (South Africa), a study conducted by Munyai et al. (2023) whose results had significant differences in metal concentrations (i.e., Ca, Mn, Fe) and non–metals (i.e., C, S) across sediment depths. Bai et al. (2014) and Munyai et al. (2023) state that heavy metals accumulate in surface soils compared to deeper soils but have no potential detrimental effects on the aquatic systems could be observed. The findings of a study conducted by Raun et al. (2006) highlight that a total concentration of Cu, Zn, Pb, Cr, and Cd were identified, followed by an examination of the vertical distribution of each element in each profile. The findings revealed that heavy metal enrichment was observed in the uppermost level of the soil in a natural forest setting, lacking any anthropogenic intervention. This phenomenon provided further evidence that the heavy metals originated from atmospheric deposition (Raun et al., 2006). However, according to Dalu et al. (2020), high metal concentrations of Cu, Fe, and Mn might have adverse ecological implications for benthic fauna and flora. Moreover, Ouma et al. (2022) state that the adverse effects of metal contamination include water pollution, atmospheric air contamination, land degradation, and threats to biodiversity.

The accumulation of nutrient (P) in granite and sandstone between 40—60cm depth and that of iron (Na) between 40—60cm depth are consistent with previous studies, which documented high nutrient levels in lower soil due to soil organic matter and microbial pathways (Lehmann and Kleber, 2015). However, based on the findings, the accumulation of nutrients and minerals could be due to elephants trampling on pans, compressing them to move down. Moreover, changes in iron concentration (Fe) found in sediments variety helps to emphasize dynamic soil properties in the overall soil profile, several mechanisms could influence the distribution of Fe. The decrease in iron concentration, in particular (Fe), with increasing depth can be caused by several mechanisms, such as leaching of iron in mineral soils, precipitation, or iron uptake by microbial communities (Kabata—Pendias and Pendias, 2001). Several factors can affect depth–related nutrient dynamics, factors such as land use activities which contributes to a load of nutrient (Smith et al., 1999), the stability of the water column which has a significant impact on the distribution and stratification of nutrients (Šolić et al.,2020) and organic matter decomposition, nutrient leaching, and root activity (Jobbágy and Jackson, 2001).

The extent of carbon sequestration and carbon accumulation exhibited variability among different geological formations. The potential for carbon storage in basalt and granite soils is evident in both surface and subsurface layers, where varied depth intervals play significant roles in soil organic accumulation. According to Dalu et al. (2022), wetlands physical and biological processes are fundamental to the distribution and structuring of organic matter in sediments. The physical processes consist of sedimentation, erosion, hydrology and flocculation and the biological processes are decomposition, bioturbation, and plant-mediated processes. Collectively, these activities are of utmost importance in determining the distribution, content, and conservation of organic matter within wetland sediments, thereby impacting the overall functioning and ecological services offered by wetland ecosystems. In addition, the carbon sequestration pattern observed in rhyolite soils exhibits a multifaceted nature, potentially impacted by the distribution of organic matter and interactions with minerals. Moreover, the build–up of carbon sequestration in sandstone soils was limited, which according to Lal (2004) could be due to their course–textured character.

2.5 Conclusion

The understanding of the vertical distribution of nutrients, metals, non–metals, and carbon sequestration in soils across diverse geological formations and sediment depths holds substantial implications for the management and preservation of ecosystems. The maintenance of soil health and the availability of nutrients are crucial factors in ensuring the sustainability of plant development and the productivity of ecosystems. Furthermore, to effectively address climate change through carbon sequestration, it is crucial to possess an in-depth understanding of geological factors and the subsequent environmental and biological consequences. This involves the identification of geological formations that possess the necessary characteristics to effectively store carbon over long periods of time, while avoiding any potential release of stored carbon dioxide. This knowledge serves a dual purpose by facilitating the attainment of net-zero emission objectives and fostering the advancement of economically viable carbon capture and storage technology. It provides guidance for policy frameworks, directing rules for the purpose of monitoring and ensuring compliance. Significantly, protecting ecological systems, reducing disruptions and the risk of pollution, while also potentially promoting diversity.

CHAPTER 3: ASSESSING THE SPATIAL DISTRIBUTION AND VARIATION OF MACROINVERTEBRATES DIVERSITY IN RELATION TO WATER AND SEDIMENT CHEMISTRY

3.1 Introduction

Aquatic macroinvertebrates refer to a diverse group of larger invertebrates, measuring over 500 µm, that inhabit various marine and freshwater environments such as oceans, rivers, streams, springs, lakes, ponds, lagoons, wetlands, and transitional ecosystems (Bonacina et al., 2023). They are highly diverse and abundant organisms found in freshwater systems, playing a crucial role in the functioning of aquatic ecosystems (Hendrey, 2001; Dalu et al., 2017; Hauer and Resh, 2017). The diverse taxa include invertebrates that are functionally essential to aquatic ecosystems, aquatic invertebrates regulate primary production, decomposition, water quality, thermal stratification, and nutrient cycling in lakes, streams, and rivers (Stephenson et al., 2020). They are widely acknowledged as a crucial food source for amphibians, fish, and other invertebrates, making them indispensable elements of aquatic food webs (Colnurn et al., 2008; Hölker et al., 2015; Dalu et al., 2017). Niedrist and Füreder (2017), Vaughn and Hoellein (2018) and Allan et al. (2021) state that macroinvertebrates play diverse functional roles within aquatic ecosystems, including but not limited to shredding, grazing, detritus consumption, filter–feeding, and predation. According to Dalu and Wasserman (2021), it is the effects of macrophytes that are known to increase the abundance and diversity of macroinvertebrates in temporary pans by providing structural complexity and food. However, de Necker (2016) explains that macroinvertebrate diversity in pans is great, with most species being opportunistic and possessing specific adaptations to withstand many drying seasons every year.

Freshwater macroinvertebrates can be categorized as either fully aquatic, meaning they complete their entire life cycle within water, or semi–aquatic, as described by Wasserman et al. (2018). Their migration and relocation are constrained by their restricted mobility in freshwater habitats, making them a suitable representative of the sampled region and an ease to identification and counts for water quality investigations (Ollis et al., 2006; Chi et al., 2022). They generally exhibit responses to changes in water quality; however, their sensitivity levels exhibit significant variation (Rasifudi et al., 2018). As indicators of the biological health of waterways, Dalu and Wasserman (2021) highlight that macroinvertebrates are the most extensively employed for assessing aquatic habitats, especially temporary pans, among the possible bioindicators available for aquatic biomonitoring. They are among the most vulnerable animals in aquatic ecosystems, making them helpful for determining ecosystem health (Dalu et al., 2021).

Ferreira et al. (2012) state that these communities are vital as they have been recognized as potential markers of the ecological integrity of wetland ecosystems. They are specialized to certain habitats, substrate types, temperatures, and dissolved oxygen concentrations that aquatic macroinvertebrates can serve as indicators of disturbance (Victor, 2013). In wetland communities, their abundance and diversity are highly influenced by hydro period, dissolved oxygen, pH, nutrients, and salinity (Dalu and Wasserman, 2021). Several studies (Schäfer, 2019; Dalu et al., 2021; Bonacina et al., 2023) state that macroinvertebrate taxa can reflect changes in water levels, and they result in water quality alterations that could potentially have an impact on aquatic ecosystem functioning through shifting community composition.

According to Merritt and Cummins (1996), macroinvertebrates diverse ecological requirements and their susceptibility to environmental stressors serve as valuable tools for evaluating water quality and assessing the overall health of ecosystems. Rosenberg and Resh (1993) indicate that fluctuations in water levels have a direct impact on macroinvertebrate communities by causing changes in dissolved oxygen levels, nutrient concentrations, and substrate composition. Alterations in community composition resulting from fluctuations in water levels can disrupt crucial ecological processes, therefore exerting an influence on the overall functionality of the aquatic ecosystem (Statzner et al., 2001). However, Victor (2013) suggests that macroinvertebrates and zooplankton populations may be able to help conservationists better understand the sensitivity of these species and their habitants.

3.2 Materials and methods

3.2.1 Study area

The same 12 pan wetlands selected in the previous chapter were used for this chapter (see Chapter 2 for details).

3.2.2 Data collection methods during high and low hydro period

3.2.2.1. Sediment chemistry variables

During each pan and season, two sediment samples were collected in a random manner using a plastic hand shovel. These samples were then carefully transferred into new Zip-lock bags, which were properly labelled. The bags were then packed on ice in a cooler box to maintain their integrity during transportation to the laboratory for subsequent. In the laboratory, the sediment samples underwent an oven drying process at a temperature of 70 °C for a duration of 48 h. Subsequently, the sediment samples were selectively isolated to obtain the fine sand fraction, while disregarding the larger fractions present within the sand. The isolated fraction was then subjected to disaggregation using a porcelain mortar, followed by straining through a sieve with a mesh size of 0.05 mm. This sieving process effectively eliminated plant roots and other debris from the samples. The sediment samples were sent to BEMLAB, a laboratory certified by the South African National Accreditation System (SANAS), for analysis.. The following were analysed: potassium(K), calcium Ca, boron B, magnesium Mg, sodium Na, iron Fe, zinc Zn, manganese Mn, and copper Cu. In detail, nutrient N and P were analysed using a SEAL Auto Analyzer, while metals were identified using an inductively coupled plasma atomic emission spectroscope instrument.

3.2.2.2. Water quality sampling

In situ measurements were taken for the following parameters at each pan: electrical conductivity (µS cm^{-1}), salinity (ppt), pH, water temperature (°C), dissolved oxygen (DO) (mg L^{-1}), and total dissolved solids (TDS) (mg L^{-1}) from two distinct locations on either side of the pan using a Cyberscan Series portable handheld multiparameter meter (Eutech Instruments, Singapore). Without disrupting the sediment, two 250 mL plastic bottles were utilized to collect samples of water from each site for analysing nutrients content, specifically phosphate, nitrate and ammonium. Then the samples were packed on ice in a cooler box to maintain their integrity during transportation to the laboratory. In the laboratory, following the filtration of water samples for the purpose of determining pelagic chlorophyll–*a* concentration, the concentrations of ammonium, nitrate and phosphate were analysed. The ammonium concentration was determined using a freshwater ammonium test kit (HI3824) with a range of 0–2.5 mg L^{-1} and a resolution of 0.5 mg L^{-1}. The phosphate concentration, on the other hand, was measured using the Hanna phosphate high range checker (HI717) from Hanna Instruments, Romania. This instrument has a range of 0–30 mg L^{-1} and a resolution of 0.1 mg L^{-1} and for nitrate concentration was measured using the nitrate test kit with range of 0–30 mg L^{-1} and resolution of 0.1 mg L^{-1}.

3.2.2.3. Invertebrate sampling

Two macroinvertebrate samples were obtained at each pan and in high and low water period using a conventional SASS net. The net, made of aluminium rim, has dimensions of 30 × 30 cm. It is equipped with a mesh size of 1 mm and is affixed to a handle measuring 1.5 m in length. The net was fully immersed in the pan, and the collection of macroinvertebrates was conducted by systematically sweeping a defined transect measuring 10 meters in length. The methodology involved traversing the water while utilizing a sampling net, as well as employing physical force to displace aquatic macrophytes, vegetation, sand, and boulders at various locations. This action was undertaken to displace the macroinvertebrates that had been attached. The nylon sample net was expeditiously retrieved from the aquatic environment to minimize the potential elopement of live organisms. Subsequently, it was transferred into a receptacle and subjected to a thorough cleansing process using a coarse mesh filter, thereby eliminating sizable organic debris while ensuring the retention of macroinvertebrates. The macroinvertebrates were subsequently transferred into plastic containers with a volume of 500 mL and were preserved using a solution of 70% ethanol. The laboratory analysis involved the use of a dissecting macroscope to conduct macroinvertebrate counting and identification up to the family taxonomic level. The relative percentage abundance of each taxon was determined using the algorithm provided below. The computation of macroinvertebrate common matrices was undertaken to evaluate the environmental integrity across the rainfall water periods.

The percentage of Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundance, the percentage of Ephemeroptera, Trichoptera, Odonata, and Coleoptera (ETOC) abundance, the percentage of Diptera abundance, and the percentage of Chironomidae and Oligochaeta abundance were analysed. The metrices utilized to assess diversity and abundance at the family level included evenness, Simpson, and the Shannon–Wiener diversity index.

$$\textit{Relative abundance of species}\ (\%) = \textit{Total number of individuals for species} / \textit{Total number of individuals of all species} \times 100$$

3.2.4. Data analysis

Prior to multivariate analysis, sediment metal and non–metal concentrations, and water physico–chemical variables (except for pH) were log–transformed to meet two basic assumptions of an ANOVA (i.e., homogeneity and normality). The differences in sediment metal and non– metal concentrations, and environmental variables between the two–sampling water period (high period and low water period) and pans (1–12), and their interaction, were assessed using a two–way ANOVA analysis, after testing for homogeneity of variance and normality of distribution using SPSS version 25. Tukey's post–hoc analysis was employed to assess the significance of differences of environmental variables across geological types (sandstone, granite, basalt and rhyolite).

A Distance–Based Permutational Analysis of Variance (PERMANOVA; Anderson 2001) was conducted using Bray–Curtis and Euclidean distance dissimilarities. The analysis included 9999 permutations with Monte Carlo tests. The purpose of this analysis was to examine differences in community structure based on macroinvertebrate data across multiple sites (1–12) and water period (high and low). The PERMANOVA+ for PRIMER version 6 software (Anderson et al., 2008) was used for this analysis. The differences in macroinvertebrate taxa composition and environmental variables between the water period were analyzed using a two–way analysis of similarity (ANOSIM; Clarke, 1993). The statistical test was performed with 9999 permutations using the PRIMER v6 add–on package PERMANOVA+ (Anderson et al., 2008). The factors utilized for the analysis included the geological types: granite, sandstone, basalt, and rhyolite. The ANOSIM statistic R is derived from the comparison of mean ranks between groups and within groups. The range of values for this variable is between –1 and +1, with a value of 0 indicating random grouping.

Macroinvertebrate community datasets in PAST version 2.0 were used to calculate diversity matrices (i.e., Evenness, Abundance, Simpson, Shannon–Weiner) to assess for differences in species diversity among pans and seasons (Dalu et al., 2020). Common macroinvertebrate metrics were used to assess the environmental integrity: %Ephemeroptera abundance, %Trichoptera abundance and %Diptera abundance, and Shannon–Wiener diversity index. Two–way ANOSIM and SIMPER testing groups was employed on macroinvertebrates communities during high and low water period in pan wetlands across four geological types.

To investigate the potential impact of environmental variables, such as water and sediment, on the structure (abundance and diversity) of macroinvertebrate communities, an initial detrended canonical correspondence analysis (DCCA) was conducted. This analysis aimed to determine whether linear or unimodal analysis methods should be utilized, following the approach outlined by Šmilauer and Leps (2014). The lengths of the gradients obtained from the DCCA output were analyzed. As the longest gradient exceeded a value of 4, a linear canonical correspondence analysis (CCA) model was chosen to investigate the relationship between macroinvertebrate communities and environmental variables across different geological types and water periods (Šmilauer and Leps, 2014). A Canonical correspondence analysis (CCA) was employed to investigate the correlation between macroinvertebrates and environmental variables. Prior to conducting multivariate analysis, the data underwent a log transformation (excluding pH) using the statistical package version 12.0. This transformation was performed to stabilize the variances.

3.3. Results

3.3.1. Water chemistry

Table 1 highlights the results for the physico–chemical parameters from high and low water period. The results show that (chl–*a*) concentration during the high–water period was consistent across the geological types, with values around 0.1 mg m^{-3} and during the low water period mean range 0.1–0.4 mg m^{-3}. The water temperature varied across geological types, with high water period range from 26–28.3 °C and the low water period mean range from 20.9–28.3 °C (Table 1). The pH levels across the various geological types were relatively consistent, falling within a mean range of 6.7 to 7.1 while in the low water period there was a variation, with a mean range 5.9–7.4. The total dissolved solids (TDS) levels showed significant variations across geological types and water periods with mean range 148.5– 262.9 mg m^{-2} in the high–water period and mean range 185.5– 410.5 mg m^{-2} in low water period (Table1). Conductivity levels were highest in granite during both high and low water periods. However, the mean range for high water period was 281.8– 312.8 µS cm^{-1} and 288.8– 774.1 µS cm^{-1} during low water period (Table 1). Salinity levels varied across geological types, and water period, the high period means range 138.3–229.7 ppm and low period mean range 171.0–427 ppm (Table 1).

Five environmental variables (i.e., temperature, pH, TDS, conductivity, and salinity) showed significant differences ($p < 0.05$) amongst seasons (Table 2). However, significant ($p < 0.001$) differences among both seasons and geology for temperature were observed (Table 2). With regards to nutrients concentrations, Ammonium ranged from 1.8 – 2 mg L^{-1} and 1.3–3.1 mg L^{-1} in high water period and in low water period, respectively. The highest reading of ammonium was recorded in low water period in the rhyolite geology (Table 1). Phosphates concentration ranged from 0.6–1.4 mg L^{-1} in high water period and 1.6–4.1 mg L^{-1} in low water period (Table 1). The nitrate levels exhibited variations across geological types, were concentrations ranged from 1–4.9 mg L^{-1} in the low water period and 1.6–2.4 mg L^{-1} in the high–water period (Table 2). Among seasons, Phosphate showed a significant difference ($p < 0.001$), with ammonia having a significant difference among geology ($p < 0.003$) (Table 2).

3.3.2. Sediment chemistry

Table 3.1 show results for metal concentrations in sediment samples collected during high and low water period. The results exhibit benthic (chl–*a*) concentration ranging from 11.1–131.9 mg m^{-3} during the high–water period mean range of 101.5–509.3 mg m^{-3} in low water period. Sediment pH ranged from 4.8–5.1 and 4.2–5.4 in high and low water period respectively (Table1). The mean concentration of H^+ ranged from 0.7–1.7 cmol kg^{-1} in the high–water 2 period and 0.7– 2.7 cmol kg^{-1} in the low water period (Table1). In resistivity, the mean range were 926.7–2316.7 Ω in high water period and 650–1522.5 Ω in low water period (Table 1). For stone the mean concentrations ranged from 5.1–11.7 % and 1.6–22.2 % in high and low water period respectively (Table 1). However, the sediment metal concentrations for P range were 13.4–44.5 mg kg^{-1} in high water period and 10.8–112.4 mg kg^{-1} in low water period (Table1). Concentrations for K ranged from 249.2–325.5 mg kg^{-1} in high water and 440.5–878.5 mg kg^{-1} in low water period (Table1). However, another concentration of K ranged from 0.6–0.8 cmol kg^{-1} and 1.1–2.2 mg kg^{-1} in high and low water period respectively (Table1). The concentration of Na range was 0.2–0.5 cmol kg^{-1} high water and 0.2–0.7 cmol kg^{-1} in low water period. The mean concentration of Cu range in sediments were 4.4–7.7 mg kg^{-1} in high water period and 8.6–12.2 mg kg^{-1} in low water period. For Zn the mean concentration was 0.8–2.7 mg kg^{-1} and 1.1–5.3 mg kg^{-1}, mean range was 103.2–211.9 mg kg^{-1} and 125.4–306.5 mg kg^{-1} for Mn, for B it ranged from 0.2–0.4 mg kg^{-1} and 0.2–0.7 mg kg^{-1}, for Fe it was

185.3–534.4 mg kg^{-1} and 1390–1358.5 mg kg^{-1}, in C the mean range was 0.5–1.3 % and 0.7–2.1 %, and for S the concentration mean range was 4.3–12.7 mg kg^{-1} and 7.4–19.8 mg kg^{-1} in high and low water period, respectively.

Most of the sediment variables were found to be statistically significant (ANOVA; $p<0.05$) in both the seasons and geology (Table 2). Concentration levels in benthic chl–*a*, potassium (K), boron (B), iron (Fe) and sulphur (S) were significantly different ($p < 0.001$), stone and resistivity ($p = 0.017$), then carbon (C, $p = 0.040$), copper (Cu; $p = 0.002$), zinc (Zn; $p = 0.003$), manganese (Mn; $p = 0.026$), and, phosphate (P; $p = 0.043$) were found significant among seasons (Table 2). However, only pH, H^+, P, Na, Mn, and B, C, S, Zn, stone and resistivity were statistically significant ($p < 0.05$) among geology (Table 2).

Table 1. Mean (± standard deviation) for water and sediment variables recorded from 12 pan wetlands across four geological types (sandstone, granite, basalt and rhyolite) during high and low water period in the central Kruger National Park, South Africa. Abbreviations: chl–*a* – chlorophyll–*a*, TDS – total dissolved solids.

Variables	Units	High water period				Low water period			
		Sandstone	Granite	Basalt	Rhyolite	Sandstone	Granite	Basalt	Rhyolite
Water									
Pelagic chl–*a*	mg m^{-3}	0.1 ± 0	0.1± 0	0.1 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0	0.4 ± 0.4
Ammonium	mg L^{-1}	1.8 ± 0.5	2.0 ± 0.6	1.8 ± 0.4	1.8 ± 0.3	1.3 ± 0.4	2.2 ± 0.5	1.4 ± 0.4	3.1 ± 0.2
Phosphates	mg L^{-1}	1.1 ± 0.2	0.8 ± 0.8	1.4 ± 1.4	0.6 ± 0.8	2.0 ± 0.6	1.6 ± 0.6	4.1 ± 2.2	2.7 ± 0.9
Nitrate	mg L^{-1}	2.2 ± 1.7	1.9 ± 1.2	2.3 ± 1.1	1.6 ± 0.6	3.7 ± 3.7	4.9 ± 5.7	2.4 ± 1.7	1 ± 0.2
Temperature	°C	26.3 ± 1.3	28.3 ± 0.7	27.2 ± 0.8	26.0 ± 1.3	21.9 ± 1.2	27.7 ± 2.2	28.1 ± 2.3	20.9 ± 0.3
pH		6.8 ± 0.1	7.1 ± 0.2	6.9 ± 0.2	6.7 ± 0.6	5.9 ± 0	5.9 ± 0.1	6.0 ± 0.1	7.4 ± 2.1
TDS	Mg/L	200.7 ± 17.5	160.8 ± 108.9	148.5 ± 126.7	262.9 ± 226.5	331.3 ± 217.9	410.5 ± 76.8	185.5 ± 29.2	341.3 ± 19
Conductivity	µS cm^{-1}	287.8 ± 29.7	281.8 ± 188.9	287.8 ± 250.7	312.8 ± 329	228.8 ± 103.2	743.6 ± 131.5	334.3 ± 73.5	774.1 ± 2.8
Salinity	ppt	188.2 ± 13.9	138.3 ± 93.4	139.8 ± 125.8	229.7 ± 209.1	372.2 ± 239.7	427.9 ± 169.4	171.0 ± 28.3	401.6 ± 31.7
Restivity	Ω	4135.0 ± 477.8	3979.0 ± 1654.6	5374 ± 3078.1	3767.1 ± 3407.3	2507.5 ± 1326.7	3499.5 ± 4474.8	2682.0 ± 629.5	1478.5 ± 75.6
Area	m^2	8975.4 ± 1012.6	867.4 ± 1203.3	2120.2 ± 2005.7	25817.2 ± 54917.7	2227.7 ± 3589.8	455.3 ± 267.7	233.5 ± 157	797.5 ± 662.5
Sediment									
Benthic chl–*a*	mg m^{-2}	115.0 ± 23.0	131.9 ± 164.5	29.2 ± 27.1	11.1 ± 4.6	413.1 ± 234.3	101.5 ± 107.3	509.3 ± 520.3	185.5 ± 113.8
pH		5.0 ± 0.5	5.1 ± 0.1	5.0 ± 0.3	4.8 ± 0.4	5.4 ± 0.1	5.3 ± 0.2	4.9 ± 0.2	4.2 ± 0.1
Resistivity	Ω	1573.8 ± 560.6	2316.7 ± 1266.1	1358.3 ± 484.1	926.7 ± 245.4	1522.5 ± 1038.1	1005.0 ± 173.6	1002.5 ± 282	650 ± 0
H+	cmol kg^{-1}	1.0 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	1.7 ± 1.4	0.7 ± 0.3	0.7 ± 0.2	1.0 ± 0.1	2.7 ± 0.3
Stone	Vol %	6.9 ± 0.7	5.1 ± 3.2	6.4 ± 13.2	11.7 ± 11.9	1.6 ± 1	8.8 ± 0.8	21.8 ± 17.5	22.2 ± 0.8
P	mg kg^{-1}	36.3 ± 39.1	44.5 ± 10.1	18.9 ± 15.6	13.4 ± 10.7	112.4 ± 26.5	54.2 ± 3	46.8 ± 5.5	10.8 ± 1.2
K	mg kg^{-1}	302.6 ± 85.1	286.0 ± 100.1	249.2 ± 65.5	325.5 ± 36.4	440.5 ± 355.6	535.8 ± 138.5	531.3 ± 91	878.5 ± 6.3

Ca	cmol kg^{-1}	4.7 ± 2.3	4.5 ± 2.4	4.5 ± 1.4	5.5 ± 1.3	4.8 ± 4.2	5.1 ± 1.3	7.4 ± 2	5.4 ± 0.1
Mg	cmol kg^{-1}	3.0 ± 1.9	2.3 ± 1.3	2.9 ± 1	4.4 ± 3.2	2.3 ± 1.6	2.6 ± 0.6	5.6 ± 1.2	3.0 ± 0.1
K	cmol kg^{-1}	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	0.8 ± 0	1.1 ± 0.9	1.4 ± 0.3	1.4 ± 0.2	2.2 ± 0.1
Na	cmol kg^{-1}	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
Cu	mg kg^{-1}	6.2 ± 3.4	7.7 ± 5.4	4.4 ± 1.6	5.6 ± 0.3	8.6 ± 6.7	12.2 ± 6.7	10.3 ± 1.3	11.1 ± 1.4
Zn	mg kg^{-1}	1.6 ± 0.2	1.3 ± 0.6	0.8 ± 0.6	2.7 ± 3.1	2.1 ± 1.1	2.6 ± 0.5	1.1 ± 0.2	5.3 ± 0.8
Mn	mg kg^{-1}	155.5 ± 40.7	171.4 ± 67.5	103.2 ± 53.7	211.9 ± 147.9	125.4 ± 107.1	306.5 ± 96.2	130.0 ± 20	298.5 ± 0.7
B	mg kg^{-1}	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0	0.2 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
Fe	mg kg^{-1}	453.3 ± 180.1	284.5 ± 147.8	185.3 ± 78.5	534.4 ± 649.7	1358.5 ± 754.5	1079.2 ± 246.2	878.5 ± 162.7	1390 ± 183.8
C	%	0.8 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	1.3 ± 1.1	0.7 ± 0.4	0.8 ± 0.2	0.8 ± 0.1	2.1 ± 0
S	mg kg^{-1}	7.7 ± 0.7	4.3 ± 1	5.6 ± 2	12.1 ± 9.6	12.7 ± 7.3	17.3 ± 2.7	7.4 ± 1.8	19.8 ± 6.5

Table 2. Two–way analysis of variance (ANOVA) results for water and sediment variables across geological types and water periods (high and low) measured from 12 pan wetlands located in the central Kruger National Park, South Africa. The bold values emphasise significant differences at $p < 0.05$.

Variables	Season			Geology			Season × Geology		
	df	F	*p*	*df*	F	*p*	*df*	F	*p*
Water									
Pelagic chl–*a*	**1**	**4.220**	**0.049**	3	0.553	0.650	3	1.501	0.234
Ammonia	1	0.717	0.404	3	3.326	0.033	**3**	**8.514**	**<0.001**
Phosphate	**1**	**26.834**	**<0.001**	3	2.738	0.061	3	0.611	0.613
Nitrate	1	0.440	0.512	3	0.866	0.469	3	0.689	0.566
Temperature	**1**	**32.383**	**<0.001**	**3**	**23.242**	**<0.001**	**3**	**12.051**	**<0.001**
pH	**1**	**14.261**	**0.001**	3	2.640	0.068	**3**	**5.467**	**0.004**
TDS	**1**	**11.102**	**0.002**	3	1.451	0.248	3	0.401	0.753
Conductivity	**1**	**5.471**	**0.026**	3	0.648	0.590	3	1.542	0.224
Salinity	**1**	**16.808**	**<0.001**	3	1.747	0.179	3	0.545	0.655
Restivity	1	0.292	0.593	3	0.681	0.571	3	0.162	0.921
Area	1	1.787	0.191	3	1.121	0.356	3	0.595	0.623
Sediment									
Benthic chl–*a*	**1**	**29.556**	**<0.001**	3	0.734	0.540	3	2.681	0.065
pH	1	2.578	0.119	**3**	**12.314**	**<0.001**	3	1.849	0.160
Resistivity	1	**6.422**	**0.017**	**3**	**3.059**	**0.043**	3	0.921	0.443
H+	1	3.869	0.058	**3**	**10.184**	**<0.001**	3	0.769	0.520
Stone	**1**	**6.372**	**0.017**	**3**	**4.546**	**0.010**	3	1.530	0.227
P	**1**	**4.487**	**0.043**	**3**	**15.855**	**<0.001**	3	1.560	0.220
K	**1**	**23.562**	**<0.001**	3	2.543	0.075	3	2.136	0.116
Ca	1	1.342	0.256	3	1.358	0.274	3	0.562	0.644
Mg	1	0.693	0.412	3	2.736	0.061	3	1.287	0.297
K	**1**	**31.674**	**<0.001**	3	2.732	0.061	3	2.067	0.126
Na	1	1.174	0.287	**3**	**8.634**	**<0.001**	3	0.429	0.734
Cu	**1**	**11.300**	**0.002**	3	0.960	0.424	3	0.329	0.805
Zn	**1**	**10.804**	**0.003**	**3**	**5.258**	**0.005**	3	0.872	0.466
Mn	**1**	**5.508**	**0.026**	**3**	**8.005**	**<0.001**	3	0.195	0.899
B	**1**	**18.603**	**<0.001**	**3**	**11.726**	**<0.001**	3	2.900	0.051
Fe	**1**	**38.230**	**<0.001**	3	1.246	0.310	3	0.359	0.783
C	**1**	**4.592**	**0.040**	**3**	**7.679**	**0.001**	3	0.766	0.522
S	**1**	**26.120**	**<0.001**	**3**	**4.281**	**0.013**	3	2.858	0.054

Table 3. Tukey's post–hoc results highlighting environmental variables that were found to be significant ($p < 0.05$) across four geological types (sandstone, granite, basalt and rhyolite) in pan wetlands located in Kruger National Park, South Africa.

Variable	Geology	*p*
Temperature	Sandstone vs Granite	<0.001
	Sandstone vs Basalt	0.001
	Granite vs Sandstone	0.000
	Granite vs Rhyolite	0.000
	Basalt vs Rhyolite	0.001
Sed–pH	Sandstone vs Basalt	0.001
	Sandstone vs Rhyolite	<0.001
	Rhyolite vs Granite	0.016
	Rhyolite vs Basalt	0.333
Sed–Resistivity	Granite vs Rhyolite	0.020
H+	Basalt vs Rhyolite	0.023
	Rhyolite vs Sandstone	<0.001
	Rhyolite vs Granite	0.002
	Rhyolite vs Basalt	0.023
Stone	Sandstone vs Rhyolite	0.023
P	Sandstone vs Basalt	<0.001
	Sandstone vs Rhyolite	<0.001
	Granite vs Basalt	0.050
	Granite vs Rhyolite	<0.001
Na	Granite vs Rhyolite	<0.001
	Basalt vs Rhyolite	0.001
	Rhyolite vs Sandstone	0.002
Zn	Basalt vs Rhyolite	0.015
Mn	Sandstone vs Granite	0.002
	Sandstone vs Rhyolite	0.004
B	Sandstone vs Basalt	0.020
	Sandstone vs Rhyolite	0.001
	Granite vs Basalt	0.024
	Granite vs Rhyolite	0.001
C	Sandstone vs Rhyolite	0.002
	Granite vs Rhyolite	0.003
	Basalt vs Rhyolite	0.018
S	Basalt vs Rhyolite	0.017

2.3.3. Distribution of macroinvertebrate community structures

A total of 5145 individual macroinvertebrates and 41 macroinvertebrates species from 9 orders were identified across 12 pan wetlands with different geological types during high water period and low water period. With high rainfall period having the high number of species ($n = 29$) and low rainfall period having a low number of species ($n = 12$). The nine orders were Hemiptera, Coleoptera, Crustacea, Mollusca, Annelida, Diptera, Odonata, Trichopteran and Ephemeroptera. Therefore, the mean relative abundance (%) of the macroinvertebrates and the total number of identified species collected from each pan are shown in Tables. The Hemiptera taxa make up (57.0 %) of all the total sample, and other taxa included with Crustacea (24.6 %), Diptera (9.1 %), Mollusca (3.3 %), Trichopteran (2.0 %), Coleoptera (1.5 %) Annelida (1.4 %), Odonata (0.9 %) and Ephemeroptera (0.1 %).

Based on PERMANOVA analysis, significant differences in macroinvertebrate community structure among geological types (Pseudo–F = 11.925, $df = 1$, $p < 0.001$) and seasons (Pseudo–F = 1750 $df = 3$, $p = 0.027$) were observed. However, no significant ($p > 0.05$) combinations were observed on PERMANOVA pairwise comparisons based on geological types for macroinvertebrate communities.

The % ETOC and %EPT were high during the high–water period with a mean range of 12.5–67 and 7.5–54 respectively and low during the low water period with mean range of 1.5–9.7 and 0–14.1 respectively. The % Chironomidae + Oligochaeta was only observed in the low water period with a mean range of 4.5–73. The Shannon–Wiener diversity index was high during low water period with a mean range 0.93–1.49 and low during the high–water period with a mean range 0.97–1.27. Species abundance was high during the low water period with a mean range of 56.7–682.3 and low during the low water period with a mean range of 37.2–

59.8. With the Simpson index, high number of dominant species were observed during the low water period with a mean range of 0.50–0.64 and a low number observed during the low water period with mean range of 0.52–0.6. The evenness index observed was high during the high–water period compared with low water period with mean range of 0.55–0.67 and 0.39–0.65, respectively.

Table 4. Mean (± standard deviation) of macroinvertebrate abundances and community metrices observed in high and low water periods across pan wetlands with different geological types (sandstone, granite, basalt and rhyolite) located in the central Kruger National Park, South Africa.

Taxa	**High water period**				**Low water period**			
	Sandstone	**Granite**	**Basalt**	**Rhyolite**	**Sandstone**	**Granite**	**Basalt**	**Rhyolite**
Annelida								
Salifidae	8.4 ± 16.6		1.6 ± 2.5					
Aliolimnatis sp.	1.2 ± 2.5	5.1 ± 12.5			0.5 ± 1.1	0.5 ± 0.9	3.8 ± 2.9	0.3 ± 0.4
Oligochaeta								2.1 ± 0.3
Crustacea								
Branchiopodidae	15.5 ± 26.6	3.7 ± 9.2	11.5 ± 16.6	22.2 ± 35.8	24.3 ± 28.1	2 ± 4		
Cypridoidea	31.2 ± 30.1	39.2 ± 32.8	42.9 ± 28.2	18.1 ± 28.5	31.1 ± 23.6	4.2 ± 8.2		18.7 ± 10.8
Lepidurus apus		1.2 ± 3.1	0.8 ± 2.1	13.7 ± 16.1				
Potamonautes sp.				25.8 ± 28.4				
Coleoptera								
Acidocerinae		0.6 ± 1.5	0.2 ± 0.5					
Hydrocanthus sp.			0.4 ± 1.0					
Hydrochus sp.	0.5 ± 0.8	1.2 ± 3.1						
Rhantus sp.	1.1 ± 2.2	2.9 ± 3.3	1.4 ± 2.2		0.3 ± 0.6	0.6 ± 0.5		0.3 ± 0.5
Regimbartia sp.		3.0 ± 4.8	0.4 ± 0.9	1.2 ± 2.1	0.5 ± 1.1	0 ± 0.1		
Copelatus sp.				0.1 ± 0.3				
Hydrophilus aculeatus		0.4 ± 1	0.4 ± 1.1					
Berosus sp.			0.8 ± 1.2					
Stenelmus sp			0.2 ± 0.5					
Spercheus sp.			0.2 ± 0.4					
Cybester sp.					0.5±0.7			
Hyditicus exclamatioms							2.1 ± 4.1	
Diptera								
Ochthera sp				0.8 ± 2.1				0.3 ± 0.5

Culex sp.							0.3 ± 0.6	
Chrysops sp.					9.5 ± 19			
Chironominae					9.7 ± 12.9	36.4 ± 41.7	2.1 ± 3.7	3.2 ± 0.9
Ephemeroptera								
Atroptilum sudafricanum							5.2 ± 10.4	
Hemiptera								
Appasus sp.		1.3 ± 1.6		0.3 ± 0.7	0.1 ± 0.2	0.1 ± 0.1		
Enithares sp.	14.2 ± 12.1	24.6 ± 22.2	1.6 ± 1.3	6.7 ± 13.2	9.7 ± 12.2	33.4 ± 26.6	37.9 ± 8.3	14.1 ± 15.5
Hydrometra sp.		0.5 ± 1.3						
Laccotrephes sp.	20.6 ± 21.2	1.6 ± 1.8						
Ranatra sp.	0.6 ± 1.5							0.3 ± 0.4
Sigara sp.	0.5 ± 1.3					1.1 ± 0.7	0.3 ± 0.6	1.2 ± 1.8
Anisops sp.					10.2 ± 9.4	17.7 ± 19.7	48.2 ± 13.2	38.6 ± 47.4
Neomecrocons								0.6 ± 0.9
Mollusca								
Lymnaea truncatula		2.1 ± 5.1	18.1 ± 19.1	1.8 ± 2.9				
Pisidium sp.	3.1 ± 7.6			1.0 ± 2.5				
Pseudosuccinea columella			0.4 ± 1.1		2.6 ± 4.3	3.6 ± 4.5		
Melanoids tubarcellata								9.8 ± 11.6
Bellamya capillata					0.2 ± 0.4			
Trichoptera								
Ecnomus sp.	2.4 ± 5.1	11.6 ± 8.3	17.9 ± 9.4	6.8 ± 11				
Odonata								
Anax speratus		0.3 ± 0.8	0.2 ± 0.5	0.9 ± 2.2				
Pseudogrion sp.			0.4 ± 0.9		0.7 ± 1.5			8.6 ± 7.7
Pinheyschna sp.								1.0 ± 0.3
Metrices								
No. of EPT individuals	15 ± 8.5	3 ± 4.2	30. 5± 2.1	4±4.2	0 ± 0	0 ± 0	2,5 ± 3,5	0±0
No. of ETOC individuals	25.5 ± 0.7	4.5 ± 4.9	39.5 ± 0.7	9±0	10 ± 14.1	4 ± 4.2	0 ± 0	15±12.7

% EPT	35 ± 21.2	7.5 ± 10.6	54 ± 16.7	20.5±24.7	0 ± 0	0 ± 0	10 ±14.1	0 ± 0
% Chironomidae+ Oligochaeta	0 ± 0	0 ± 0	0 ± 0	0 ± 0	73 ± 4.2	19.5 ± 10.6	4.5±0	5.5 ± 0.7
% Corixidae	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
%ETOC	65.5 ± 9.2	12.5 ± 5.8	67 ± 18.4	25 ± 26.9	1.5 ± 0.7	3.5 ± 4.9	1 5± 19.8	9.7 ± 7.1
Abundances	45.3 ± 37.9	37.2 ± 15.0	59.8 ± 21.7	38.8 ± 37.1	205.5 ± 242.9	682.25 ± 580.79	56.75 ± 45.64	140.5 ± 20.5
Shannon–Wiener	1.19 ± 0.31	1.23 ± 0.31	1.27 ± 0.42	0.97 ± 0.33	1.21 ± 0.16	0.93 ± 0.12	1.04 ± 0.28	1.49 ± 0.55
Simpson	0.58 ± 0.15	0.59 ± 0.13	0.6 ± 0.19	0.52 ± 0.15	0.64 ± 0.03	0.50 ± 0.08	0.59 ± 0.11	0.64 ± 0.24
Evenness	0.62 ± 0.16	0.61 ± 0.14	0.55 ± 0.08	0.67 ± 0.23	0.63 ± 0.13	0.39 ± 0.08	0.65 ± 0.16	0.42 ± 0.17

The SIMPER analysis indicated similarities for the high water (mean 42.0 %) and low water (mean 42.1 %) periods. For the high–water period, Cypridoidea (28.3 %), *Enithares* sp. (18.7 %), *Ecnomus* sp. (16.9 %) and *Laccotrephes* sp. (10.2 %) were the dominant taxa, whereas for the low water period, *Enithares* sp. (32.2 %), *Anisops* sp. (29.3 %) and Chironominae (10.9 %) were the dominant taxa.

For the different geological types, *Laccotrephes* sp. (28.5 %), *Enithares* sp. (25.0 %), Cypridoidea (22.0 %) and Branchiopodidae (11.7 %) were the dominant taxa within the sandstone, whereas *Enithares* sp. (34.7 %), Cypridoidea (22.8 %), *Ecnomus* sp. (15.9 %) were dominant in granite areas. Within the basalt soils, Cypridoidea (30.8 %), *Ecnomus* sp. (20.2 %), *Enithares* sp. (15.3 %), *Anisops* sp. (12.6 %) and *Lymnaea truncatula* (10.2 %) were the dominant taxa. Lastly, within the rhyolite soils, *Potamonautes* sp. (32.1 %) and *Lepidurus apus* (21.0 %) were the dominant taxa within the pans.

Table 5. Two–way ANOSIM and SIMPER testing groups on macroinvertebrates communities during high and low water period in pan wetlands across four geological types located in the central Kruger National Park.

Groups	**Global Test R**	**Pairwise test R**	***p***	**Dissimilarity distance**	**Main dissimilatity contribute taxa**
Geological types	0.8		**<0.001**		
High water × Low water		0.8	**<0.001**	86.9	*Anisops* sp. (16.4 %), *Enithares* sp. (15.0 %), Cypridoidea (12.9 %)
Seasons	**0.39**		<0.001		
Sandstone × Granite		**0.2**	**0.032**	66.1	Cypridoidea (15.2 %), *Enithares* sp. (14.1 %), Branchiopodidae (11.0 %)
Sandstone × Basalt		0.62	**<0.001**	70.6	Cypridoidea (16.2 %), Branchiopodidae (12.4 %), *Enithares* sp. (10.7 %),
Sandstone × Rhyolite		0.41	**0.004**	78.9	Cypridoidea (15.0 %), Branchiopodidae (13.0 %), *Laccotrephes* sp. (11.11 %), *Potamonautes* sp. 10.7 %)
Basalt × Granite		0.36	**0.001**	62.2	*Enithares* sp. (16.5 %), Cypridoidea (11.9 %)
Rhyolite × Granite		0.3	**0.009**	74.9	Cypridiodea (16.2 %), *Enithares* sp. (13.9 %), *Potamonautes* sp. (11.1 %)
Rhyolite × Basalt		0.53	**<0.001**	72.8	Cypridoidea (20.4 %), *Lymnaea truncatula* (10.3 %), Branchiopodidae (10.0 %)

2.3.4 Relationship between macroinvertebrate community structure and physicochemical variables

The canonical correspondence analysis (CCA) axes 1 and 2 explained 35.8 % of the fitted cumulative variation in the macroinvertebrate community structure and physicochemical variables across different geological types and seasons. The macroinvertebrate communities were clearly distinguished between high water and low water periods across axis 2 (Figure 2). About nineteen variables i.e., sediment–resistivity, temperature, sediment–pH, nitrate (N), manganese (Mn), zinc (Zn), sulphur (S), total dissolved solids (TDS), salinity, iron (Fe), Stone, potassium (K), copper (Cu), phosphorus (P), benthic chl–*a*, boron (B), phosphate, sediment organic carbon (SOC), and hydrogen (H^+), were significant in structuring macroinvertebrate community (Figure 2a). The low water period pans were negatively associated with the CCA axis 1 and were characterized by benthic chl–*a* phosphate, stone, Cu, Fe, C, B, P and H^+ (Figure 2a). Examples of macroinvertebrates species that were associated with the samples include Oligochaeta, *Hyditicus exclamationis*, *Cybester* sp., *Culex* sp., *Ranatra* sp., *Anisops* sp., *Atroptilum Sudafricanum*, *Pinheyschna* sp., *Pseudogrion* sp., *Enithares* sp., and *Melanoids tubarcellata*. High water period was passively associated with axis 1 and it was characterized by sediment–pH, temperature, nitrate, salinity, total dissolved solids (TDS), Mn, Zn, S (Figure 2a). The examples of macronvertebrates that were associated with the water period included *Ecnomus* sp., *Anax speratus*, *Pseudosuccinea columella*, *Neomecrocons* sp., *Sigara* sp., *Laccotrephes* sp., *Appasus* sp., *Chironomiae*, *Chrysops* sp., *Rhantus* sp., *Hydrochus* sp., *Aliolimnatis* sp., *Berosus* sp., Salifidae, Cypridoidea, *Copelatus* sp., *Regimbartia* sp., *Hydrometra* sp., and *Lymnaea truncatula* (Figure 2b).

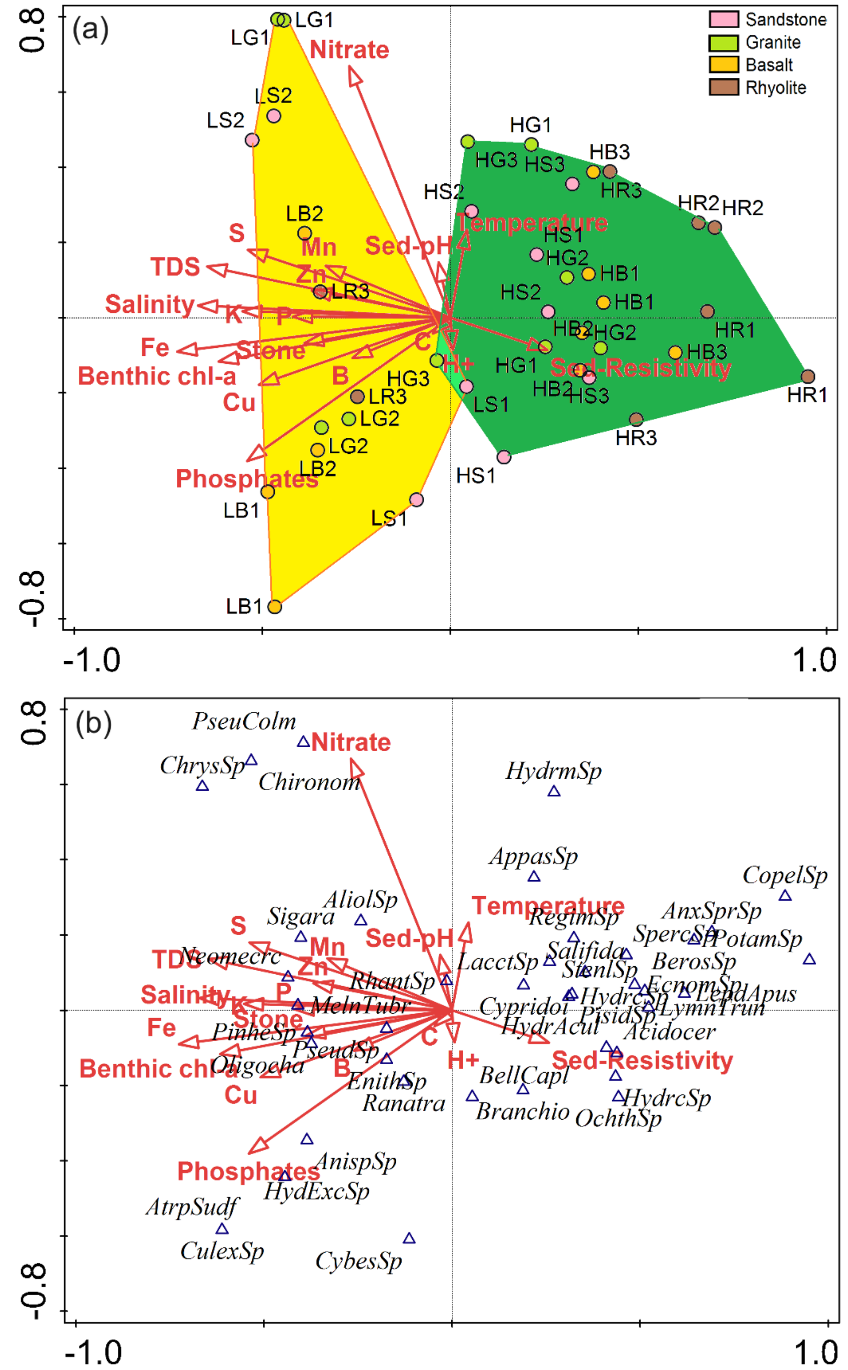

Figure 2. Canonical correspondence analysis of macroinvertebrate community structure and environmental variables for the sampled pan wetlands highlighting the relationships between the variables and study sites in central Kruger National Park: (a) samples and environmental variables, and (b) species and environmental variables. The abbreviations (figure 2a and 2b): L– low water period, H–high water period and the letters next to L and H indicates the geological types (granite, basalt, sandstone and rhyolite.

Using Pearson correlations, a significant and weak negative relationship between macroinvertebrate abundances and water pH (r = –0.34, p = 0.040) was observed, whereas weak positive relationship was observed for macroinvertebrate abundances and conductivity (r = 0.32, p = 0.049), salinity (r = 0.33, p = 0.044), Cu (r = 0.32, p = 0.050), Fe (r = 0.35, p = 0.033) and S (r = 0.40, p = 0.012).

2.4 Discussion

As per the studies objective to assess spatial distribution and variation of macroinvertebrates' diversity and abundances during high and low water period in relation to water and sediment chemistry variable in pan wetlands located across different geological types in Kruger National Park. The results indicated that water period played a significant role in macroinvertebrates diversity and abundance. Macroinvertebrates were more diverse during high water period and more abundant during the low water period. physicochemical variables (i.e., sediment–resistivity, temperature, sediment–pH, N, Mn, Zn, S, TDS, salinity, Fe, Stone, K, Cu, P, benthic–chl–*a*, B, phosphates, C, and H^+) during both the low and high–water period had an influence on the structure of macroinvertebrates. The findings of this study provide further support to previous research indicating that alterations in certain parameters of water quality can have a significant impact on the composition of the macroinvertebrate community (Halabowski and Lewin, 2021). According to Masina et al. (2023) various macroinvertebrates exhibit distinct preferences for specific ranges of environmental characteristics.

In a study conducted by Masina et al. (2023) indicated that water (i.e., temperature, dissolved oxygen, pH, salinity, conductivity), physical (i.e., stone composition) and sediment (i.e., sulphur, sodium) parameters were found to have a significant impact on the macroinvertebrate communities. Moreover, Dalu and Chauke (2020) in a study on macroinvertebrate communities in relation to environmental variables: the case of Sambandou wetlands, Vhembe Biosphere Reserve found that variables such pH, phosphate, temperature, ammonium, macrophyte cover, conductivity and water depth, which were significant in structuring macroinvertebrate community. These results were in line with the hypothesis that high macroinvertebrate diversity will be during the high–water period and abundance will be during the low–water period due to habitat suitability and reproductive opportunities.

Macroinvertebrates common matrices were applied to assess the pan wetlands integrity. Significant differences in matrices for Shannon–Wiener, Simpson diversity index, Ephemeroptera, Plecoptera and Trichoptera (%EPT), %ETOC, and %Chironomidae + Oligochaeta were observed across different geological types in both water periods. High Shannon–Wiener index and the Simpson diversity index values were observed during high water period. The EPT metric index pertains to three distinct orders of aquatic macroinvertebrates that demonstrate heightened susceptibility to water pollution (Masina et al., 2023). This taxon is sensitive to pollution and degradation; hence, it is used to assess the health of an ecosystem (Hickey and Clements, 1998; Girgin et al., 2010). Batty et al. (2005) and Masina et al. (2023) state that low values of the EPT in aquatic systems indicate a stressful condition and high values indicate less stressful conditions. The results indicated high values in EPT during high water period across all geological types, whereas in low water period there were only identified in the basalt. These results contrast with what a study conducted by Batty et al. (2005) examining nine water treatment wetlands located in the northeast region of England. According to Ab–Hamid and Rawi (2017), the presence of EPT species denotes that those parameters in the habitat is within the tolerance limit of the species.

Furthermore, Chironomidae (Chironominae) were only identified in abundance during the low water period. These results correspond with the findings of a study conducted by Masina et al. (2023) who observed an abundance of Diptera (Chironominae) during winter season (low water period). However, they contrast with the findings of Odume and Muller (2011), Dalu et al., (2017) Dalu and Chauke (2020), who observed the presents of Chironomidae in summer season. Significant alterations were observed in the macroinvertebrate fauna between the high and low water period, with the high–water period was dominated by Cypridoidea, *Enithares* sp., *Ecnomus* sp. and *Laccotrephes* sp. taxa and the low water period dominated by *Enithares* sp., *Anisops* sp. and Chironominae taxa. The observed variations in the response of macroinvertebrate species composition and abundance can be due to taxa's significant response to environmental factors (Ferreira et al., 2014; Aschalew and Moog, 2015). Although a weak negative relationship between macroinvertebrate abundances and water pH was observed and a weak positive relationship was observed for macroinvertebrate abundances, conductivity, and salinity. The pH sensitivity of macroinvertebrates exhibits species–specific variations, with certain species demonstrating susceptibility to adverse effects under acidic circumstances. These findings align with a study carried out by Courtney and Clements (1998), which identified that the main factor contributing to the decrease in macroinvertebrate abundance was the reduction in the population of mayflies (Ephemeroptera), known for their high sensitivity level.

A slightly high benthic chl–*a* concentration was observed during low water period. Dalu and Chauke (2020) state that a high concentration of chl–*a* in wetlands is an indicator of potential eutrophication which turns to harm aquatic species and reduce biodiversity. Moreover, high concentration of nutrients (i.e., ammonium, phosphate and nitrate) was observed in the low water period. The presence of high nutrients may be due to a decrease in water volume, restricted water flow, and continuous biological activity. The phenomenon of dilution effect reduction results in the concentration of nutrients within a reduced volume of water, whereas slow water flow contributes to the concentrated accumulation of nutrients. In addition, the breakdown of organic matter, limited absorption of nutrients by plants, and the addition of nutrients from external sources also contribute to the observed abundance of nutrients.

Active involvement nutrients promote primary productivity which leads to the growth and development of algae and aquatic plants (Ansari et al.,2011). Naeem et al. (2014) state that when there is a high abundance of these nutrients, they can function as constraining elements, facilitating the proliferation of phytoplankton, which serve as the principal producers in aquatic environments. In addition, the concentration of chl-a, the photosynthetic pigment necessary for the survival of phytoplankton, rises in tandem with their population growth (Naeem et al,2014). Consequently, greater concentrations of ammonium and phosphates frequently result in heightened chlorophyll-a levels, indicating the heightened productivity and biomass of phytoplankton populations (Naeem et al., 2014).

These results are consistent with the findings by Dalu and Chauke (2020) indicating an increase of chl–*a* concentration along a nutrient gradient of ammonium and phosphates. Their study revealed a change in macroinvertebrate community structure and abundance. Therefore, based on this study, it can be inferred that as the pan wetland dry up due to declines in ground water levels and potentially rainfall, concentration of nutrients within the pans increases.

2.5 Conclusions

The findings of the study revealed that macroinvertebrate communities were influenced by environmental variables and water period. As the pan wetland are dependent on the supply of ground water, it was observed that during the low water period there was an abundance of macroinvertebrate, metal and nutrients concentration in the pan. Consequently, in poor water quality. Therefore, correlation between low water periods and poor water quality underscores the necessity of implementing focused conservation and management strategies. These include maintaining minimum groundwater levels, restoring wetlands using natural hydrological patterns and native vegetation, reducing sedimentation and erosion, establishing buffer zones, monitoring ecological indicators, raising public awareness, enacting protective legislation, and encouraging collaborative management approaches. Moreover, since macroinvertebrates are crucial for assessing water quality and serve as indicators of the overall health of the pan wetland ecosystem. Their abundance and diversity are significant factors in this assessment.

.

CHAPTER 4: GENERAL SYNTHESIS

4.1. Summary

The thesis provides valuable insights into the intricate dynamics of floodplain pan wetland ecosystems. The chapters covered examined carbon sequestration, metal concentrations, and macroinvertebrate diversity and abundance across various geological types. This synthesis consolidates the findings of the interrelationships between biotic and abiotic factors in pan wetlands located in Kruger National Park, South Africa.

The study emphasized the influence of pH on the availability of nutrients and microbial activity, highlighting variations across different geological types in soil mineralogy. The presence of metals and non–metals in the upper layers of soil, along with the impact of elephants on the distribution of nutrients and minerals, had valuable insights into the intricacies of soil dynamics within these wetland ecosystems. Furthermore, the study has provided valuable insights into the variability of carbon sequestration across different geological formations. It highlighted the crucial role of wetland physical and biological processes in influencing the distribution of organic matter in a soil profile. Moreover, the spatiotemporal fluctuations in macroinvertebrate diversity and abundance within pan wetlands indicated the significant influence that water period had on the structure of macroinvertebrate communities. Greater diversity during periods of high water and increased abundance during periods of low water was observed. Several environmental variables were found to have a significant impact on macroinvertebrate structure. These variables included sediment resistivity, temperature, and various physicochemical parameters.

The evaluation of pan wetland integrity through the utilization of various metrics, including Shannon–Wiener and Simpson diversity indices, %EPT, %ETOC, and %Chironomidae + Oligochaeta, provided a comprehension understanding of the ecological well–being of these wetlands. The study emphasized the responsiveness of specific macroinvertebrate taxa to water quality, which has significant implications for the evaluation of ecosystem health.

The thesis provides valuable insights into the intricate dynamics of floodplain pan wetland ecosystems. The chapters covered examined carbon sequestration, metal concentrations, and macroinvertebrate diversity and abundance across various geological types. This synthesis consolidates the findings of the interrelationships between biotic and abiotic factors in pan wetlands located in Kruger National Park, South Africa.

The study emphasized the influence of pH on the availability of nutrients and microbial activity, highlighting variations across different geological types in soil mineralogy. The presence of metals and non–metals in the upper layers of soil, along with the impact of elephants on the distribution of nutrients and minerals, had valuable insights into the intricacies of soil dynamics within these wetland ecosystems. Furthermore, the study has provided valuable insights into the variability of carbon sequestration across different geological formations. It highlighted the crucial role of wetland physical and biological processes in influencing the distribution of organic matter in a soil profile. Moreover, the spatiotemporal fluctuations in macroinvertebrate diversity and abundance within pan wetlands indicated the significant influence that water period had on the structure of macroinvertebrate communities. Greater diversity during periods of high water and increased abundance during periods of low water was observed. Several environmental variables were found to have a significant impact on macroinvertebrate structure. These variables included sediment resistivity, temperature, and various physicochemical parameters.

The evaluation of pan wetland integrity through the utilization of various metrics, including Shannon–Wiener and Simpson diversity indices, %EPT, %ETOC, and %Chironomidae + Oligochaeta, provided a comprehension understanding of the ecological well–being of these wetlands. The study emphasized the responsiveness of specific macroinvertebrate taxa to water quality, which has significant implications for the evaluation of ecosystem health.

4.2. General conclusions and recommendations

In summary, the collective chapters shed light on crucial aspects within wetland ecosystems, emphasizing their intricate interconnections. Understanding the challenges posed by low water periods and their impact on macroinvertebrate communities offers a roadmap for proactive conservation strategies aimed at bolstering the ecological health of pan wetlands. Additionally, the imperative to address climate change through carbon sequestration necessitates a deep dive into geological factors and their environmental ramifications. Identifying geological formations with optimal carbon storage capabilities, while mitigating risks of carbon dioxide release, becomes paramount. This dual-purpose knowledge not only aids in achieving net-zero emission goals but also propels the development of economically viable carbon capture and storage technologies.

These insights are instrumental in crafting effective policy frameworks, guiding monitoring mechanisms, and ensuring compliance. Ultimately, they underscore the importance of safeguarding ecological systems, reducing disruptions, mitigating pollution risks, and potentially fostering biodiversity. Furthermore, based on the research problem and the research findings of the study, it is recommended that for a protected area like Kruger National Park must be able to locate and understand the pan wetlands that exist in the space. This is important because they do not only serve as biodiversity hotspots but as watering points for inland animals. For their distribution to be known, the park must treat these wetlands as an ecological asset or infrastructure that needs to go into their existing asset management register. With that being said, the management of the park needs to identify practical opportunities which integrate ecological infrastructure into formal asset management processes toward internalising, the benefits and costs of the flow of natural benefits from ecosystem.

REFERENCES

Ab Hamid, S., and Rawi, C.S.M. 2017. Application of aquatic insects (Ephemeroptera, Plecoptera and Trichoptera) in water quality assessment of Malaysian headwater. *Tropical Life Sciences Research*, 28(2), pp.143.

Adams, W.A., 1973. The effect of organic matter on the bulk and true densities of some uncultivated podzolic soils. *Journal of Soil Science*, *24*(1), pp.10–17.

Adeeyo, A.O., Ndlovu, S.S., Ngwagwe, L.M., Mudau, M., Alabi, M.A., and Edokpayi, J.N. 2022. Wetland resources in South Africa: threats and metadata study. *Resources*, *11*(6), pp.54.

Aldous, A.R. and Bach, L.B., 2014. Hydro–ecology of groundwater–dependent ecosystems: applying basic science to groundwater management. *Hydrological Sciences Journal*, *59*(3–4), pp.530–544.

Allan, J.D., Castillo, M.M. and Capps, K.A., 2021. *Stream Ecology: Structure and Function of Running Waters*. Springer Nature.

Anderson, M., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. *Primer–E Limited.*

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral ecology*, 26, pp.32–46.

Aschalew, L., Moog, O., 2015. Benthic macroinvertebrates based new biotic score "ETHbios" for assessing ecological conditions of highland streams and rivers in Ethiopia. *Limnological* 52, 11–19.

Ayeni, O.O., Ndakidemi, P.A., Snyman, R.G. and Odendaal, J.P., 2010. "Chemical, biological and physiological indicators of metal pollution in wetlands," *Scientific Research and Essays*, vol. 5(15), pp. 1938–1949.

Bai, J., Xiao, R., Zhang, K., and Gao, H., 2012. "Arsenic and heavy metal pollution in wetland soils from tidal freshwater and salt marshes before and after the flow–sediment regulation regime in the Yellow River Delta, China," *Journal of Hydrology*, 450(451), pp. 244–253.

Bai, J., Zhang, G., Zhao, Q., Lu, Q., Jia, J., Cui, B., and Liu, X. 2016. Depth–distribution patterns and control of soil organic carbon in coastal salt marshes with different plant covers. *Scientific Reports, 6(1),* pp *34835.*

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A. and Dar, S.A., 2020. Concerns and threats of contamination on aquatic ecosystems. *Bioremediation and biotechnology: sustainable approaches to pollution degradation*, pp.1–26.

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A. and Dar, S.A., 2020. Concerns and threats of contamination on aquatic ecosystems. *Bioremediation and biotechnology: sustainable approaches to pollution degradation*, pp.1–26.

Bassi, N., Kumar, M.D., Sharma, A., and Pardha–Saradhi, P. 2014. Status of wetlands in India: A review of extent, ecosystem benefits, threats and management strategies. *Journal of Hydrology: Regional Studies*, *2*, pp. 1–19.

Batty, L.C., Atkin, L., and Manning, D.A.C. 2005. Assessment of the ecological potential of mine–water treatment wetlands using a baseline survey of macroinvertebrate communities. *Environmental Pollution*, 138, pp.412–419.

Batzer, D.P., Palik, B.J., and Buech, R. 2004. Relationships between environmental characteristics and macroinvertebrate communities in seasonal woodland ponds of Minnesota. *Journal of the North American Benthological Society*, 23, pp.50–68.

Bêche, L.A. and Statzner, B., 2009. Richness gradients of stream invertebrates across the USA: taxonomy–and trait–based approaches. *Biodiversity and Conservation*, *18*, pp.3909–3930.

Bernal, B. and Mitsch, W.J., 2012. Comparing carbon sequestration in temperate freshwater wetland communities. *Global Change Biology*, *18*(5), pp.1636–1647.

Bonacina, L., Fasano, F., Mezzanotte, V. and Fornaroli, R., 2023. Effects of water temperature on freshwater macroinvertebrates: a systematic review. *Biological Reviews*, *98*(1), pp.191–221.

Bonada, N., Prat, N., Resh, V.H., and Statzner, B. 2006. Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, 51, pp.495–523.

Brady, N.C. and Weil, R.R., 2008. The soils around us. *The nature and properties of soils, 14th ed Pearson Prentice Hall, New Jersey and Ohio*, pp.1–31.

Bridgham, S.D., Megonigal, J.P., Keller, J.K., Bliss, N.B. and Trettin, C., 2006. The carbon balance of North American wetlands. *Wetlands*, *26*(4), pp.889–916.

Cheng, Q., Wang, R., Huang, W., Wang, W. and Li, X., 2015. Assessment of heavy metal contamination in the sediments from the Yellow River Wetland National Nature Reserve (the Sanmenxia section), China. Environmental science and pollution research, 22, pp.8586–8593.

Chi, J., Xu, G., Yang, Q., and Sun, J. 2022. Water quality mediated community variations and niche differentiation of macroinvertebrates in Qingyijiang River Basin, China. *Ecological Indicators*, *138*, p.108830.

Chibuike, G.U. and Obiora, S.C., 2014. Heavy metal polluted soils: effect on plants and bioremediation methods. *Applied and environmental soil science*, *2014*:1–12.

Clarke, K.R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18, pp.117–143.

Courtney, L.A. and Clements, W.H., 1998. Effects of acidic pH on benthic macroinvertebrate communities in stream microcosms. *Hydrobiologia*, *379*(1–3), pp.135–145.

Dalu, T. and Wasserman, R.J. eds., 2021. *Fundamentals of tropical freshwater wetlands: from ecology to conservation management*. *Elsevier Science*.

Dalu, T., and Chauke, R. 2020. Assessing macroinvertebrate communities in relation to environmental variables: the case of Sambandou wetlands, Vhembe Biosphere Reserve. *Applied Water Science,* 10(1), pp.1–11.

Dalu, T., Dlamini, P., Wasserman, R.J., Mokgoebo, M.J., Mutshekwa, T., Dondofema, F., and Cuthbert, R.N. 2021. Effects of environmental variables on littoral macroinvertebrate community assemblages in subtropical reservoirs. *Chemistry and Ecology*, 37(5), pp.419–436.

Dalu, T., Tshivhase, R., Cuthbert, R.N., Murungweni, F.M. and Wasserman, R.J. 2020. Metal distribution and sediment quality variation across sediment depths of a subtropical Ramsar declared wetland. *Water*, *12*(10), pp. 2779.

Dalu, T., Wasserman, R.J., 2022. Tropical freshwater wetlands: an introduction. In: Dalu, T., Wasserman, R.J. (Eds.), Fundamentals of Tropical Freshwater Wetlands: From Ecology to Conservation Management. Elsevier, Cambridge, pp. 1–22

Dalu, T., Wasserman, R.J., Tonkin, J.D., Alexander, M.E., Dalu, M.T., Motitsoe, S.N., Manungo, K.I., Bepe, O., and Dube, T. 2017. Assessing drivers of benthic macroinvertebrate community structure in African highland streams: an exploration using multivariate analysis. *Science of the Total Environment*, *601*, pp.1340–1348.

Daryadel, E., and Talaei, F. 2014. Analytical study on threats to wetland ecosystems and their solutions in the Framework of the Ramsar Convention. *International Journal of Environmental and Ecological Engineering*, *8*(7), pp.2108–2118.

de Necker, L., Dyamond, K., Greenfield, R., van Vuren, J. and Malherbe, W., 2023. Aquatic invertebrate community structure and functions within a Ramsar wetland of a premier conservation area in South Africa. *Ecological Indicators*, *148*, p.110135.

Dickens, C.W., and Graham, P. 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* 27(1), pp.1–10.

Esmaeilzadeh, M., Karbassi, A. and Moattar, F., 2016. Assessment of metal pollution in the Anzali Wetland sediments using chemical partitioning method and pollution indices. *Acta Oceanological Sinica*, *35*, pp.28–36.

Fennessy, M.S., Wardrop, D.H., Moon, J.B., Wilson, S., and Craft, C. 2018. Soil carbon sequestration in freshwater wetlands varies across a gradient of ecological condition and by ecoregion. *Ecological Engineering*, 114, pp.129–136.

Ferreira, W.R., Ligeiro, R., Macedo, D.R., Hughes, R.M., Kaufmann, P.R., Oliveira, L.G., and Callisto, M. 2014. Importance of environmental factors for the richness and distribution of benthic macroinvertebrates in tropical headwater streams. *Freshwater Science*, 33, pp.860–871.

Gargouri, D., Gzam, M., Kharroubi, A. and Jedoui, Y., 2018. Use of sediment quality indicators for heavy metals contamination and ecological risk assessment in urbanized coastal zones. *Environmental Earth Sciences*, *77*, pp.1–8.

Gaur, V.K., Gupta, S.K., Pandey, S.D., Gopal, K., and Misra, V. 2005. Distribution of heavy metals in sediment and water of river Gomti. *Environmental Monitoring and Assessment*, 102, pp.419–433.

Gerber, A., and Gabriel, M.J.M. 2002. Aquatic invertebrates of South African rivers field guide. Institute for Water Quality Studies, Department of Water Affairs and Forestry (DWAF). Pretoria, South Africa.

Gibbs, R.J. 1967. The geochemistry of the Amazon River system: Part I. The factors that control the salinity and the composition and concentration of the suspended solids. *Geological Society of America Bulletin*, 78(10), pp.1203–1232.

Girgin, S., Kazanci, N., and Dügel, M. 2010.: Relationship between aquatic insects and heavy metals in an urban stream using multivariate techniques. *International Journal of Environmental Science and Technology*, 7(4), pp. 653–664.

Halabowski, D., and Lewin, I. 2021. Triggers for the Impoverishment of the Macroinvertebrate Communities in the Human–Impacted Rivers of Two Central European Ecoregions. *Water Air Soil Pollution*, *232*, 55.

Hauer, F.R., and Resh, V.H. 2017. Macroinvertebrates. In Richard, F. Hauer and Gary A. Lamberti (Eds.). In *Methods in Stream Ecology, 1(3)*, pp. 297–319. Academic Press.

Helson, J.E. (2012). Macroinvertebrate Community Structure and Function in Seasonal, *Low–land, Tropical Streams across a Pristine–rural–Urban Land–use Gradient*. (Doctoral dissertation, Department of Ecology and Evolutionary Biology, University of Toronto).

Hickey, C.W. and Clements, W.H., 1998. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. *Environmental Toxicology and Chemistry: An International Journal*, *17*(11), pp.2338–2346.

Hoffmann, W.A., Geiger, E.L., Gotsch, S.G., Rossatto, D.R., Silva, L.C., Lau, O.L., Haridasan, M., and Franco, A.C. 2012. Ecological thresholds at the savanna-forest boundary: how plant traits, resources and fire govern the distribution of tropical biomes. *Ecology letters*, *15*(7), pp.759–768.

Hölker, F., Vanni, M.J., Kuiper, J.J., Meile, C., Grossart, H.P., Stief, P., Adrian, R., Lorke, A., Dellwig, O., Brand, A., and Hupfer, M. 2015. Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. *Ecological Monographs*, *85*(3), pp.333–351.

Hose, G. C., Dabovic, J., Nelson, T., Pollitt, L., and Korbel, K. L. 2022. Groundwater dependent aquatic and terrestrial ecosystems. In Tockner, K. and Mehner, T. (Eds.), *Encyclopedia of Inland Waters* 2(3), pp. 339–347. Elsevier.

Hu, C., Deng, Z.M., Xie, Y.H., Chen, X.S. and Li, F., 2015. The risk assessment of sediment heavy metal pollution in the East Dongting Lake Wetland. *Journal of Chemistry*, *2015*.

Jansson, J.K. and Hofmockel, K.S., 2020. Soil microbiomes and climate change. *Nature Reviews Microbiology*, *18*(1), pp.35–46.

Jobbagy, E.G. and Jackson, R.B., 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. *Biogeochemistry*, *53*, pp.51–77.

Johnson, R.K., Wiederholm, T., and Rosenberg, D.M. 1993. Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. *Freshwater Biomonitoring and Benthic Macroinvertebrates*, *40*, pp.158.

Kabata–Pendias, A., 2001. Trace metals in soils–a current issue in Poland. *Acta Universitatis Wratislaviensis. Prace Botaniczne*, *79*, pp.13–20.

Kløve, B., Ala–Aho, P., Bertrand, G., Boukalova, Z., Ertürk, A., Goldscheider, N., Ilmonen, J., Karakaya, N., Kupfersberger, H., Kvœrner, J., and Lundberg, A. 2011. Groundwater dependent ecosystems. Part I: Hydroecological status and trends. *Environmental Science and Policy,* 14(7), pp.770–781.

Kouamé, M.K., Dietoa, M.Y., Edia, E.O., Da Costa, S.K., Ouattara, A., and Gourène, G. 2011. Macroinvertebrate communities associated with macrophyte habitats in a tropical man–made lake (Lake Taabo, Côte d'Ivoire). *Knowledge and Management of Aquatic Ecosystems*, 400, pp. 03.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *science*, *304*(5677), pp.1623–1627.

Lehmann, J. and Kleber, M., 2015. The contentious nature of soil organic matter. *Nature*, *528*(7580), pp.60–68.

Li, Z., Jiang, W., Wang, W., Chen, Z., Ling, Z., and Lv, J. 2020. Ecological risk assessment of the wetlands in Beijing–Tianjin–Hebei urban agglomeration. *Ecological Indicators*, *117*, pp.106677.

Lindsay, W.L., 1979. *Chemical equilibria in soils*. *John Wiley and Sons* Ltd.

Mabidi, A., Bird, M.S., and Perissinotto, R. 2017. Distribution and diversity of aquatic macroinvertebrate assemblages in a semi–arid region earmarked for shale gas exploration (Eastern Cape Karoo, South Africa). *PLoS One*, 12(6), pp. e0178559.

Masina, F.M., Wasserman, R.J., Wu, N., Mungenge, C.P., Dondofema, F., Keates, C., Shikwambana, P. and Dalu, T., 2023. Macroinvertebrate diversity in relation to limnochemistry in an Austral semi–arid transboundary aquifer region pan system. *Science of The Total Environment*, *878*, p.163161.

Mann, L.K. (1986.) Changes in soil carbon storage after cultivation. *Soil Science*, *142*(5), pp.279–288.

Mann, L.K. 1990 Changes in soil organic carbon and nitrogen as a result of cultivation. In: *Bouwman AF (ed) Soils and the Greenhouse Effect*. *John Wiley and Sons*, New York.

Merritt, R. W., and Cummins, K. W. (Eds.). (1996). An Introduction to the Aquatic Insects of North America. Kendall Hunt Publishing, United States of America.

Merritt, R.W. and Cummins, K.W. eds., 1996. *An introduction to the aquatic insects of North America*. Kendall Hunt.

Mitsch, W. J., and Gosselink, J. G. 2015. Wetlands. John Wiley and Sons.

Morales–García, S.S., Meza–Olvera, E., Shruti, V.C. and Sedeño–Díaz, J.E., 2020. Assessment of metal contamination and their ecological risks in wetland sediments of the former Texcoco saline lake, Mexico. *Journal of Soils and Sediments*, *20*, pp.2912–2930.

Moomaw, W.R., Chmura, G.L., Davies, G.T., Finlayson, C.M., Middleton, B.A., Natali, S.M., Perry, J.E., Roulet, N. and Sutton–Grier, A.E., 2018. Wetlands in a changing climate: science, policy and management. *Wetlands*, *38*(2), pp.183–205.

Munyai, L.F., Mugwedi, L., Wasserman, R.J., Dondofema, F., Riddell, E., Keates, C., and Dalu, T. (2023). Metal and non–metal dynamics and distribution in soil profiles across selected pans in the Ramsar declared subtropical within a national protected area. *Chemistry and Ecology*, pp.1–21.

Nag S.K., Das Ghosh, B., Nandy, S. *et al.* Comparative assessment of carbon sequestration potential of different types of wetlands in lower Gangetic basin of West Bengal, India. *Environmental Monitoring and Assessment* 195, 154 (2023).

Nahlik, A.M. and Fennessy, M.S., 2016. Carbon storage in US wetlands. *Nature Communications* 7, pp. 13835.

Niedrist, G.H. and Füreder, L., 2017. Trophic ecology of alpine stream invertebrates: current status and future research needs. *Freshwater Science*, *36*(3), pp.466–478.

Odume ON, Muller WJ (2011) Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River, South Africa. *Physics and Chemistry of the Earth* 36, pp.929–938.

Ollis, D.J., Dallas, H.F., Esler, K.J., and Boucher, C. 2006. Bioassessment of the ecological integrity of river ecosystems using aquatic macroinvertebrates: an overview with a focus on South Africa. *African Journal of Aquatic Science*, 31, pp.205–227.

O'Neill, B.J. and Thorp, J.H., 2014. Untangling food-web structure in an ephemeral ecosystem. *Freshwater Biology*, *59*(7), pp.1462–1473.

Ouma, K.O., Shane, A. and Syampungani, S., 2022. Aquatic ecological risk of heavy–metal pollution associated with degraded mining landscapes of the Southern Africa River Basins: A review. *Minerals*, *12*(2), pp.225.

Pérez Hoyos, I.C., Krakauer, N.Y., Khanbilvardi, R., and Armstrong, R.A. 2016. A review of advances in the identification and characterization of groundwater dependent ecosystems using geospatial technologies. *Geosciences*, 6(2), pp.17.

Phillips, D.P., Human, L.R.D. and Adams, J.B., 2015. Wetland plants as indicators of heavy metal contamination. *Marine pollution bulletin*, *92*(1–2), pp.227–232.

Post, W.M., and Kwon, K.C. 2000. Soil carbon sequestration and land-use change: processes and potential. *Global change biology*, *6*(3), pp.317–327.

Ragot, S.A., Huguenin–Elie, O., Kertesz, M.A., Frossard, E. and Bünemann, E.K., 2016. Total and active microbial communities and phoD as affected by phosphate depletion and pH in soil. *Plant and soil*, *408*, pp.15–30.

Rasifudi, L., Addo–Bediako, A., Bal, K. and Swemmer, T.M., 2018. Distribution of benthic macroinvertebrates in the Selati River of the Olifants River system, South Africa. *African Entomology*, *26*(2), pp.398–406.

Richardson, S., Irvine, E., Froend, R., Boon, P., Barber, S., and Bonneville, B. 2011. Australian groundwater dependent ecosystems toolbox part 1: assessment framework. *Waterlines report, National Water Commission*, *Canberra*. [Accessed 23 June 2022]

Rohde, M.M., Froend, R. and Howard, J., 2017. A global synthesis of managing groundwater dependent ecosystems under sustainable groundwater policy. Groundwater 55: 293–301. *Study on adaptive management approaches of GDE, challenges and opportunities*.

Ruan, X.L., Zhang, G.L., Zhao, Y.G., Yuan, D.G., and Wu, Y.J. 2006. Distribution and migration of heavy metals in soil profiles by high–resolution sampling. *Huan Jing ke Xue= Huanjing Kexue*, *27*(5), pp.1020–1025.

Schäfer, R.B., 2019. Responses of freshwater macroinvertebrates to pesticides: insights from field studies. *Current Opinion in Environmental Science and Health*, *11*, pp.1–7.

Shen, Q., Wu, M. and Zhang, M., 2022. Accumulation and relationship of metals in different soil aggregate fractions along soil profiles. *Journal of Environmental Sciences*, *115*, pp.47–54.

Siebert, F., Siebert, S.J. and Eckhardt, H.C., 2010. The vegetation and floristics of the Letaba exclosures, Kruger National Park, South Africa. *Koedoe: African Protected Area Conservation and Science*, *52*(1), pp.1–12.

Sinclair, A.R., and Walker, B. (2003). The Kruger experience: ecology and management of savanna heterogeneity. Island Press: status and future research needs. Freshwater Science, 36(3), pp.466–478.

Six, J., Bossuyt, H., Degryze, S. and Denef, K., 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and tillage research*, *79*(1), pp.7–31.

Šmilauer, P., Lepš, J., 2014. Multivariate Analysis of Ecological Data Using CANOCO 5. Cambridge University Press, CA

Šolić, M., Šantić, D., Šestanović, S., Bojanić, N., Grbec, B., Jozić, S., Vrdoljak, A., Ordulj, M., Matić, F., Kušpilić, G. and Gladan, Ž.N., 2020. Impact of water column stability dynamics on the succession of plankton food web types in the offshore area of the Adriatic Sea. *Journal of Sea research*, 158, p.101860.

Statzner, B., Bêche, L. A., and Herouin, E. 2001. Toward a better use of the saprobic system for diagnosing organic pollution. *Freshwater Biology*, 46(2), pp.205–213.

Stephenson, P.J., Ntiamoa–Baidu, Y., and Simaika, J.P. 2020. The use of traditional and modern tools for monitoring wetlands biodiversity in Africa: challenges and opportunities. *Frontiers in Environmental Science*, 8, pp.61.

Suir, G.M., Sasser, C.E., DeLaune, R.D., and Murray, E.O. 2019. Comparing carbon accumulation in restored and natural wetland soils of coastal Louisiana. *International Journal of Sediment Research*, *34*(6), pp.600–607.

Tooth, S. and McCarthy, T.S., 2007. Wetlands in drylands: geomorphological and sedimentological characteristics, with emphasis on examples from southern Africa. *Progress in Physical Geography*, *31*(1), pp.3–41.

Trettin, C.C., Kolka, R.K., Marsh, A.S., Bansal, S., Lilleskov, E.A., Megonigal, P., Stelk, M.J., Lockaby, G., D'Amore, D.V., MacKenzie, R.A. and Tangen, B., 2020. Wetland and Hydric Soils. *Forest and Rangeland Soils of the United States Under Changing Conditions*, pp.99.

Van Rooyen, N., Theron, G.K., and Grobbelaar, N. 1981. A floristic description and structural analysis of the plant communities of the Punda–Milia–Wambiya area in the Kruger National Park, Republic of South Africa: 3. The *Colophospermum mopane* communities. *Journal of South African Botany,* 47(4), 585–626.

Vanschoenwinkel, B., Mergeay, J., Pinceel, T., Waterkeyn, A., Vandewaerde, H., Seaman, M., and Brendonck, L. 2011. Long distance dispersal of zooplankton endemic to isolated mountaintops–an example of an ecological process operating on an evolutionary time scale. *PLoS One*, *6*(11), p.e26730.

Vaughn, C.C., and Hoellein, T.J. 2018. Bivalve impacts in freshwater and marine ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 49, pp.183–208.

Victor, W. (2013). Macroinvertebrate assemblage changes as an indicator of water quality of perennial endorheic reed pans on the Mpumalanga Highveld, South Africa. *Journal of Environmental Protection,* 4, pp 10–21.

Villa, J.A., and Bernal, B. (2018). Carbon sequestration in wetlands, from science to practice: An overview of the biogeochemical process, measurement methods, and policy framework. *Ecological Engineering*, 114, pp.115–128.

Wang, X.W., Liu, H.Y., Gu, X.F., Tu, Y., Yu, E.J., and Wu, P. 2022. Distribution Characteristics of Heavy Metals in Soils Affected by Different Land Use Types in a Superimposed Pollution Area with High Geological Background. *Huan jing ke xue= Huanjing kexue*, *43*(4), pp.2094–2103.

Wasserman, R.J., Weston, M., Weyl, O.L., Froneman, P.W., Welch, R.J., Vink, T.J. and Dalu, T., 2018. Sacrificial males: the potential role of copulation and predation in contributing to copepod sex-skewed ratios. *Oikos*, *127*(7), pp.970–980.

Were, D., Kansiime, F., Fetahi, T., Cooper, A., and Jjuuko, C. 2019. Carbon sequestration by wetlands: a critical review of enhancement measures for climate change mitigation. *Earth Systems and Environment*, *3*, pp.327–340.

Wachniew, P., Witczak, S., Postawa, A., Kania, J., Żurek, A., Różański, K. and Dulliński, M., 2014. Groundwater dependent ecosystems and man: Conflicting groundwater uses. *Geological Quarterly*, *58*(4), pp.695–706.

Whigham, D.F., 1999. Ecological issues related to wetland preservation, restoration, creation and assessment. *Science of the Total Environment*, *240*(1–3), pp.31–40.

Wu, J., Zhao, Y., Qi, H., Zhao, X., Yang, T., Du, Y., Zhang, H. and Wei, Z., 2017. Identifying the key factors that affect the formation of humic substance during different materials composting. *Bioresource technology*, *244*, pp.1193–1196.

Xu, C.M., Holmgren, E.H., Van Nes, F.T., Maestre, S., Soliveres, M., Berdugo, S., Kéfi, P. A., Marquet, S., Abades, S., and Scheffer, M. 2015. Can we infer plant facilitation from remote sensing? a test across global drylands. *Ecological Applications,* 25, pp.1456–1462.

Yadav, N., Singh, S., Saini, O. and Srivastava, S., 2022. Technological advancement in the remediation of heavy metals employing engineered nanoparticles: a step towards cleaner water process. *Environmental Nanotechnology, Monitoring and Management*, pp.100757.

Zedler, J.B., and Kercher, S. 2005. Wetland resources: Status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, 30, pp.39–74.

Zhang, S., Pan, S., Li, G. and Liu, Z. 2022. Spatial variation, sources, and potential ecological risk of metals in sediment in the northern South China Sea. *Marine Pollution Bulletin*, 181, pp.113929.