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Spatio-seasonal variation in wetland water quality, heavy metal pollution and macroinvertebrate communities in the Waterberg Mountain Complex

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ABSTRACT

Wetlands in semi-arid southern Africa are increasingly threatened by combined climatic and anthropogenic stressors, yet seasonal data on water quality and biota remain scarce. We assessed 11 wetlands in the Waterberg Mountain Complex, Limpopo Province sampling during early rains and late rains. At each site we measured in-situ physico-chemical variables, quantified water-sediment heavy metals and collected benthic macro-invertebrates. Dissolved oxygen was the only physico-chemical variable showing a significant seasonal decline (11.7 mg/l early rains to 5.8 mg/l in late rains; $p < 0.05$). Iron exceeded Canadian guidelines in 64% of LR samples (max = 22 mg/l). Cadmium exhibited the greatest seasonal increase in sediments ($p < 0.01$). Diptera dominated macroinvertebrate assemblages particularly at the most metal-enriched site whereas Ephemeroptera, Trichoptera and Odonata were abundant in wetlands with higher oxygen and lower metal loads. Canonical correspondence analysis linked turbidity, conductivity and temperature with tolerant taxa (Hemiptera, Hydracarina), whereas redundancy analysis indicated zinc and cadmium strongly structured communities at polluted sites. These findings highlight oxygen limitation and localized Fe–Cr–Cd enrichment as key stressors influencing macroinvertebrate diversity. As the first integrated seasonal assessment for Waterberg wetlands, the study provides a baseline for monitoring systems facing intensifying land-use and climate pressures and underscores the need for continued multi-season biomonitoring to guide adaptive management.

1. Introduction

Wetlands, once dismissed as unproductive wastelands are now globally recognized as vital ecosystems that sustain biodiversity, regulate hydrological cycles and deliver critical ecological services (Kingsford et al., 2016; Sharma and Naik, 2024). Despite their ecological and socioeconomic importance wetlands face escalating anthropogenic pressures such as agricultural encroachment, urbanisation, mining and pollution (Kundu et al., 2024). These pressures degrade their structure and compromise services such as biogeochemical cycling, flood attenuation and water quality enhancement (Gardner and Finlayson, 2018; Rojas et al., 2021; Salimi et al., 2021). Alarmingly, over 80% of wetlands have been lost or severely degraded in densely populated regions of developing nations (Banda et al., 2023). These global losses are mirrored in Africa, where limited governance and sparse monitoring exacerbate wetland degradation (Davidson et al., 2019; Simaika et al., 2021). For example, South Africa has lost approximately 50% of its original wetland area leaving about 300 000 wetlands remaining. The remaining wetlands make up only 2.4% of the country's total area and only about 11% are well protected (Skowno et al., 2019). This decline underscores the urgent need for improved conservation and management efforts at national and regional levels.

One region exemplifying these challenges is the Waterberg Mountain Complex (WMC) in Limpopo Province, South Africa. The WMC harbours numerous wetlands that provide habitat for diverse species and provide critical ecosystem services (Metwane, 2023). However, wetlands in the region are experiencing accelerated degradation driven by localized stressors from surrounding land use (Ballut-Dajud et al., 2022; Dalu and Chauke, 2020). Given their importance, sustainable management of these freshwater ecosystems was deemed imperative for socioeconomic

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development, poverty alleviation and environmental protection. This priority was underscored by continental initiatives like the Africa Water Vision 2025 and the UN Sustainable Development Goals (SDGs) (Cosgrove and Loucks, 2015; Mangadze et al., 2019).

Effective wetland management and conservation require robust monitoring of ecological health. Therefore, biomonitoring with use of benthic macroinvertebrates has emerged as a powerful tool. These organisms act as bioindicators, reflecting environmental quality through their presence, abundance and sensitivity to disturbances (Mangadze et al., 2016; Matlou et al., 2017). Their relatively long-life cycles, sedentary nature, and varied pollution tolerance (pollution-sensitive Ephemeroptera vs. tolerant Chironomidae) make them ideal for detecting ecosystem changes (Malherbe et al., 2018; Niba and Sakwe, 2018; Addo-Bediako, 2023). Combining biological indicators along with physico-chemical variables allows for more comprehensive monitoring of wetlands (Dube et al., 2017). This integrated approach aligns with growing emphasis on defining ecological thresholds and using monitoring frameworks to guide resource management (Bertule et al., 2018; Dickens et al., 2022; Mabhaudi et al., 2022). However, there is limited empirical understanding of how sediment-bound heavy metals influence macroinvertebrate assemblages in African wetlands. Sediment-bound contaminants like lead, cadmium and arsenic pose long-term risks to aquatic food webs and human health yet monitoring remains sporadic in Africa (Sonone et al., 2021; Yap and Al-Mutairi, 2022). Although several studies have examined water quality and macroinvertebrates independently, few have integrated seasonal and spatial analyses that link sediment-associated contaminants to community structure. (Dahms et al., 2017; Matlou et al., 2017; Dalu and Chauke, 2020; Addo-Bediako et al., 2021). Therefore, the lack of integrated, fine-scale assessments constrains the ability to identify pollution-tolerant taxa and evaluate ecological thresholds for wetland biota under combined chemical and hydrological stressors (Dalu et al., 2020; Sonone et al., 2021).

Against this backdrop, this study investigates how spatio-seasonal patterns in water quality and sediment-bound heavy metals influence macroinvertebrate community composition across wetlands in the Waterberg Mountain Complex. By linking ecological responses to contaminant gradients, the study contributes critical baseline data to guide wetland conservation and biomonitoring in semi-arid African systems.

2. Materials and methods

2.1. Study area

The Waterberg Mountain Complex, locally known as *Thaba Meetse* (translated as "Mountain of Water") is a prominent mountainous massif in northern Limpopo Province, South Africa. Characterized by peaks reaching up to 2,000 meters above sea level and an average elevation of 600 meters, this biodiverse region encompasses a mosaic of ecosystems including grasslands, savannas and critical wetland habitats (Baber et al., 2003). Farming (particularly cattle) and crop production dominate the local economy alongside thriving game farms that support wildlife conservation and ecotourism (Netshipale et al., 2022). Additionally, mining operations for tin, chromium and fluorspar between Mookgopong and Mokopane contribute to the region's economic profile though they pose potential risks to wetland integrity through contamination and habitat fragmentation (Ahiakwo et al., 2018). The Nylsvlei floodplain which is a Ramsar-designated wetland has been extensively studied for its ecological significance (Greenfield, 2007; Jay & Greenfield, 2018; Rowberry et al., 2011; Saddam, 2015), while the broader wetlands across the Waterberg remain under studied. These wetlands are hydrologically dynamic with an average annual rainfall of less than 600 mm concentrated primarily between November and February (Baber et al., 2003; Netshipale et al., 2022; Pool-Stanvliiet, 2014). To address this knowledge gap, 11 wetlands across five game reserves Kaingo (WMC-01 to WMC-04), Lindane (WMC-05 to WMC-07), Jembisa (WMC-08), Leobo (WMC-09) and Syringa Sands (WMC-10 to WMC-11) were selected for a comprehensive assessment. Sampling trips conducted during the early rains (November 2022) and late rains (March 2023) collected water, sediment, and macroinvertebrate specimens to evaluate spatial and seasonal variations in biotic communities and water quality (Fig. 1).

2.2. Physico-chemical, water and sediment sampling

Water quality parameters such as temperature, turbidity, conductivity, total dissolved solids (TDS), salinity and dissolved oxygen (DO) were measured in situ using a calibrated YSI 556™ Multi Probe System (MPS) following standardized protocols for freshwater ecosystems (Méndez-Barroso et al., 2020). Water samples were collected in acid-pretreated (10% HNO_3) polypropylene bottles (100 mL) preserved at -20°C and transported to WaterLab (Pretoria, South Africa) for chemical analysis to minimize post-sampling degradation (USEPA, 2010). Surface sediment samples (0–10 cm depth) were collected using a stainless-steel hand shovel with five subsamples per site homogenized to create a composite sample (Adomat & Grischek, 2021). Sediments were stored in pre-acidified (10% HNO_3) glass bottles frozen at -20°C and transported to WaterLab to prevent microbial activity and metal leaching.

2.3. Water and sediment analyses

All analyses were conducted at WaterLab (Pty) Ltd, an ISO/IEC 17025:2017-accredited facility. Water and sediment samples were oven-dried at 60°C for 24 h, digested with 68% HNO_3 and 40% HCl (3:1 v/v) and filtered through 0.45 μm membrane filters (USEPA Method 3050B; APHA, 2017). Heavy metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) were quantified using inductively coupled plasma-optical emission spectrometry (ICP-OES; PerkinElmer Optima 2100 DV) with concentrations expressed as mg/kg dry weight. Analytical accuracy was verified using certified reference materials (De Bruyn Spectroscopic Solutions 500 MUL20-50STD2) achieving recovery rates of 90–110% (Bervoets & Blust, 2003).

2.4. Benthic macroinvertebrate sampling

Benthic macroinvertebrates were sampled using the kick net sampling technique where three replicates per site were collected by disturbing substrates (vegetation, gravel, sand, mud). Specimens were preserved in 99% ethanol and identified to family level using taxonomic keys from the Field Guide to Freshwater Macroinvertebrates of Southern Africa, supplemented by stereo microscopy (Leica M205C) at the SANBI-National Zoological Gardens.

2.5. Statistical and multivariate analyses

The spatial and temporal variation in water quality and ecological responses were examined across 11 wetland sites in the Waterberg Mountain Complex, a suite of multivariate and univariate statistical techniques was employed using R software (version 4.3.1) (R Core Team, 2020). Paired t-tests were conducted using the *stats* package to assess seasonal differences in physico-chemical parameters between the Early Rain (ER) and Late Rain (LR) periods. For heavy metal analyses, one-way ANOVA was applied using the *car* package to determine if sediment metal concentrations differed significantly between seasons. To assess contamination levels, Geoaccumulation Index (Igeo) and Enrichment Factor (EF) calculations were performed following methods by Muller (1969) and Sutherland (2000), respectively. To assess biodiversity, macroinvertebrate community metrics including Shannon-Wiener Index (H′), Simpson's Diversity Index (1–D), taxa richness and total abundance were calculated. Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) were used to

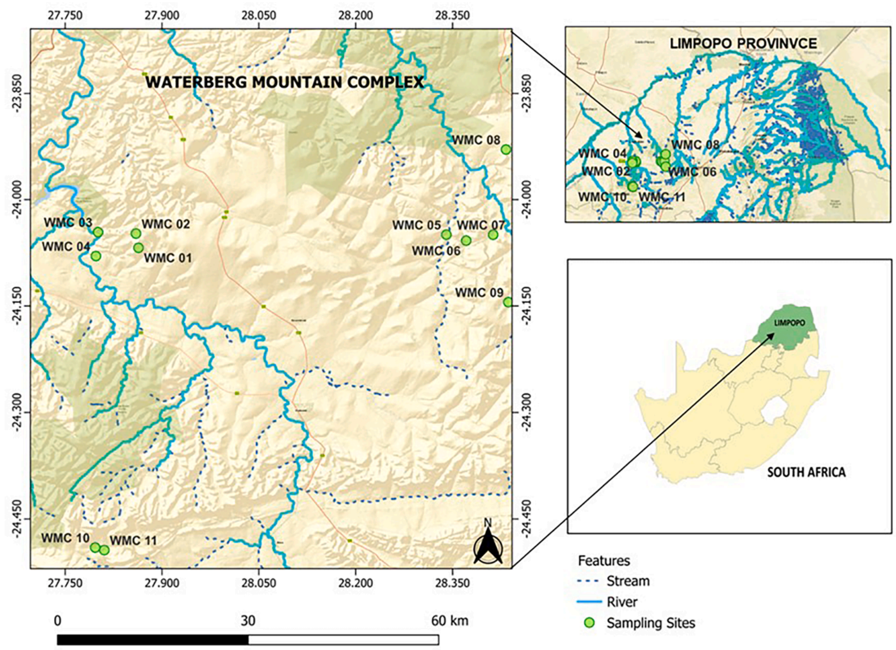

Fig. 1. Location of sampling sites within the Waterberg Mountain Complex, Limpopo Province, South Africa. Green circles indicate the positions of water quality sampling sites (WMC-01 to WMC-11). The inset maps shows the location of the study area within the Limpopo Province and the broader context of South Africa.

explore the influence of physico-chemical and heavy metal gradients on macroinvertebrate community structure.

3. Results

3.1. Physico-chemical parameters

Seasonal comparison of physico-chemical parameters across 11

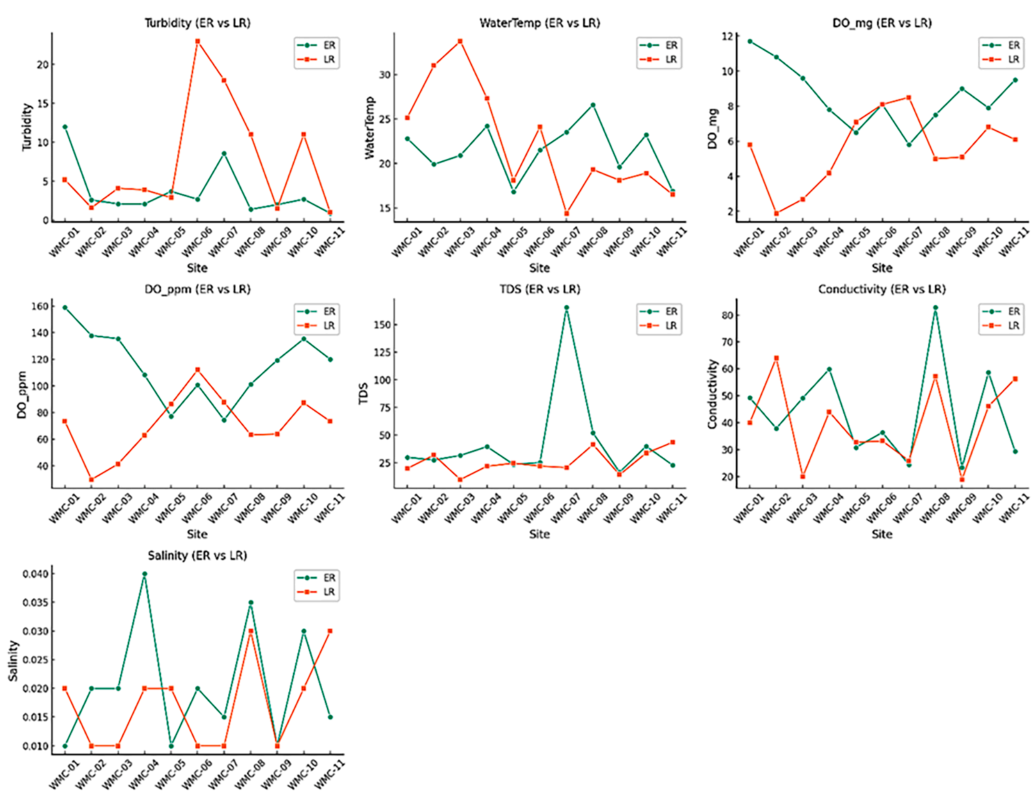

Fig. 2. Seasonal comparison of water quality parameters (Early Rain vs Late Rain) across 11 monitoring sites (WMC-01 to WMC-11). Each subplot shows the temporal variation between Early Rain (ER) and Late Rain (LR) for key physico-chemical parameters: turbidity, water temperature, dissolved oxygen (mg/L and ‰), total dissolved solids (TDS), conductivity, and salinity. ER and LR trends are represented using distinct markers and colours.

wetland sites in the Waterberg Mountain Complex revealed both spatial and temporal variability, particularly between the ER and LR seasons (Fig. 2; Table 1). Visual plots indicated increases in turbidity during LR at sites like WMC-06 and WMC-07 but this trend was not statistically significant (t = -1.72, $p = 0.116$). Similarly, water temperature appeared elevated at certain sites in LR (WMC-03 and WMC-04) however, this change was also not statistically significant (t = -0.48, $p = 0.643$). In contrast, dissolved oxygen (DO) levels showed significant seasonal differences with higher mean concentrations in ER (WMC-01: 11.7 mg/l) dropping markedly in LR (WMC-01: 5.8 mg/l).

DO in mg/l declined significantly (t = 2.89, $p = 0.016$) and percent saturation (‰) followed a similar trend (t = 3.49, $p = 0.006$). Other parameters like TDS and conductivity, statistical tests indicated no significant seasonal difference (TDS: t = 1.31, $p = 0.218$; conductivity: t = 0.73, $p = 0.482$), despite high values at some sites (WMC-07: TDS = 165.4 mg/L during ER). Salinity levels remained low across all sites (< 0.04) with no significant seasonal effect (t = 0.98, $p = 0.351$) affirming the freshwater character of the wetland system. Collectively, only DO saturation showed statistically significant seasonal variability and other physico-chemical variables while spatially heterogeneous did not shift consistently across seasons.

3.2. Water and sediment heavy metal concentrations

Heavy metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) were measured but only Iron (Fe), Manganese (Mn) and Lead (Pb) were detectable (Table 2). The water heavy metal results indicated that Fe concentrations consistently exceeded the Canadian Council of Ministers of the Environment (CCME) guideline value of 0.33 mg/l, particularly during the LR season. Seasonal variations were pronounced with higher mean Fe concentrations during LR (5.02 mg/l) compared to ER (2.37 mg/l). Pb concentrations ranging from 0.002 mg/l at WMC-01 during LR to 0.003 mg/l at WMC-07 & 08 during ER and LR, respectively, but were slightly above the South African Water Guidelines for aquatic ecosystems of ≤ 0.0002 mg/l – 1.2 mg/l.. Mn levels ranging from 0.034 mg/l at WMC- 02 during ER to 0.430 mg/l at WMC-04 during LR. The LR of 0.430 mg/l at WMC-04 was above the target water quality range for Mn according to the South African Water Guidelines for aquatic ecosystems indicating chronic effect. Pb and Mn both indicated significant seasonal increase ($p < 0.05$) from ER to LR.

For the sediment, the same heavy metals as the water where measured and only Mercury was not detected. The seasonal bar plots in Fig. 3 reveal notable patterns in heavy metal concentrations across the 11 monitored sites. Fe exhibited the highest concentrations, averaging 9 000 mg/kg in ER and rising above 15 000 mg/kg in LR, especially at WMC-04 and WMC-08. Zinc (Zn) and Mn also showed elevated levels at sites such as WMC-08 and WMC-07 with Zinc peaking at 30 mg/kg (ER) at WMC-08.

In contrast, Cadmium (Cd) remained below detection limits in most ER samples but showed slight increases in LR, notably 0.04 mg/kg at WMC-08. Arsenic (As) ranged from 0.0 to 1.7 mg/kg with the highest values recorded at 1.68 mg/kg at WMC-02 during LR. Chromium (Cr) presented modest spatial variation remaining well above CCME guidelines (37.3 mg/kg) at all sites between all seasons. Copper (Cu) also presented modest spatial variation but only exceeded CCME guidelines (35.7 mg/kg) at WMC-08 during ER. Nickel (Ni) and Pb followed a similar pattern as Cr and Cu but there are no CCME guidelines for Ni and Pb remained well below the guidelines (35 mg/kg). The highest Ni concentration of 28 mg/kg was at WMC-08 during ER and for Pb was 21 mg/kg at WMC-08 during ER.

A one-way ANOVA was conducted to assess seasonal differences in sediment heavy metal concentrations between ER and LR seasons across 11 monitoring sites (Table 3). Among the heavy metals analysed (excluding mercury), only Cd showed a statistically significant variation between seasons (F = 8.239, p = 0.00946). In contrast, As, Cr, Cu and Fe exhibited no significant seasonal changes.

On a similar trend, Pb, Mn, Ni and Zn showed no statistically significant seasonal variation despite localized fluctuations at certain sites. These findings highlight Cd as the most seasonally responsive pollutant in the study.

Table 1
Results of paired t-tests comparing Early Rain (ER) and Late Rain (LR) seasons across water quality parameters.

Parameter	T-statistic	P-value	Interpretation
Turbidity	-1.72	0.116	No significant seasonal difference
Water Temp	-0.48	0.643	No significant seasonal difference
DO_mg/l	2.89	0.016	Significant seasonal difference (ER > LR)
DO_‰	3.49	0.006	Significant seasonal difference (ER > LR)
TDS	1.31	0.218	No significant seasonal difference
Conductivity	0.73	0.482	No significant seasonal difference
Salinity	0.98	0.351	No significant seasonal difference

Table 2
Water heavy metal concentrations (mg/L) across sites with mean, standard deviation (SD) (Early Rain vs Late Rain), and comparison to CCME guidelines.

Sites	Fe (ER)	Fe (LR)	Pb (ER)	Pb (LR)	Mn (ER)	Mn (LR)
WMC-01	0.53	16.00	<0.001	0.002	0.073	0.045
WMC-02	1.39	1.03	<0.001	<0.001	0.034	0.030
WMC-03	0.225	0.394	<0.001	<0.001	<0.025	0.034
WMC-04	0.766	1.98	<0.001	<0.001	0.090	0.430
WMC-05	0.772	0.757	<0.001	<0.001	0.058	0.035
WMC-06	5.64	1.79	<0.001	<0.001	0.044	<0.025
WMC-07	1.15	22.00	<0.001	0.003	0.051	<0.025
WMC-08	4.36	0.677	0.003	<0.001	0.083	<0.025
WMC-09	0.785	6.86	<0.001	<0.001	0.036	0.154
WMC-10	0.478	1.27	<0.001	<0.001	<0.025	<0.025
WMC-11	2.36	6.91	<0.001	<0.001	<0.025	0.114

Metal	Mean (ER)	SD (ER)	Mean (LR)	SD (LR)	CCME Guideline
Fe	**2.37**	1.89	5.02	6.40	0.33 mg/L
Pb	<0.001	-	0.001	0.002	0.007 mg/L
Mn	0.05	0.02	0.09	0.04	0.18 mg/L

3.3. Igeo and EF

The Geoaccumulation Index (Igeo) was used to evaluate the extent of heavy metal pollution in the sediment across 11 monitoring sites during both the ER and LR seasons. This index was calculated using mean heavy metal concentrations relative to average shale background levels, provides insight into the influence of both natural geochemical variability and potential anthropogenic inputs (Fig. 4). The results revealed that the majority of measured heavy metals including AS, Cu, Fe, Pb, Mn, Ni and Zn exhibited Igeo values well below zero across all sites and both seasons. During the ER season, Cr concentrations yielded values ranging from 0.19 to 0.83, with the highest values recorded at WMC-04 and WMC-01. According to Müller's classification, these values correspond to Class 1–2, indicating unpolluted to moderately polluted conditions. In the LR season, Cr values ranged from −0.11 to 0.82, with a slight decrease at several sites (WMC-08 and WMC-09) and increases at others (WMC-04 and WMC-11). Despite these shifts, most sites remained within Class 1. Overall, the Igeo analysis demonstrates that most of the study area remains geochemically stable and unimpacted by heavy metal pollution, with Cr standing out as a metal of concern due to its deviation from natural baselines at certain hotspots.

Enrichment Factor (EF) analysis revealed site-specific and metal-specific variation in anthropogenic influence across the study area (Fig. 5). Cr exhibited the highest enrichment, with EF values exceeding 25 at WMC-10 and WMC-11 in both seasons, indicating very severe enrichment. Pb also showed elevated EF values, particularly at WMC-11 (EF > 10). Moderate enrichment (EF = 3–5) was observed for Cu, Ni and Zn at WMC-08 and WMC-11. In contrast, Cd, As and Mn generally exhibited EF values < 1. These patterns underscore the persistent

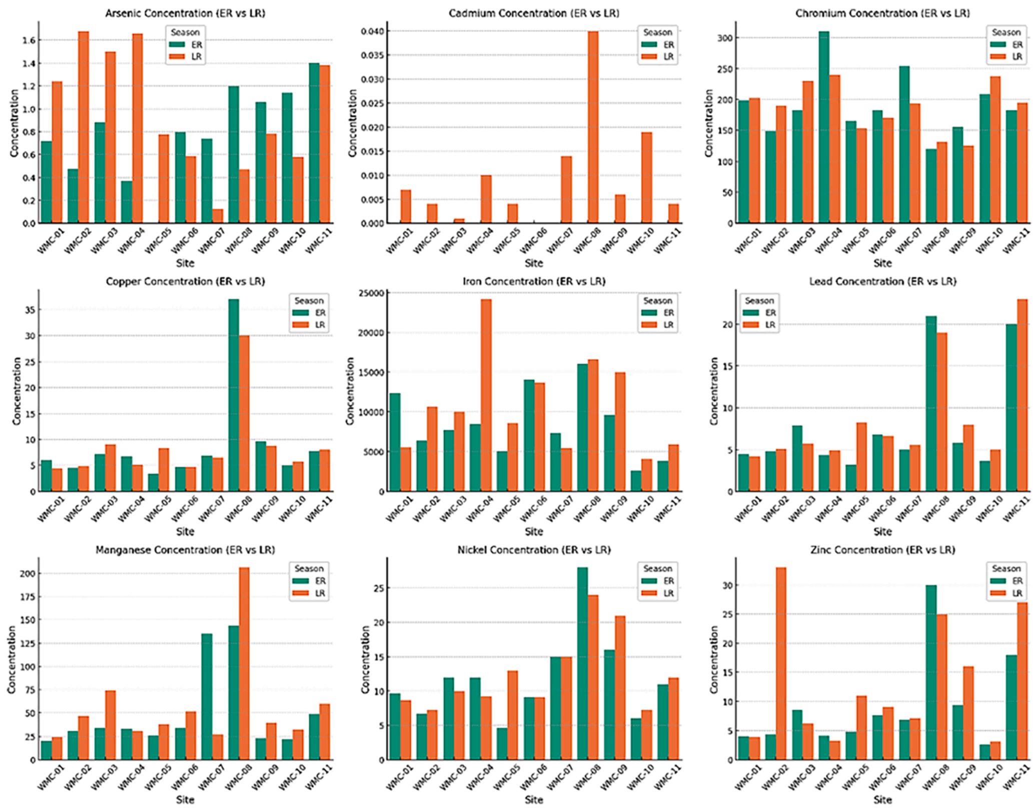

Fig. 3. Seasonal variation in heavy metal concentrations (mg/L) across 11 monitoring sites during Early Rain (ER) and Late Rain (LR) seasons. Bar plots illustrate differences for Arsenic, Cadmium, Chromium, Copper, Iron, Lead, Manganese, Nickel, and Zinc.

Table 3
One-way ANOVA results comparing Early Rain (ER) and Late Rain (LR) seasons for heavy metal concentrations across 11 sites.

Metal	F-statistic	P-value	Significance
Arsenic	0,806	0,37995	Not Significant
Cadmium	8,239	**0,00946**	Significant
Chromium	0,033	0,85671	Not Significant
Copper	0,007	0,9333	Not Significant
Iron	1,141	0,29818	Not Significant
Lead	0,078	0,78304	Not Significant
Manganese	0,124	0,72802	Not Significant
Nickel	0,051	0,82316	Not Significant
Zinc	1,013	0,32628	Not Significant

anthropogenic signature at specific wetlands, particularly WMC-10 and WMC-11.

3.4. Macroinvertebrate diversity

The macroinvertebrate abundance indicates that Diptera (true flies) were the most abundant order across the dataset, particularly dominant at WMC-11 where their count exceeded 1,200 individuals (Supplementary data, Fig. 1). Odonata (dragonflies and damselflies) and Ephemeroptera (mayflies) which are typically sensitive to water quality changes were also abundant at WMC-04 and WMC-11. In contrast, sites such as WMC-01 through WMC-05 exhibited lower overall abundance and reduced representation of Ephemeroptera and Trichoptera. The intermediate representation of Coleoptera (beetles) and Hemiptera (true bugs) across most sites reflects their generalist nature and tolerance to a range of aquatic habitats. The elevated abundances of Trichoptera at sites WMC-10 and WMC-04 were notable, given that this group is generally sensitive to sedimentation and pollution.

Macroinvertebrate diversity assessed using the Shannon-Wiener Index (H′) and Simpson's Diversity Index ($1 - D$), exhibited distinct spatial and seasonal patterns across the 11 monitoring sites (Fig. 6). Shannon index values ranged from 1.2 to 2.9 with notably higher diversity observed during the LR season at most sites. Sites WMC-03, WMC-07, WMC-08, and WMC-09 displayed the highest H′ values (≥ 2.7) during LR. In contrast, ER diversity was substantially lower at several sites (WMC-01, WMC-04 & WMC-05). Simpson's Diversity Index ($1 - D$), which emphasizes dominance structure, also showed consistently higher values in the LR season across all sites with most indices exceeding 0.85. Sites such as WMC-09, WMC-10 and WMC-11 approached the upper diversity limit ($1 - D > 0.95$) during LR. Taxa richness representing the number of distinct macroinvertebrate families per site-season, was highest at WMC-09 to WMC-11 (median >30 taxa). In contrast, WMC-06 exhibited the lowest richness overall, with a median around 10 taxa. Total macroinvertebrate abundance varied by over an order of magnitude among sites. WMC-11 exhibited the highest abundance (peaking >3000 individuals), followed by WMC-04, WMC-09, and WMC-10. The lowest densities were recorded at WMC-01, WMC-05, and WMC-06, sites which also corresponded to lower diversity and richness metrics.

3.5. Physico-chemical CCA

The Canonical Correspondence Analysis (CCA) was conducted to investigate the influence of key physico-chemical parameters on the spatial distribution of macroinvertebrate taxa across monitoring sites. The CCA biplot (Fig. 7) illustrates the strength and direction of environmental gradients and how different taxa and sites respond to these factors. The first two CCA axes explained 29.4% and 18.7% of the total constrained variation, respectively, accounting for a cumulative 48.1% of species-environment relationships. The CCA model was statistically significant ($p = 0.02$, based on 999 permutations), indicating that the observed macroinvertebrate distribution patterns were meaningfully

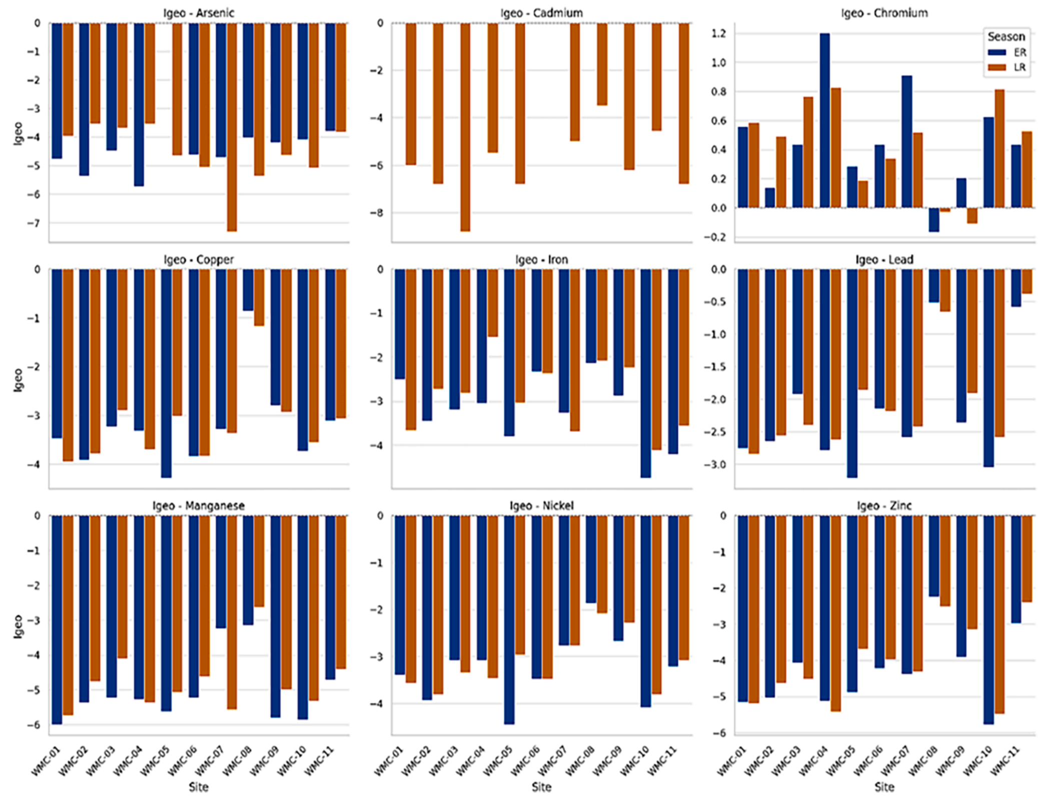

Fig. 4. Bar plots of the Geoaccumulation Index (Igeo) for heavy metals across 11 monitoring sites during Early Rain (ER) and Late Rain (LR) seasons. The dashed line at Igeo = 0 marks the threshold between unpolluted and enriched conditions.

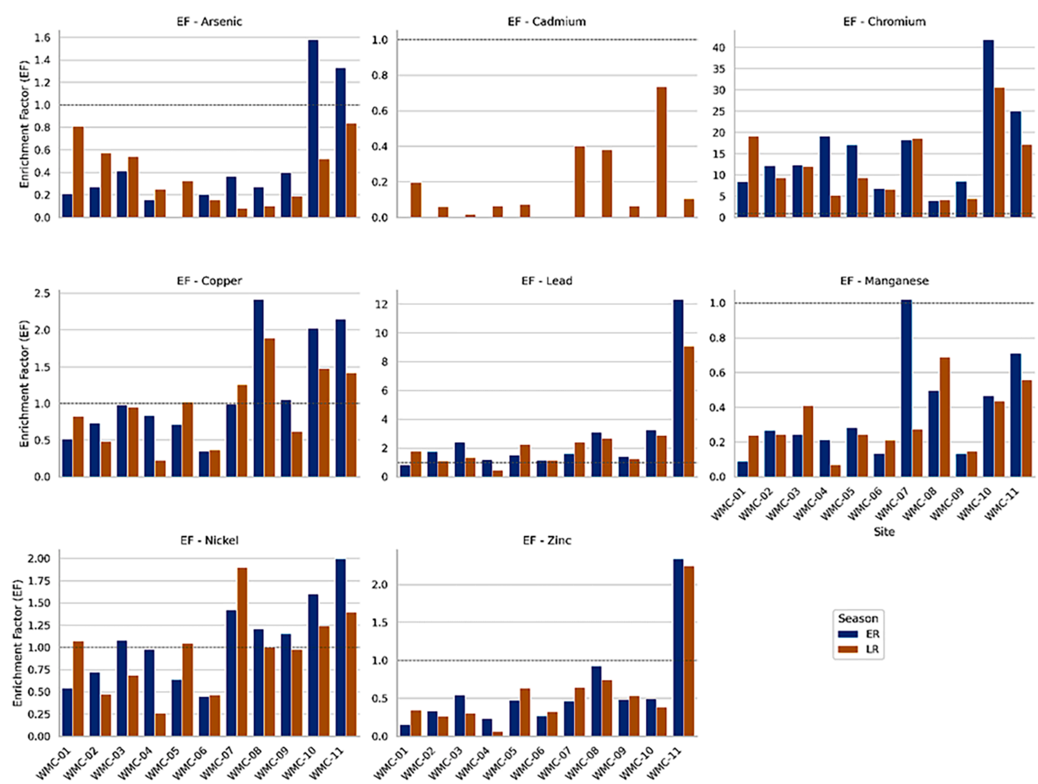

Fig. 5. Enrichment Factor (EF) values for heavy metals in the sediment across 11 monitoring sites during Early Rain (ER, blue) and Late Rain (LR, orange) seasons. Iron (Fe) was used as the reference element, and average shale values served as background concentrations. The dashed line at EF = 1 represents the threshold between natural and anthropogenic enrichment.

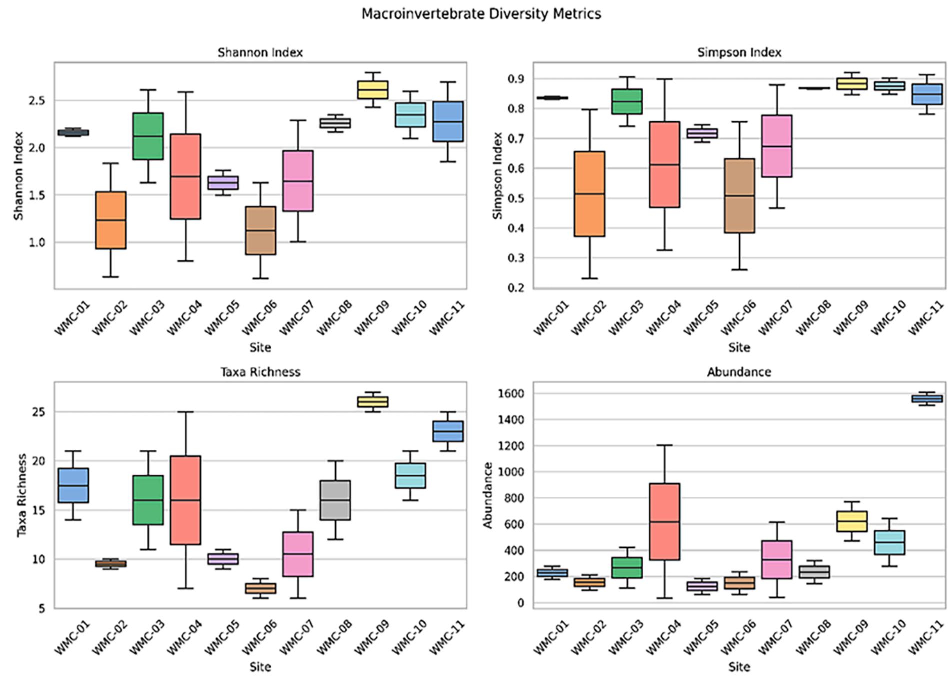

Fig. 6. Comparison of macroinvertebrate diversity indices across wetland monitoring sites using Shannon-Wiener Index (H′), Simpson's Index (1–D), Taxa Richness, and Abundance.

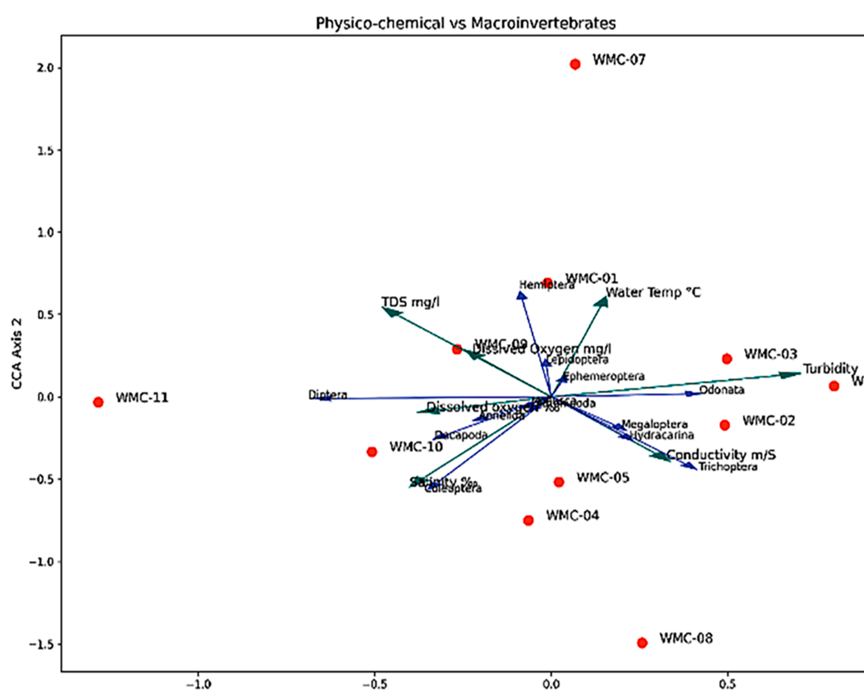

Fig. 7. Canonical Correspondence Analysis (CCA) biplot illustrating the influence of physico-chemical parameters (green arrows) on macroinvertebrate community composition (blue arrows) across wetland sampling sites (orange points). The direction and length of arrows indicate the magnitude and gradient of influence of variables such as turbidity, dissolved oxygen, and conductivity.

associated with variation in environmental conditions. The first axis (CCA Axis 1) largely reflects a gradient associated with increasing turbidity, conductivity, and temperature with WMC-06 and WMC-03 aligning most strongly along this axis. On the other hand, WMC-01 and WMC-07 are positively associated with higher temperatures and dissolved oxygen. The second axis (CCA Axis 2) appears to distinguish sites based on levels of TDS and oxygen saturation, with WMC-09 and WMC-11 positioned toward opposite ends of the gradient.

Taxa exhibited distinct responses to these environmental gradients. For instance, Odonata, Trichoptera and Ephemeroptera which are generally regarded as indicators of good water quality (Deacon, 2020) were closely associated with sites exhibiting higher dissolved oxygen and conductivity. Hemiptera and Hydracarina appeared in close proximity to sites with elevated temperature and turbidity. Conversely, Diptera and Decapoda were located toward the left side of the plot showing a weak association with the main environmental gradients. Overall, the CCA results underscore the pivotal role of turbidity, conductivity, temperature and TDS in shaping macroinvertebrate community structure across the study sites. The strong alignment of certain taxa with specific environmental vectors highlights the diagnostic value of macroinvertebrates as bioindicators.

3.6. Heavy metals RDA

The RDA biplot demonstrates that several metals, notably Zn, Cd and Ni exert strong directional influence on macroinvertebrate community structure (Fig. 8). The first two RDA axes explained a substantial proportion of the constrained variance, with RDA Axis 1 accounting for 38.2% and RDA Axis 2 for 17.6%, yielding a cumulative explanation of 55.8%. A permutation test confirmed the statistical significance of the model ($p = 0.01$, 999 permutations), indicating that the observed patterns were unlikely due to chance. The biplot revealed distinct gradients in species responses to metal contamination. Zn represented by a long arrow aligned with the positive end of RDA Axis 1, showed the strongest gradient, indicating its dominant role in shaping biological assemblages particularly at WMC-11 which was positioned furthest along this axis. The high separation of WMC-11 suggests significant environmental pressure from Zn. Similarly, Cd also showed a strong positive correlation with RDA Axis 1 and was spatially associated with WMC-09 indicating its probable role in influencing the biotic composition at that site.

Macroinvertebrate taxa responded variably to metal gradients. Ephemeroptera, Trichoptera and Odonata which are generally sensitive to pollution, were positioned closer to the origin and in the direction opposite to high metal vectors, suggesting these taxa do thrive in less contaminated environments. Conversely, Diptera and Hemiptera showed closer alignment with moderate levels of metals such as Cr, As and Ni indicating a broader tolerance to contaminated conditions. Coleoptera displayed a mixed association aligning moderately with As and Ni potentially reflecting species-specific adaptability or site-specific responses. Overall, the RDA analysis highlights the differential impact of specific heavy metals on macroinvertebrate distribution, with Zn and Cd exerting the most pronounced influence on community composition.

4. Discussion

This pilot study provides critical insights into the spatial and seasonal variability of physico-chemical water quality in the wetlands of the Waterberg Mountain Complex (WMC), a semi-arid and ecologically sensitive region in South Africa that remains largely understudied.

4.1. Spatial and temporal dynamics of physico-chemical parameters

The results confirm that DO was the only parameter exhibiting significant seasonal change, with higher values during the ER season and marked declines in the LR period. This counterintuitive pattern of higher DO in the warmer, peak-rainfall period than in the cooler season mirrors observations from other semi-arid freshwater ecosystems (Lacson et al., 2019; Nelson et al., 2017; Schliemann et al., 2021), where elevated temperatures and increased biological activity during high-flow events can paradoxically drive hyperoxic conditions despite the theoretical expectation of reduced oxygen solubility in warmer water. The pronounced drop in DO levels in LR, especially at sites like WMC-01 and WMC-04 poses risks to oxygen-sensitive macroinvertebrates and may signal eutrophication processes influenced by land-use activities and runoff (Bonacina et al., 2023; Corry et al., 2012; Dalu et al., 2019). However, in highly seasonal rivers and wetlands, communities often include more tolerant groups (certain Diptera and Oligochaeta) that can withstand episodic hypoxia, meaning that low DO is likely to restructure assemblages rather than cause uniform declines across all taxa. In contrast, other in situ parameters such as turbidity, temperature, conductivity, TDS, and salinity, showed no consistent seasonal shift, but did vary markedly among sites (Gachie, 2020; Schliemann et al., 2021). For

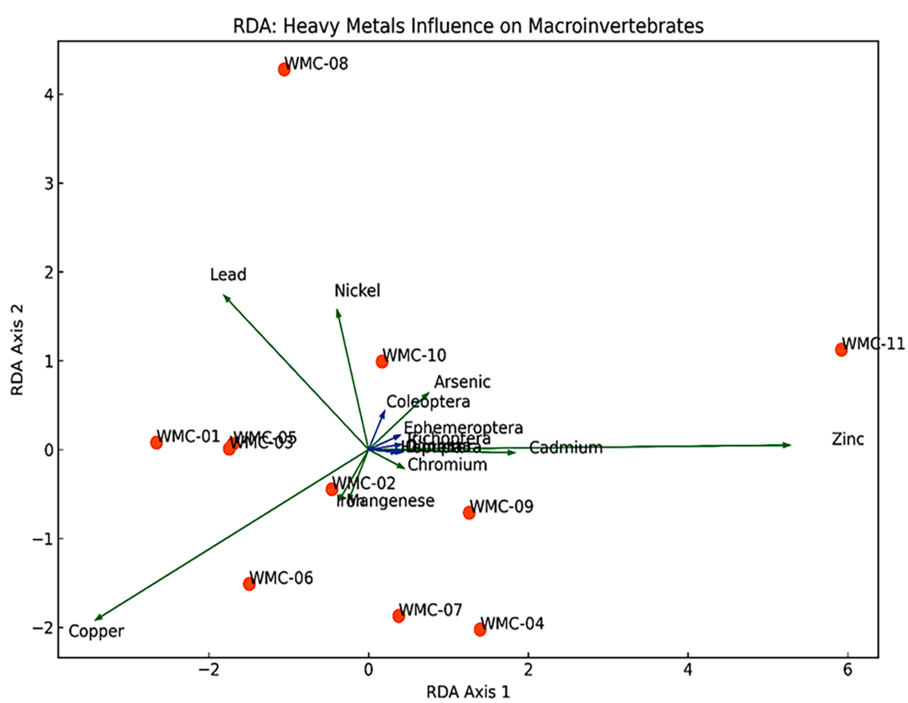

Fig. 8. Redundancy Analysis (RDA) biplot illustrating the influence of sediment heavy metal concentrations (green arrows) on macroinvertebrate community structure (blue arrows) across wetland sampling sites (orange circles) in the Waterberg Mountain Complex. Arrows indicate the strength and direction of associations. Sites positioned along the direction of certain heavy metals (e.g., Chromium, Iron) show corresponding biological patterns, revealing environmentally driven gradients in community composition.

example, elevated turbidity at WMC-06 and WMC-07 during LR may be attributed to sediment-laden surface runoff (Xu et al., 2022), whereas higher conductivity and TDS at WMC-04 and WMC-08 suggest solute enrichment from surrounding agricultural zones, echoing patterns observed in similar African systems (Kibena et al., 2014). Throughout the study, salinity remained uniformly low, confirming that these systems. Even so, small fluctuations in ionic composition could still impose osmotic stress on aquatic fauna (Herbert et al., 2015). Overall, given the increasing water scarcity in semi-arid regions like Limpopo province, understanding baseline water quality and seasonal dynamics is crucial for wetland conservation. This study highlights the vulnerability of these wetlands to climatic and anthropogenic disturbances and establishes a baseline for future hydrological and ecological assessments.

4.2. Heavy metal distribution and pollution indicators

The Geoaccumulation Index (Igeo) and Enrichment Factor (EF) analyses provided complementary evidence on both the degree of contamination and the probable sources of heavy metals in the WMC wetlands. Igeo values showed that iron (Fe), chromium (Cr), and cadmium (Cd) were the most ecologically relevant contaminants, though their risk profiles differed. Chromium exhibited particularly elevated Igeo values at WMC-03 and WMC-04, consistent with localized anthropogenic inputs such as agrochemical residues, soil amendments, or intermittent wastewater discharges (McClain and Maher, 2016; Ndlovu, 2023; Oruko et al., 2021). These concentrations approach or exceed sediment quality guideline thresholds, suggesting potential sublethal or chronic toxicity to benthic macroinvertebrates and other sediment-associated biota. Although cadmium concentrations were generally low, several late-rain samples displayed Igeo values indicative of moderate contamination, important given Cd's high mobility, strong bioaccumulation potential, and low-effect thresholds in aquatic organisms (Edokpayi et al., 2017). EF values helped clarify whether the observed metals predominantly reflected geological background or human influence. Metals such as Cr and Pb exhibited higher EF values at WMC-11 and WMC-10, categorizing them as severely enriched and strongly indicative of anthropogenic sources (Muller, 1969; Curtis et al., 2024). These results align with land-use patterns that include smallholder agriculture, informal settlements, and road-adjacent runoff. In contrast, Mn and Fe showed EF values, consistent with derivation from parent geology and natural sediment weathering (McClain and Maher, 2016). Moderate enrichment (EF 3–5) of Cu, Ni, and Zn at WMC-08 and WMC-11 likely reflects diffuse pollution from mixed land uses, including livestock activity, fertilizer inputs, and low-intensity peri-urban development (Sutherland, 2000).

4.3. Macroinvertebrate community structure and diversity

Macroinvertebrate assemblages clearly reflected the water quality gradients. Overall diversity (Shannon, Simpson indices), taxa richness and abundance were significantly higher during LR, suggesting that seasonal flooding created new habitat and resources for colonization. This pattern was consistent with the intermediate disturbance hypothesis and with other African wetland studies (Bird et al., 2013; Dalu and Chauke, 2020; Gleason et al., 2018), where flood pulses reset communities and open niches, allowing a surge in taxa (Dong et al., 2021; Mathers et al., 2023; Matlou et al., 2017). For example, generalist and fast-reproducing taxa likely exploited the brief abundance of detritus and algae after rains. Aquatic macroinvertebrates in semi-arid regions may synchronize emergence, reproduction and recruitment with early-rain inflows and elevated temperatures, which may accelerate growth rates and enhance propagule availability (Steward et al., 2022). Thus, the LR increase in diversity reflects not only disturbance-driven habitat renewal but also seasonal life-history pulses typical of warm-season assemblages . The taxonomic composition also varied with pollution gradients. Site WMC-11, which had the highest turbidity, salinity, conductivity and heavy metal levels (especially Fe–Cr–Cd), was overwhelmingly dominated by Diptera. The chironomid midges and mosquito larvae were particularly found in high numbers. High Diptera abundance (>1200 individuals at WMC-11) was typical of eutrophic or organically enriched waters, because many dipterans (like Chironomidae) are tolerant detritivores that thrive under low oxygen and high nutrient conditions (Odume et al., 2016). This matched a study by Courtney et al. (2017), who found that Diptera proliferation was a hallmark of hypoxic and polluted wetlands. In sharp contrast, wetlands with higher DO and lower metal loads (WMC-08, WMC-10, WMC-04) supported richer assemblages of pollution-sensitive taxa: Ephemeroptera (mayflies), Trichoptera (caddisflies) and Odonata (dragonflies/damselflies) were most abundant at those sites. These insects require clean, well-oxygenated water and complex habitat (riparian vegetation or stable substrates) and are well-known bioindicators of good water quality (Mabidi et al., 2017; Masese et al., 2023a; Nhiwatiwa et al., 2017). Their presence suggested that WMC-08, WMC-10 and WMC-04 currently functioned as relatively healthy wetlands. Finer taxonomic and functional nuances were observed, for example, predatory Odonata nymphs (Coenagrionidae, Libellulidae) occurred mainly where DO was high and sedimentation low. Filter-feeding Hydracarina (water mites) and certain Hemiptera (water bugs) were more often associated with mineral-rich or slightly impaired waters, consistent with their wider tolerance (Múrria et al., 2020; Zawal et al., 2017). In disturbed wetlands, higher abundance of collector-gatherer Diptera and absence of scrapers or sensitive detritivores, indicating shifts in trophic structure was noted. Overall, the macroinvertebrate patterns aligned with bioassessment studies from South Africa, Kenya and Zimbabwe (Banda et al., 2023; Dalu et al., 2022; Masese et al., 2023b; Ndichu et al., 2023): tolerant, opportunistic taxa dominate polluted sites, whereas diverse EPT assemblages indicate intact wetland functioning. These results reinforce the utility of macroinvertebrates as indicators of wetland health in the region.

4.4. Multivariate analysis of environmental drivers

Multivariate analyses (CCA and RDA) confirmed that key physicochemical gradients and heavy metal contaminants strongly structure macroinvertebrate communities in the Waterberg Mountain Complex (WMC), Limpopo Province. The canonical correspondence analysis (CCA) separated sites along a continuum from clear, oxygen-rich wetlands to turbid, metal-enriched ones. In the CCA biplot, Trichoptera and Hydracarina aligned strongly with higher conductivity, reflecting tolerance of mineral-rich or ionically stressed waters, while Ephemeroptera and Hemiptera associated with high dissolved oxygen and moderate temperatures. This pattern suggests that mayflies and true bugs depend on well-oxygenated conditions (Bonacina et al., 2023) whereas some caddisflies and mites tolerate ionic stress (Múrria et al., 2020; Zawal et al., 2017). The position of WMC-06 near the turbidity variable reflects disturbed habitat conditions, possibly due to sediment runoff and this influenced community composition toward more tolerant taxa like Odonata (Vilenica et al., 2024). Conversely, WMC11 was and outlier on the negative side of CCA Axis 1, reflecting distinct environmental conditions. During the LR season, WMC-11 exhibited elevated turbidity, salinity, conductivity, and heavy metal concentrations, along with reduced dissolved oxygen conditions indicative of increased runoff, erosion, and potential pollution inputs. These degraded conditions coincided with a notably high abundance of Diptera (>1,200 individuals), a group known for its tolerance to stress and pollution (Docile et al., 2015; Odume et al., 2016). The combination of impaired physico-chemical conditions and Diptera dominance supports WMC11's separation on the ordination, signalling pronounced ecological variation relative to other sites. Overall, the CCA biplot demonstrates that physico-chemical variables play a critical role in structuring macroinvertebrate assemblages, highlighting the importance of maintaining optimal water quality for biodiversity conservation in wetlands (Dallas and Rivers-Moore, 2014).

The Redundancy Analysis (RDA) biplot further partitioned the influence of heavy metal contaminants on macroinvertebrate community composition across different wetland monitoring sites in the Waterberg Mountain Complex, South Africa. Sites such as WMC-09 and WMC-11 were strongly associated with elevated concentrations of Cd and Zn respectively, suggesting metal enrichment likely due to anthropogenic inputs or geogenic sources. The placement of WMC-06 near the Cu and WMC-01 near Pb suggests site-specific contamination potentially impacting macroinvertebrate diversity and community structure (Ryan et al., 2019). A study by (Dalu et al., 2020) at the Nylsvley floodplain also revealed that metal concentration differences could be highly site-specific and likely linked to additional factors such as anthropogenic point sources and local bedrock. Notably, WMC-04 and WMC-08 were positioned in extreme quadrants of the RDA, yet both showed minimal influence from measured heavy metals. This suggests that despite their distinct environmental profiles, they may represent relatively undisturbed or reference-like sites with low direct metal contamination. Macroinvertebrate groups like Ephemeroptera and Trichoptera are positioned away from the variables for Cd and Cu, indicating their sensitivity to these metals. (Bere et al., 2016), found that trace metals accumulate differently in aquatic macroinvertebrates and can significantly reduce sensitive taxa abundance. Conversely, taxa like Diptera and Odonata which are more resilient to pollution, are located closer to or within the influence zones of multiple metals such as Cr, As and Fe, consistent with findings by (Ouma et al., 2022) which demonstrated that tolerant taxa can persist in metal-contaminated waters. The congruence of CCA and RDA highlights the utility of macroinvertebrates as bioindicators for metal pollution in wetland systems and underscores the ecological risk posed by heavy metal contamination.

Integrative Management and Conservation Implications These findings established a vital seasonal baseline for wetland management in the Waterberg region, Limpopo Province, South Africa. Clear links between water quality, contaminants and macroinvertebrate communities highlight the need to manage both hydrological regimes and pollutant sources. Degraded sites may require targeted remediation, while intact wetlands should be prioritized for conservation. Multi-season biomonitoring of water chemistry and invertebrates is essential, as encouraged by South Africa's National Wetland Monitoring Programme and SDG 6.5.1 commitments. The ecological indices and thresholds provided here support adaptive management in line with the National Water Act, National Biodiversity Framework, and Wetland Management Strategy, enabling informed decisions to protect freshwater ecosystem integrity amid increasing environmental pressures.

5. Conclusion

This pilot study contributes novel insights into the ecological functioning and environmental pressures facing wetlands in the Waterberg Mountain Complex, Limpopo Province, South Africa, a semi-arid and hydrologically sensitive landscape. By integrating physico-chemical assessment, heavy metal analysis, and macroinvertebrate biomonitoring, the study demonstrates how multiple stressors shaped wetland ecological condition. The results highlighted the diagnostic value of macroinvertebrates as indicators not only of general water quality but also of contaminant exposure, reinforcing their relevance for wetland health assessments across semi-arid systems. A key contribution of this work lies in establishing a baseline ecological dataset for a region where long-term monitoring programs are scarce. The multivariate patterns captured here illustrated how semi-arid wetlands responded to interacting natural and anthropogenic pressures and underscored the need for conservation strategies that account for seasonal variability and localised impacts. These insights have direct management relevance: safeguarding high-condition wetlands, improving land-use practices in vulnerable sub-catchments, and strengthening pollution controls. This work will be essential for maintaining biodiversity and sustaining ecosystem services, including water security for rural communities. Furthermore, the findings emphasised the importance of continuous, long-term biomonitoring to track ecological responses to climatic shifts, land-use change, and increasing pressure on water resources. Future studies should expand taxonomic resolution, incorporate functional trait approaches, and evaluate sediment–water interactions to refine our understanding of stressor pathways. As an early but integrative assessment, this study demonstrates the utility of macroinvertebrate-based indices and multivariate tools for guiding evidence-based wetland policy and management in Africa's semi-arid landscapes, offering a foundation for more comprehensive conservation planning in the Waterberg region.

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CRediT authorship contribution statement

Katlego S. Matlou: Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Abe Addo-Bediako:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization. **Kwabena K. Ayisi:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Monica Mwale:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

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Supplementary materials

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