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Macroinvertebrate diversity in relation to limnochemistry in an Austral semi–arid transboundary aquifer region pan system

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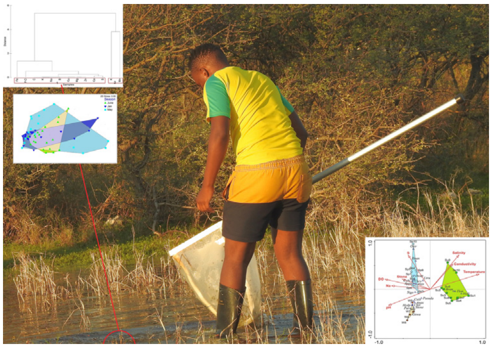
HIGHLIGHTS

- All metals and nutrients were significant seasonal with the exception for P and K concentrations.
- Toxic risk index values indicated low toxic risk across all seasons.
- 41 macroinvertebrates species belonging to 7 orders were identified.
- First two CCA axes accounted for 76.1 % of the total explained fitted cumulative variance.
- Relationships between macroinvertebrates and environment are crucial in understanding ecosystem functioning.

GRAPHICAL ABSTRACT



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ABSTRACT

Pan–wetland systems are one of the world's essential and productive ecosystems and are considered important, unique and complex ecosystems. Anthropogenic activities around the temporary pans in the Khakhea Bray Transboundary Aquifer region are increasingly becoming a big issue of concern as this may affect pan biodiversity. The study specifically aimed to investigate spatial and temporal distributions of metal and nutrient concentrations within the pans in relation to land use, identify potential pollution sources in this water–scarce region, and assess macroinvertebrate diversity and distribution in relation to pan limnochemistry using a combination of multivariate analyses from 10 pans across three seasons. Environmental and anthropogenic variables influence water quality and the distribution of metals concentration in Khakhea–Bray pan systems. Anthropogenic activities such as animal grazing, infrastructure degradation, water withdrawal and littering have resulted in poor water quality within temporary pans, which may strongly influence macroinvertebrate diversity and distribution. Forty–one macroinvertebrate species from 5 insect orders (i.e., Coleoptera, Hemiptera, Odonata, Ephemeroptera, Diptera), Crustacea and Mollusca were identified. Significant differences were observed across the seasons for macroinvertebrate taxa, with high and low species richness being observed in autumn and winter, respectively. 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. Therefore, understanding the relationships between

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macroinvertebrates and their environment is crucial in understanding how the ecosystem taxa are structured and is vital for informing conservation managers on how to properly manage and protect these systems from further degradation.

1. Introduction

Pan wetland systems (hereafter referred to as “pans”) are common in arid zone basins (De Klerk et al., 2016). These systems are generally shallow, variable in size, and typically well mixed by wind (Goudie and Thomas, 1985). Characterised by closed drainage basins which accumulate rainwater inflows and- often above the groundwater table, pans are subject to seasonal and/or perennial surface water inundation (Shaw and Thomas, 1989). These systems exhibit considerable variability due to changes in characteristics such as origin, geology, size, morphology, hydroperiod, and the relative importance of surface and groundwater inputs (De Klerk et al., 2016). Pan systems are productive aquatic ecosystems (Verones et al., 2013), that occupy approximately 7.6 % of the earth's surface. They are considered complex ecosystems due to the variety of their unique terrestrial and aquatic conditions (Clarkson et al., 2013). Pans provide a variety of crucial ecological functions and services, ranging from flood protection to groundwater recharge and discharge, carbon storage, water quality maintenance and soil components (Barbier et al., 1997). They provide habitats for diverse flora and fauna which aid in tourism potential in properly managed systems (Helson, 2012; Nhiwatiwa et al., 2017; Kochi et al., 2020; Dalu and Wasserman, 2022). Despite their significance, however, these ecosystems are often compromised by anthropogenic impacts such as pollution and habitat degradation due to developments (Dalu et al., 2017a, 2017b; Dalu and Wasserman, 2022). Anthropogenic effects on temporary pans are, therefore, a major concern (Riato et al., 2018), as they affect pan biodiversity (Habib and Yousuf, 2015).

In temporary pan ecosystems aquatic macroinvertebrate community dynamics are complex and regulated by the hydroperiod (Bird et al., 2019). The hydroperiod or inundation period can be highly variable, resulting in varying frequency and duration of the dry phases between inundation events (Waterkeyn et al., 2008). The aquatic faunal groups successfully exploiting these ecosystems have therefore evolved mechanisms to deal with these fluctuating environments. Aquatic macroinvertebrates that exploit temporary pan ecosystems are comprised of both wholly aquatic organisms which emerge from dormant cysts in the sediment and colonising semi–aquatic invertebrates with mobile adult life stages (Wasserman et al., 2018). The wholly–aquatic community are typically dominated by crustaceans and molluscs while the semi–aquatic community is dominated by insects, most of which are predatory and utilise these habitats for foraging in the benthic environment and the water–column (Batzer and Boix, 2016; Wasserman et al., 2016b). While these organisms all typically respond to water quality changes, their sensitivity levels vary considerably (Rasifudi et al., 2018). Macroinvertebrates are no exception and have been widely used as bioindicators in assessing aquatic ecosystem health (Mathers and Wood, 2016; Haggag et al., 2018; Mangadze et al., 2019).

Aquatic macroinvertebrate communities are comprised of a variety of taxa with different tolerance levels to pollution (Gerber and Gabriel, 2002; Dallas and Day, 2004; Wolmarans et al., 2014). While this has been well explored in permanent water systems, these dynamics have been less explored in endorheic temporary wetlands. The physicochemical conditions of pan water and sediments are intrinsically intertwined, making it impossible to ignore sediment flora and fauna dynamics if one is to fully understand these aquatic habitats (Bulte and Van Soest, 2001). For example, natural physicochemical dynamics are important in determining pan sediment characteristics, with implications for overlying water (Hoy, 2001; Adesuyi et al., 2016). This is particularly important with regard to runoff, where catchment–level activities can be magnified in pan sediments (Hoy, 2001; Zhang et al., 2016). Sediment can also act as a reservoir for heavy metals, nutrients, and organochlorine pesticides that are released into the water column before entering the food chain (Helson, 2012). It therefore, plays an important direct and indirect role in various biotic and abiotic processes in pans (Zhang et al., 2016).

While metals in aquatic ecosystems can be produced naturally through the slow leaching of soil and rocks (Dalu et al., 2012), human activities such as sewage discharge can increase metals and contribute heavily to environmental and animal health deterioration (Human et al., 2018). Metal contamination of aquatic ecosystems is a burgeoning field, yet little work has been directed at small endorheic temporary pan systems (Gaur et al., 2005). Unlike other lentic systems, metals in pan ecosystems have limited avenues out of the system, as these systems are internally drained i.e., endorheic (Shibambu, 2016).

In South Africa, pans occupy an estimated 2.4 % of the total surface area. and they present several benefits to society. These include resources for local communities, habitat for pan–dependent species, water purification, and flood control (Jogo and Hassan, 2010; Macfarlane, 2016). Pans are among the most vulnerable and threatened ecosystems in South Africa, given the ongoing degradation and poor management of these systems (Van Deventer et al., 2020). It is estimated that more than half of South Africa's pans have been destroyed, and most of the remaining pans are under threat, with only a few pans being appropriately protected (Macfarlane, 2016; Mpakairi et al., 2022a). Here we investigate aquatic macroinvertebrate dynamics in relation to physico–chemical characteristics across a pan network in an understudied arid Austral region. The study specifically aimed to (a) investigate temporal and spatial distributions of metals and nutrient concentrations within pans in relationships to land use, (b) identify potential pollution sources of the pans in this water scarce region, and (c) assess macroinvertebrate diversity and distribution in relation to pan limnochemistry. It was hypothesised that (i) higher metal and nutrient concentrations would occur during the winter due to low water levels as the pans dry out and, therefore, resulting in increased deposition of metals and nutrients within the sediment, (ii) pans that are closer to anthropogenic activities (i.e., distance to kraal, households and latrines) would have more elevated metal levels and nutrient concentrations as these could be potential sources of enrichment within pans, and (iii) macroinvertebrate abundance should decrease during winter due to reduced inundation and increased concentration of ions and nutrients.

2. Materials and methods

2.1. Study area

The study was conducted in the Khakhea–Bray Transboundary Aquifer region, located on the borders of Botswana and South Africa, along the seasonal Molopo River (Fig. 1). The Khakhea–Bray is characterised by Campbell–Rand dolomites outcrops and is about 29,700 km^2 in size. The climatic of Khakhea–Bray Transboundary Aquifer region is mostly semi–arid, with low annual rainfall received during the wet season (approximately 376 mm per annum), and high evapotranspiration rates of 2050–2250 mm per annum (Murray et al., 2006; Mpakairi et al., 2022a, 2022b). The flat landscape and dolomitic geology play host to a considerable number of shallow pan wetlands that develop during the rainy season, primarily through direct rainfall into the network of small endorheic systems. These pan systems represent the only surface water for much of the year and are mainly used for human consumption and for agricultural activities such as livestock grazing, and cultivation (Eamus et al., 2016). Thus, these wetlands are a crucial resource, contributing to ecosystem integrity and many ecosystem services. The study was conducted in winter (June 2021), summer (January 2022), and autumn (May 2022). While ten pan

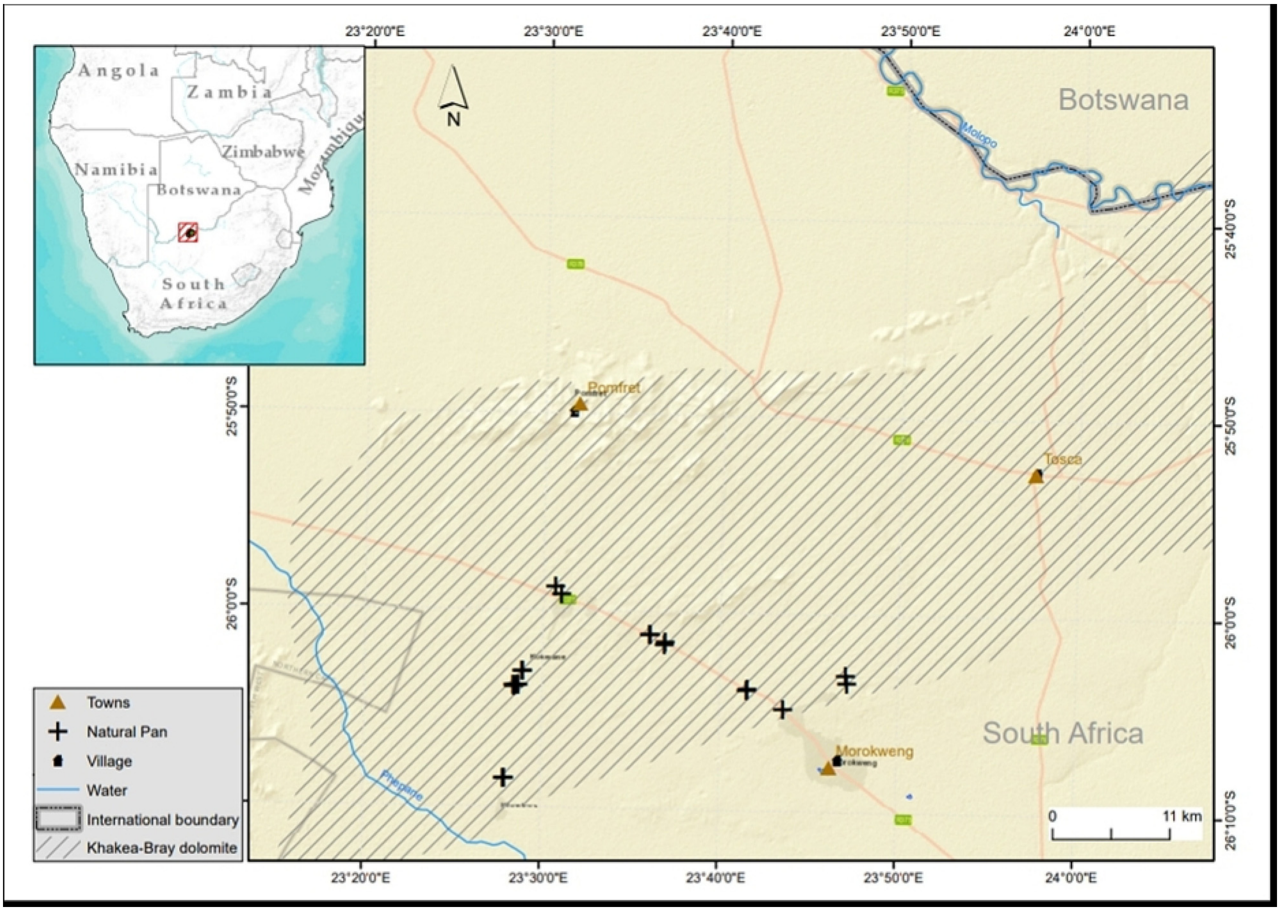

Fig. 1. Map showing the study sites within the Khakhea–Bray Transboundary Aquifer pan region, South Africa.

systems were selected across the entire Khakhea–Bray dolomite pan system area, pans 5, 10 and 8 were inundated during winter, summer, and autumn, respectively.

2.2. Environmental variables

2.2.1. Water chemistry

At each pan, conductivity ($\mu S\ cm^{-1}$), salinity (ppm), pH, water temperature (°C), dissolved oxygen (DO) ($mg\ L^{-1}$) and total dissolved solids (TDS) ($mg\ L^{-1}$) were measured in situ using a portable handheld multiparameter AquaRead probe (Model AP–700 and AP–800, AquaRead Ltd., UK) from three different locations across the pan system (i.e., mean depth 0.4 m). Water samples ($n = 2$) were collected randomly from each pan using a 250 mL container per season for nutrients analyses Ammonium (NH_4^+) with a medium range of 0–10 $mg\ L^{-1}$ and a ± 0.5 $mg\ L^{-1}$ accuracy, and phosphate (PO_4^{3-}) with a high range of 0–30 $mg\ L^{-1}$ and ± 1 $mg\ L^{-1}$ accuracy were measured using the HI 83203 multiparameter photometer (Hanna Instruments Inc., Rhode Island). Water samples were kept on ice in a cooler box and analysed within 8 h of collection. The distance to the nearest kraal (DtK), household (DtH) and pit latrine/toilet (DtL) was measured in metres (m) for each pan using Google Earth Imagery.

2.2.2. Sediment chemistry

Sediment samples ($n = 2$) were randomly collected from each of the ten study pans per each sampling event. Sediments were collected using a plastic hand shovel, placed into new labelled polyethylene Ziplock bags, stored on ice in a cooler box, and transported to the laboratory for further analysis. In the laboratory, the sediment samples were oven dried at 60 °C for 72 h before disaggregation in a porcelain mortar and sieved (mesh size 0.05 mm) to remove plant roots and other debris. Thereafter, the sediment samples were taken for analysis at BEMLAB, a South African National Accreditation System (SANAS) certified laboratory. The following were analysed: (i) *nutrients*; nitrogen (N) and phosphorus (P) using a SEAL Auto Analyzer, (ii) *cation elements*; potassium (K), calcium (Ca), boron (B), magnesium (Mg), sodium (Na) using the acid digestion method, with the metal content being determined using an ICP–OES optical emission spectrometer (Varian, Mulgrave, Australia) and (iii) *heavy metals*; iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) analyses was measured using an ICP–OES optical emission spectrometer (Varian, Mulgrave, Australia).

2.3. Sediment contamination assessment

The Buat-Menard and Chesselet (1979) formula was used to calculate the enrichment factor, which is a measure of the concentration of a certain metal in the sediment, relative to the natural background concentration. Six categories are recognised: <1 background concentration, 1–2 depletions to minimal enrichment, 2–5 moderate enrichment, 5–20 significant enrichment (hereafter referred to as high enrichment), 20–40 very high enrichment and >40 extremely high enrichment (Sutherland, 2000).

The pollution load index (PLI) derived from Tomlinson et al. (1980) allows for the standardization, with a high degree of accuracy, of metal concentrations found in the pan sediment at each pan. The pollution load index value of >1 indicates the system is polluted and <1 indicates no pollution. The toxic risk index (TRI) based on the threshold effect level (TEL), which is the measure of concentrations below which adverse effects for sediment–dwelling organisms would be infrequently expected, and the probable effect level (PEL), which is the chemical concentrations above which adverse effects are likely to occur frequently, effects for the toxic risk assessment were adapted for this study (Zhang et al., 2016). The classification of the TRI is: ≤5 – no toxic risk, >5 to ≤10 – low toxic risk, >10 to ≤15 –

moderate toxic risk, >15 to ≤20 – considerable toxic risk and >20 – very high toxic risk (Zhang et al., 2016).

To evaluate possible environmental health impact consequences of the studied metals, comparisons were made between the measured concentrations and the Canadian Sediment Quality Guidelines (SQGs; MacDonald et al., 2000) to determine the degree to which sediment–bound contaminants might adversely affect the organisms living in or near sediments (Skordas et al., 2015). We assessed two SQGs: TEL and PEL. These guidelines include safe levels not only for metals but also for nutrients i.e., the lowest effect level (LEL) indicating the contamination level which does not affect the majority of sediment–dwelling organisms, and the severe effect level (SEL) that likely affects the health of sediment–dwelling organisms (Persaud et al., 1993).

2.4. Macroinvertebrate sampling

At each pan and season, macroinvertebrates were sampled using a standard SASS net (30 × 30 cm aluminum rim, 1 mm mesh size, attached to a 1.5 m handle). The net was submerged in the water, and macroinvertebrates were collected by sweeping a demarcated 10 m transect. The macroinvertebrates were then put into 500 mL plastic containers, following the removal of organic matter and stones and preserved in 70 % ethanol. Macroinvertebrates were counted and identified to the lowest possible taxonomic level in the laboratory using a dissecting Nikon DS–L3 camera head microscope using identification keys by Fry (2022), with the relative percentage abundances calculated for each taxa. Common macroinvertebrate metrics were computed to assess the environmental integrity among the three seasons. These included % EPT abundance (Ephemeroptera, Plecoptera and Trichoptera), % ETOC abundance (Ephemeroptera, Trichoptera, Odonata and Coleoptera), % Diptera abundance, % Chironomidae + Oligocheata, species evenness, taxa richness and Shannon–Wiener diversity index.

2.5. Data analysis

Prior to multivariate analysis, sediment metal, nutrient concentrations and water physico–chemical variables (with the exception of pH) were log–transformed to stabilise the variances and were further tested for two basic assumptions of an ANOVA (i.e., homogeneity and normality). The differences in sediment metal, nutrient concentrations, and environmental variables between the three sampling seasons (winter, summer, autumn) and pans (1 − 10) were assessed using a two–way ANOVA analysis. We also tested the relationships between sediment organic carbon, metals and nutrient concentration using Pearson correlation using SPSS version 25.

Principal component analysis (PCA) with varimax rotation and hierarchical cluster analysis, (HCA) using the average group linkage method, was employed to determine whether the metal concentrations constituted natural and/or anthropogenic sources of pollution. Hierarchical cluster analysis, non–metric multi–dimensional scaling (*n*–MDS) were conducted in PC–ORD version 5.10.

Macroinvertebrate community datasets were used to calculate diversity matrices (i.e., evenness, taxa richness, Shannon–Wiener diversity index) to assess for differences in species diversity among pans and seasons in PAST version 2.0. We tested for differences in diversity matrices among seasons and study pans using a non–parametric Kruskal–Wallis test implemented in SPSS version 25.0 (SPSS Inc., 2017).

Distance–based Permutational Analysis of Variance (PERMANOVA; Anderson, 2001) based on Bray–Curtis and Euclidean distance dissimilarities and 9999 permutations with Monte Carlo tests were performed to analyse differences in community structure based on all macroinvertebrate communities' data across sites and seasons using PERMANOVA+ for PRIMER version 6 (Anderson et al., 2008). Differences in macroinvertebrate taxa composition and environmental variables among seasons were tested using one–way analysis of similarity (ANOSIM; Clarke, 1993) and the statistic test was computed after 9999 permutations were conducted using PRIMER v6 add–on package PERMANOVA+ (Anderson et al., 2008). Factors used for the analysis were seasons (summer, autumn, winter). The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups. It varies between −1 and +1 and value 0 indicates random grouping.

To examine whether environmental variables (i.e., water, sediment) and anthropogenic factors (i.e., DtH, DtK, DtL) influenced macroinvertebrate community structure, initially a detrended canonical correspondence analysis (DCCA) was employed to determine whether linear or unimodal analysis methods should be employed (Šmilauer and Lepš, 2014). The gradient lengths from the DCCA output were examined and since the longest gradient was >4, a linear canonical correspondence analysis (CCA) model was selected (Šmilauer and Lepš, 2014) to study macroinvertebrate communities and their relation to the environmental variables among sites and seasons. Canonical correspondence analysis (CCA) was used to explore the relationship between macroinvertebrates and environmental variables.

3. Results

3.1. Environmental variables

3.1.1. Water chemistry

During the study, water pH was generally alkaline throughout the seasons, with mean ranges of 9.9–11.6 (winter), 7.2–8.2 (summer), and 8.2–9.3 (autumn). The water temperature at all the pans also showed seasonal trends with higher temperatures recorded in summer (mean range 23.9–29.1 °C), and lower temperatures in winter (mean range 19.6–22.3 °C) and autumn (mean range 21.2–24.2 °C) (Table 1). Significant differences (ANOVA, $p < 0.001$) among seasons were observed for temperature and pH. Significant variation among pans were also observed for pH (ANOVA, $p = 0.004$) during the study (Table 2). The mean DO

Table 1
Mean ± standard deviation of water environmental variables recorded across ten pans and three seasons (winter, summer, autumn) in the Khakhea–Bray Transboundary Aquifer pan region of South Africa.

Environmental variables	Acron.	Winter ($n = 5$)	Summer ($n = 10$)	Spring ($n = 8$)
Water				
Temperature (°C)	Temp	20.73 ± 1.1	26.62 ± 1.5	22.26 ± 0.9
pH		10.51 ± 0.5	7.98 ± 0.4	8.94 ± 0.3
Conductivity (μS cm^{-1})	Cond	135.47 ± 39.1	191.77 ± 43.0	173.38 ± 97.9
Total dissolved solids (mg L^{-1})	TDS	129.33 ± 33.9	91.89 ± 280.6	69.21 ± 129.2
Salinity (ppm)	Sal	0.07 ± 0.02	125.70 ± 30.1	111.54 ± 64.5
oxygen saturation (%)		89.59 ± 5.0	62.60 ± 19.7	89.59 ± 12.7
Dissolved oxygen (mg L^{-1})	DO	6.83 ± 0.8	4.15 ± 1.5	7.08 ± 0.8
Phosphates (mg L^{-1})	Phos	0.70 ± 0.4	0.36 ± 0.1	0.42 ± 0.3
Ammonium (mg L^{-1})	Amm	0.83 ± 0.7	0.10 ± 0.1	0.54 ± 0.6
Sediment				
pH		7.68 ± 0.4	7.73 ± 0.3	7.72 ± 0.4
Resistance (Ω)	Res	1835.83 ± 434.9	1961.50 ± 665.1	1580 ± 737.2
Stone (%)		3.48 ± 2.2	2.39 ± 3.4	6.14 ± 4.2
Phosphorus (mg kg^{-1})	P	29.20 ± 12.3	47.28 ± 46.3	63.67 ± 70.6
Potassium (mg kg^{-1})	K	133.37 ± 57.7	139.61 ± 135.6	152.83 ± 180.6
Calcium (mg kg^{-1})	Ca	6.91 ± 3.0	8.25 ± 4.9	12.56 ± 6.4
Magnesium (mg kg^{-1})	Mg	1.21 ± 0.5	1.27 ± 0.9	1.63 ± 0.9
Sodium (mg kg^{-1})	Na	0.08 ± 0.03	0.02 ± 0.01	0.08 ± 0.04
Copper (mg kg^{-1})	Cu	1.96 ± 1.3	1.48 ± 1.1	1.50 ± 1.4
Zinc (mg kg^{-1})	Zn	0.99 ± 0.9	0.77 ± 0.5	1.22 ± 1.3
Manganese (mg kg^{-1})	Mn	135.83 ± 179.6	66.85 ± 88.4	87.24 ± 155.2
Boron (mg kg^{-1})	B	0.25 ± 0.08	0.23 ± 0.2	0.31 ± 0.17
Iron (mg kg^{-1})	Fe	51.48 ± 25.9	29.00 ± 15.2	51.28 ± 49.5
Carbon (mg kg^{-1})	C	1.39 ± 0.4	0.67 ± 0.4	0.58 ± 0.5
Sulphur (mg kg^{-1})	S	6.51 ± 3.6	5.77 ± 3.8	10.59 ± 7.1
Physical				
Distance to kraal (m)	DtK	307.64 ± 195.8	270.25 ± 169.2	266.14 ± 187.8
Distance to house (m)	DtH	899.02 ± 743.4	780.01 ± 727.4	898.59 ± 770.5
Distance to latrine (m)	DtL	919.82 ± 752.4	799.05 ± 735.2	917.28 ± 779.8

Table 2
Statistical values of water and sediment chemistry variables across three seasons (winter, summer, autumn) from pans in the Khakhea–Bray Transboundary Aquifer pan region of South Africa. Bold values indicate significance values at $p < 0.05$.

Variable	Seasons			Pans			Seasons × Pans		
	df	F	*p*	*df*	F	*p*	*df*	F	*p*
Water									
Temperature	**2**	**48.55**	**<0.001**	9	1.54	0.192	11	1.02	0.461
pH	**2**	**18.64**	**<0.001**	**9**	**3.96**	**0.004**	**11**	**3.12**	**0.010**
Conductivity	**2**	**9.22**	**0.001**	**9**	**8.81**	**<0.001**	**11**	**3.27**	**0.008**
Salinity	**2**	**25.31**	**<0.001**	**9**	**10.58**	**<0.001**	**11**	**3.83**	**0.003**
DO saturation	**2**	**31.74**	**<0.001**	**9**	**3.90**	**0.004**	**11**	**4.93**	**0.001**
DO	**2**	**64.30**	**<0.001**	**9**	**6.08**	**<0.001**	**11**	**5.95**	**<0.001**
Phosphates	**2**	**9.24**	**0.001**	**9**	**3.14**	**0.013**	11	1.83	0.107
Ammonium	**2**	**9.61**	**0.001**	9	1.25	0.316	**11**	**3.14**	**0.010**
Sediment									
P	2	1.97	0.158	**9**	**6.08**	**<0.001**	**16**	**5.88**	**<0.001**
K	**2**	**8.33**	**0.001**	**9**	**16.82**	**<0.001**	**16**	**7.84**	**<0.001**
Ca	**2**	**6.98**	**0.003**	**9**	**7.91**	**<0.001**	**16**	**2.15**	**0.037**
Mg	**2**	**6.00**	**0.007**	**9**	**16.09**	**<0.001**	**16**	**5.01**	**<0.001**
K	2	2.59	0.093	**9**	**46.39**	**<0.001**	**16**	**20.15**	**<0.001**
Na	**2**	**52.30**	**<0.001**	**9**	**6.75**	**<0.001**	**16**	**4.11**	**0.001**
Cu	**2**	**4.47**	**0.021**	**9**	**5.11**	**<0.001**	**16**	**4.10**	**0.001**
Zn	**2**	**3.75**	**0.036**	**9**	**7.95**	**<0.001**	**16**	**3.85**	**0.001**
Mn	**2**	**3.67**	**0.038**	**9**	**3.22**	**0.008**	16	1.68	0.112
B	**2**	**5.86**	**0.008**	**9**	**6.88**	**<0.001**	**16**	**4.90**	**<0.001**
Fe	**2**	**3.47**	**0.045**	**9**	**5.65**	**<0.001**	**16**	**2.05**	**0.046**
C	**2**	**56.59**	**<0.001**	**9**	**5.13**	**<0.001**	**16**	**2.68**	**0.011**
S	**2**	**15.01**	**<0.001**	**9**	**8.49**	**<0.001**	**16**	**2.30**	**0.026**

concentration recorded in this study ranged from 6.4 to 8.2 mg L^{-1} in winter, 2.2–6.3 mg L^{-1} in summer and 6.2–8.7 mg L^{-1} in autumn (Table 1), with significant variation ($p < 0.01$) observed across seasons and pans (Table 2). The mean electrical conductivity range in the pans was 89–192 μS cm^{-1} for the winter, 126–246 μS cm^{-1} for the summer and 100.3–409 μS cm^{-1} for the autumn sampling events (Table 1). Significant differences (ANOVA; $p < 0.01$) among seasons and pans were observed for conductivity observed during the study (Table 2).

With regards to the nutrient levels in the pans, the ammonium concentration ranged from 0.4–2.56 mg L^{-1}, 0.2–0.6 mg L^{-1}, to 0.2–1.9 mg L^{-1} in winter, summer, and autumn, respectively. The highest reading of ammonium was recorded in winter (Table 1). Phosphate concentration ranged from 0.4 to 1.3 mg L^{-1} in winter, 0–0.4 mg L^{-1} in summer and 0.2–1.1 mg L^{-1} in autumn (Table 1). Among seasons, ammonium and phosphates concentrations showed significant differences ($p < 0.01$), with phosphates concentrations having significant differences among pans (p = 0.013) (Table 2).

3.1.2. Sediment chemistry

Metal concentrations detected in the sediment samples collected across pans and seasons are presented in Table 3. No significant seasonal differences (ANOVA; $p > 0.05$) were found for P and K concentrations, among the pans, in this study (Table 2). All other variables were found to be significantly different (ANOVA; $p < 0.05$) among pans and seasons (Table 2). Phosphorus (P), calcium (Ca), manganese (Mn), iron (Fe) and potassium (K) concentrations were generally high across all seasons, while sodium (Na), while sodium (Na), copper (Cu), zinc (Zn), magnesium (Mg) and boron (B) concentrations were low (Table 1). The pans' mean concentrations for P were 20–31.9 mg kg^{-1} in winter, 11.8–158 mg kg^{-1} in summer and 8.2–156.5 mg kg^{-1} in autumn. Concentration for K was 73.2–229 mg kg^{-1} in winter, 80.4–406.5 mg kg^{-1} for winter and 30.4–534 mg kg^{-1} for autumn.

Table 3
Descriptive statistics (mean ± standard deviation) highlighting the enrichment factors and pollution load index in wetland sediment sampled from pans in the Khakhea–Bray Transboundary Aquifer pan region in South Africa.

Variable	Winter	Summer	Autumn
K	2099.9 ± 958.5	1939 ± 1883.6	2122.7 ± 2507.8
Ca	60.7 ± 33.1	63.5 ± 37.8	96.9 ± 49.5
Mg	18.7 ± 8.8	19.2 ± 13.2	24.7 ± 13.2
Cu	0.4 ± 0.2	0.29 ± 0.22	0.3 ± 0.3
Zn	0.1 ± 0.	0.04 ± 0.03	0.1 ± 0.6
Mn	1.3 ± 1.6	0.5 ± 0.67	0.6 ± 1.2
B	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
Fe	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Pollution load	0 ± 0	0.02 ± 0.06	0 ± 0

3.1.3. Sediment contaminant assessments

The threshold effect level (TEL), probable effect level (PEL), lowest effect level (LEL) and the severe effect level (SEL) for Zn and Cu metal were within the limits for all the pans and seasons. For Cu levels, pan 9 in summer (18.9 mg kg^{-1}), pan 3 in autumn (18.1 mg kg^{-1}), pan 4 in autumn (20.9 mg kg^{-1}) and pan 8 in autumn (16.3 mg kg^{-1}) were over the limit for LEL (16 mg kg^{-1}). The enrichment factors varied across the study pans and seasons, and most enrichment factors were within the background levels for most metals, with a few metals such as Na, Fe, Zn, Mn, Cu and B showing background to moderate enrichment across all pans. In all the seasons and all the pans, Na, Cu, Fe, Zn, Mn, and Fe showed depletion to minimal enrichment with very high enrichment for Mg, and extremely high enrichment for P, K, and Ca (Table 3). Among all pans and seasons, the pollution load index was <1 which indicates no pollution (Table 3). Toxic risk index (TRI) indicated that most pans posed no toxic risk as they were ≤5 for all seasons. The TRI values were low toxic risk during seasons, having mean ranges of 0.2–0.7 in winter, 0.2–0.8 in summer and 0.1–0.9 in autumn.

3.2. Relationships between the measured variables

Using PCA, the first two principal components (PC) accounted for 78.4 % of the total variance, with PC1 and PC2 accounting for 46.4 % and 32.0 % of the total variance, respectively (Table 4). Principal component analysis classified metals into two groups, with group 1 consisting of all metals, with the exception of P and Fe which were the only metals in group 2 (Table 4).

A correlation matrix showed that all metals in the pan sediments showed a significant positive association except for P vs Na (p = 0.205), Ca vs Cu (p = 0.364), Mn vs Mg (p = 0.673), Mn vs K (p = 0.106), Fe vs Na (p = 0.788), Fe vs B (p = 0.326) and S vs Fe (p = 0.808) (Table 5). This suggests that all metals in the studied sediments may have originated from a similar source. We observed that only Ca (p = 0.031) and Mg (p = 0.033) showed a significant relationship with distance to kraal (DtK) The remaining sediment parameters were not significantly

Table 4
Principle component analysis results for metal concentrations across all seasons within pans. Factor loadings >0.5 are highlighted in bold.

	Principal component	
	PC1	PC2
Eigenvalue	31.789	21.914
%Variance	46.39	31.98
Cum%Var	46.39	78.37
Variable	Factor loadings	
P	−0.04	**0.66**
K	**−0.79**	0.08
Ca	**−0.67**	0.47
Mg	**−0.73**	0.45
Na	**−0.56**	0.07
Cu	**−0.70**	−0.46
Zn	**−0.83**	−0.07
Mn	**−0.55**	**−0.55**
B	**−0.89**	0.13
Fe	−0.36	**−0.65**
C	**−0.62**	−0.37
S	**−0.83**	0.29

Table 5

Pearson correlation summary for the metals and nutrient variables determined in pan sediments from the Khakhea–Bray Transboundary Aquifer pan system, South Africa. The *p*–values are indicated in grey (3 decimal places), and the *r*–values in white (2 decimal places). Significant values ($p < 0.05$) are indicated in bold. Abbreviations: DtK – distance to kraal, DtH – distance to household, DtL – distance to pit latrine/toilet.

	DtK	DtH	DtL	P	K	Ca	Mg	K	Na	Cu	Zn	Mn	B	Fe	C	S
DtK	1	**0.58**	**0.57**	–0.18	–0.10	**–0.31**	**–0.31**	–0.09	–0.15	0.00	–0.10	0.09	–0.21	0.13	0.04	–0.21
DtH	**<0.001**	1	1.00	**–0.48**	**–0.52**	**–0.25**	**–0.32**	**–0.53**	**–0.04**	**–0.37**	**–0.51**	**–0.41**	**–0.46**	–0.23	**–0.34**	**–0.41**
DtL	**<0.001**	**<0.001**	1	**–0.48**	**–0.51**	–0.25	**–0.32**	**–0.52**	–0.04	**–0.36**	**–0.50**	**–0.41**	**–0.45**	–0.21	**–0.33**	**–0.41**
P	0.222	**0.001**	**0.001**	1	**0.75**	**0.54**	**0.46**	**0.77**	0.19	**0.49**	**0.84**	**0.42**	**0.73**	**0.31**	**0.48**	**0.65**
K	0.500	**<0.001**	**<0.001**	**<0.001**	1	**0.47**	**0.62**	**0.96**	**0.37**	**0.83**	**0.75**	**0.74**	**0.75**	**0.34**	**0.70**	**0.57**
Ca	**0.031**	**0.091**	0.084	**0.000**	**0.001**	1	**0.86**	**0.51**	**0.47**	0.13	**0.51**	0.06	**0.66**	**–0.44**	0.24	**0.79**
Mg	**0.033**	**0.028**	**0.028**	**0.001**	**<0.001**	**<0.001**	1	**0.63**	**0.58**	**0.41**	**0.41**	0.24	**0.68**	**–0.35**	**0.34**	**0.62**
K	0.522	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	1	**0.38**	**0.82**	**0.80**	**0.76**	**0.79**	**0.34**	**0.63**	**0.66**
Na	0.311	**0.765**	0.793	0.205	**0.009**	**0.001**	**<0.001**	**0.008**	1	**0.36**	**0.31**	0.21	**0.44**	0.04	**0.38**	**0.51**
Cu	0.986	**0.010**	**0.013**	**<0.001**	**<0.001**	0.364	**0.004**	**<0.001**	**0.012**	1	**0.48**	**0.83**	**0.57**	**0.53**	**0.56**	**0.29**
Zn	0.488	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.004**	**<0.001**	**0.033**	**0.001**	1	**0.49**	**0.74**	**0.39**	**0.66**	**0.81**
Mn	0.557	**0.004**	**0.004**	**0.003**	**<0.001**	0.673	0.106	**<0.001**	0.155	**<0.001**	**<0.001**	1	**0.53**	**0.52**	**0.49**	**0.28**
B	0.149	**0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	1	0.14	**0.56**	**0.78**
Fe	0.363	0.122	0.155	**0.031**	**0.018**	**0.002**	**0.016**	**0.018**	0.788	**<0.001**	**0.006**	**<0.001**	0.326	1	**0.35**	–0.04
C	0.798	**0.020**	**0.022**	**0.001**	**<0.001**	0.097	**0.018**	**<0.001**	**0.008**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.016**	1	**0.46**
S	0.142	**0.004**	**0.004**	**0.000**	**<0.001**	**0.000**	**<0.001**	**<0.001**	**<0.001**	**0.049**	**<0.001**	**0.050**	**<0.001**	0.808	**0.001**	1

($p > 0.05$) correlated with DtK (Table 5). However, there was a significant correlation between distance to households (DtH) and pit latrines/toilets (DtL) and most variables, with the exception of Fe (DtH, $p = 0.122$; DtL, $p = 0.155$), Ca (DtL, $p = 0.084$), and Na (DtL, $p = 0.793$) (Table 5).

The HCA dendrogram formed two clusters: *Group 1*, which consisted of Fe, P, C, K, Na, B, Cu, Mg, Zn, Ca, S and *Group 2* that consisted of K and Mn. The similarity for *Group 2* formation was not as high as that of *Group 1* (Fig. 2). The overlap illustrated in the *n*–MDS plot illustrates the lack of variation between sediment metal concentrations across the three seasons (Fig. 3).

3.3. Macroinvertebrate diversity

A total of 27,943 individual macroinvertebrates and 41 macroinvertebrates taxa belonging to 7 orders were identified from the Khakhea–Bray pan systems over three seasons (winter, summer, and autumn). The seven orders were Coleoptera, Hemiptera, Odonata, Mollusca, Diptera, Crustacea, and Ephemeroptera. Mollusca taxa comprised the highest percentage of the total sample with 31.8 %. The remaining sample comprised of Crustacea (31.1 %), Diptera (16.2 %), Hemiptera (13.1 %), Odonata (5.7 %), Coleoptera (1.5 %), and Ephemeroptera (0.6 %).

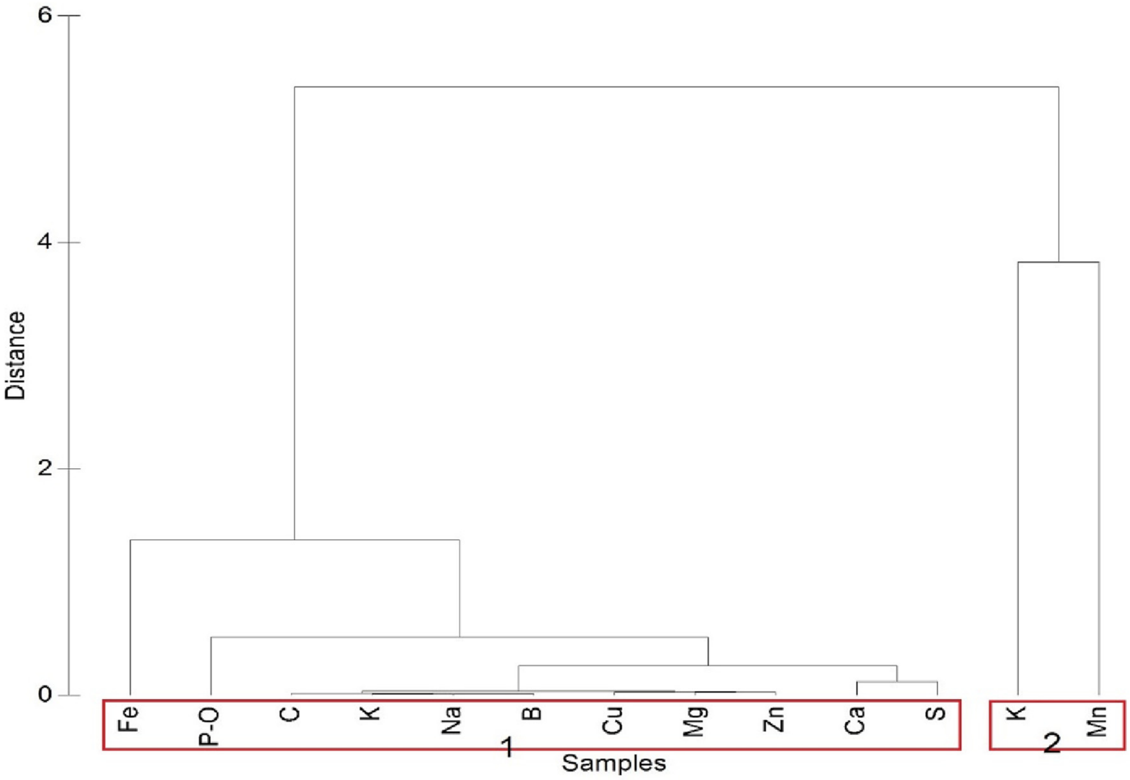

Fig. 2. Hierarchical cluster dendrogram showing the clustering of the sediment metal concentrations sampled Khakhea–Bray Transboundary Aquifer pan region in South Africa.

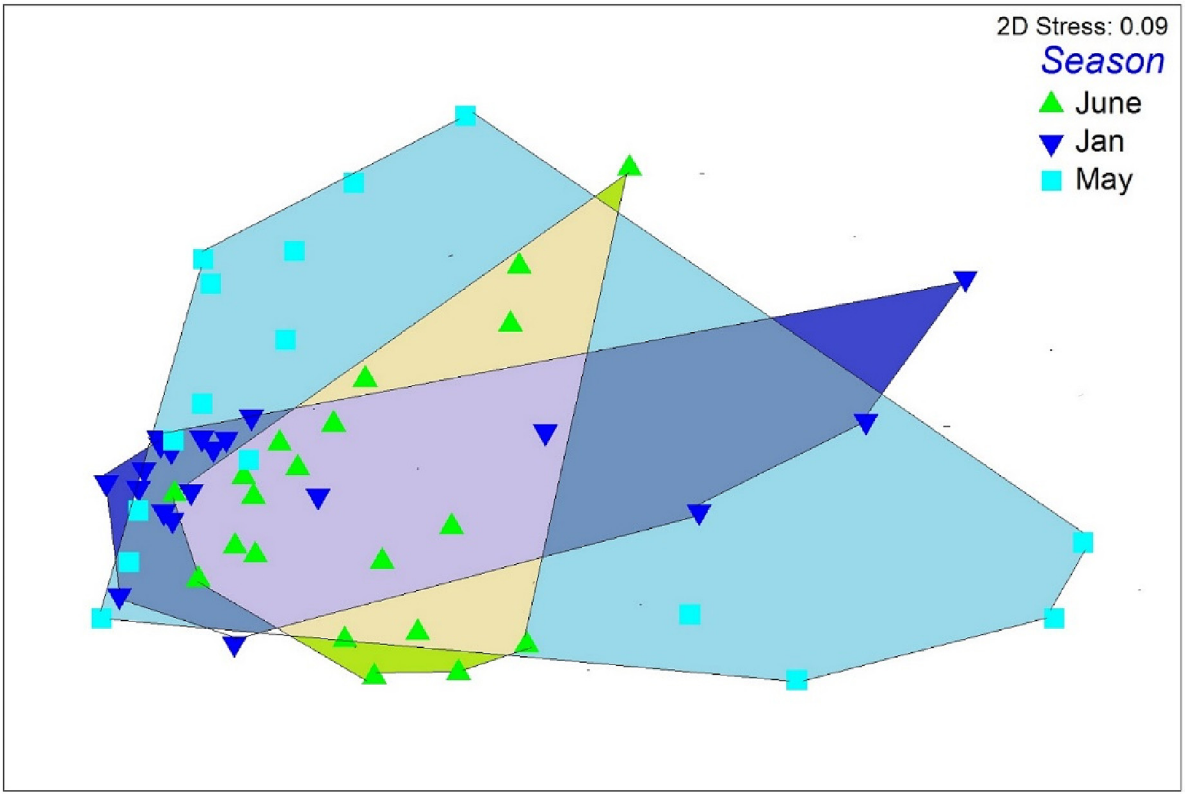

Fig. 3. The *n*–MDS ordination (stress value 0.09) illustrates the variation of sediment metal concentrations across pans and seasons. Polygons indicate the three seasons: green − winter, dark blue − summer and light blue − autumn.

Using PERMANOVA analysis, significant differences in macroinvertebrate community structure was observed across seasons (F = 45.557, $p < 0.0001$) and pans (F = 4.600, $p < 0.0001$). ANOSIM analysis indicated average similarities of 70.9 %, 71.2 % and 67.9 % for the winter, summer, and autumn seasons, respectively. These similarities were mostly driven by *Culex* spp. (23.0 %), *Lymnaea truncatula* (21.1 %), *Anax* spp. (12.0 %) and *Sigara* spp. (11.1 %) in winter, Steptocephalidae (37.8 %), *Triops granarius* (28.9 %), and L. *truncatula* (21.1 %) in summer, and *L. truncatula* (34.9 %), *Culex* spp. (14.4 %), *Anisops* spp. (12.7 %), and *Sigara* spp. (11.4 %) in autumn.

The most abundant species with the highest relative abundance in winter, summer, and autumn were *Culex* sp. (32.6 %), Streptocephalidae (53.2 %), and *L. truncatula* (48.2 %), respectively (Table 6). The macroinvertebrate taxa most widely distributed across the pans in winter were Hydroporinae, *Culex* sp., *Chironominae*, *Anisop* sp., *Sigara* sp., *Anax* sp., *Pseudagrion* sp., and *L. truncatula.* In summer they were Streptocephalidae, *T. granarius* and L. *truncatula.* In autumn, *L. truncatula*. *Lymnaea truncatula* was the only species that was widely distributed across the three seasons. Taxa alpha diversity ranged between 9 and 14 per pan in winter, 5–11 in summer, and 3–17 in autumn. Autumn had the highest number of species (n = 24), followed by summer with 22 species, and winter with 18 species (Table 6).

The %Corixidae, %Chironomidae + Oligochaeta, and %ETOC were high for the pans in winter, whereas the highest %EPT was obtained in autumn across pans. The mean %EPT recorded in this study ranged from 0 to 5.2 in autumn, whereas in summer and winter, we did not record any %EPT (Table 6). The %Chironomidae + Oligochaeta ratio was high for the pans in winter, with a mean range of 2–15.5, and low in summer and autumn, with a mean range from 0–0.9 to 0–3.7, respectively. Regarding the % ETOC ratio, winter (13.5–27.6) and autumn (2.4–24.4) had a markedly higher mean range when compared to summer (0–6.2). Lastly, the mean %Corixidae ratio ranged from 1.4 to 14.6 in winter, 0–6.3 in summer and 0–34.7 in autumn (Table 6). The Shannon–Wiener diversity index was highest during the autumn season (mean range 0.5–1.9) and lower in summer (mean range 0.6–1.5) and winter (mean range from 1.3 to 1.6). Using a non–parametric Kruskal–Wallis test, significant differences in diversity matrices for %EPT, %ETOC, %Chironomidae + Oligocheata, %Corixidae and Shannon–Wiener were observed across seasons ($p < 0.01$). However, no significant differences (p = 0.514) in evenness were observed across seasons. No significant differences ($p > 0.05$) in diversity matrices were observed across pans (Table 7).

3.4. Relationship between macroinvertebrate community and environmental variables

Based on CCAs carried out using individual variables, the following predictor variables were found to have a significant (Monte Carlo test, $p < 0.050$) impact on the macroinvertebrate communities: water temperature, dissolved oxygen, water pH, sodium (Na), salinity, sulphur, (S), stone percentage, and water conductivity. The first two axes of the species–environmental variables plot accounted for 76.1 % of the total explained fitted cumulative variance in the macroinvertebrate community, with CCA axis 1 and 2 accounting for 57.5 % and 18.6 %, of the macroinvertebrate taxa variation due to the measured environmental variables, respectively (Fig. 4). The winter pans were most negatively associated with CCA axis 1 and were characterised by pH. Examples of macroinvertebrates species that were associated with these sites include *Anax* sp., Culex sp., Chironominae, *Pseudagrion* sp., *Gerris swakopensis*, *Stenelmis* sp., and *Paraplea* sp. Summer pans were positively associated with axis 1 and were characterised by salinity, conductivity, and water temperature. Examples of macroinvertebrates species that were associated with these pans included Streptocephalidae, *Triops granarius*, and *Haliplus* sp. Autumn pans were positively associated with axis 1 and were characterised by stone, sulphur, sodium, and dissolved oxygen. Examples of macroinvertebrates species that were associated with these pans included Ostracoda, *Rhantus* sp., *Demoreptus capensis*, *Cybister* sp., *Chrysops* sp., *Appasus* sp., *Anisops* sp., and *Sigara* sp., (Fig. 4).

Table 6
Mean (±SD) of macroinvertebrates relative abundances (%) observed in temporary pans across seasons in the Khakhea–Bray Transboundary Aquifer pan region. Abbreviations: EPT – Ephemeroptera, Plecoptera, and Trichoptera taxa; ETOC – Ephemeroptera, Trichoptera, Odonata and Coleoptera taxa.

Taxa	Winter	Summer	Spring
Coleoptera			
Acidocerinae	0.08 ± 0.2		
Anaceanini sp.			
Hydrochus sp.		0.57 ± 1.0	
Pseudangronyx sp.			0.01 ± 0.02
Hydroporinae	1.99 ± 1.78	0.01 ± 0.02	0.46 ± 0.7
Rhantus sp.			1.46 ± 2.4
Hydrophilini sp.		0.41 ± 1.0	0.02 ± 0.04
Haliplus sp.		0.09 ± 0.2	
Amphiops sp.		0.05 ± 0.2	
Rhantus sp.		0.10 ± 0.2	
Cybister sp.			0.61 ± 0.9
Regimbartia sp.			0.33 ± 0.9
Loccobius sp.	0.04 ± 0.08	0.05 ± 0.2	
Stenelmis sp.	0.57 ± 0.6	0.004 ± 0.01	
Hydrochus sp.			0.05 ± 0.1
Rapnus sp.			0.01 ± 0.03
Copelatus sp.			0.05 ± 0.1
Crustacea			
Streptocephalidae		53.20 ± 23.2	0.08 ± 0.2
Triops longicaudatus		24.56 ± 22.5	
Ostracoda			2.13 ± 4.9
Diptera			
Brachydeutera sp.		0.08 ± 0.3	0.04 ± 0.1
Culex sp.	32.60 ± 26.2	1.38 ± 2.2	15.17 ± 15.6
Chironominae	5.75 ± 3.8	0.09 ± 0.2	1.01 ± 1.4
Chrysops sp.		0.02 ± 0.05	0.68 ± 0.9
Psychoda sp.			0.45 ± 1.3
Ochthera sp			0.04 ± 0.1
Hemiptera			
Anisops sp.	4.62 ± 4.7	0.70 ± 1.9	16.24 ± 15.6
Sigara sp.	7.06 ± 4.8	0.69 ± 1.3	7.47 ± 10.9
Appasus sp.	0.12 ± 0.2		0.17 ± 0.3
Gerris swakopensis	0.09 ± 0.2		
Laccocoris sp.	0.01 ± 0.03		
Paraplea sp.	0.11 ± 0.2		
Xiphoveloidae sp.	0.67 ± 1.5		
Odonata			
Anax sp.	13.57 ± 10.0	0.85 ± 2.6	3.35 ± 2.6
Pseudagrion sp.	5.34 ± 2.0	1.62 ± 4.8	1.17 ± 2.0
Ephemeroptera			
Demoreptus capensis			1.84 ± 1.7
Mollusca			
Unio tumidus	0.07 ± 0.07	0.84 ± 1.9	
Lymnaea truncatula	26.91 ± 17.5	18.90 ± 17.1	48.17 ± 26.3
Melanoides tuberculata		0.01 ± 0.02	
Matrices			
No. of EPT individuals			22.3 ± 22.6
No. of ETOC individuals	260.2 ± 113.8	19.0 ± 36.3	104.4 ± 77.0
%EPT			1.8 ± 1.7
%Chironomidae + Oligocheata	14.1 ± 12.5	0.09 ± 0.2	1.01 ± 1.4
%Corixidae	11.0 ± 12.2	0.50 ± 1.0	7.5 ± 10.9
%ETOC	22.4 ± 8.7	3.8 ± 8.7	9.4 ± 5.4
ETOC richness	4.20 ± 0.8	2.20 ± 1.8	5.00 ± 2.7
Hemiptera + Diptera richness	4.70 ± 1.6	2.40 ± 1.4	4.63 ± 2.0
EPT richness			0.75 ± 0.5
Taxa richness	9.15 ± 1.66	5.95 ± 1.70	9.63 ± 3.7
Shannon–Wiener	1.52 ± 0.22	1.04 ± 0.34	1.27 ± 0.50
Evenness	0.52 ± 0.11	0.51 ± 0.13	0.46 ± 0.21

Table 7
Statistical values of diversity matrices across three seasons (winter, summer, autumn) from pans in the Khakhea–Bray Transboundary Aquifer region of South Africa. Bold values indicate significance values at $p < 0.05$.

Metrices	Seasons	Pans
	P	*P*
%Corixidae	**<0.001**	0.616
%Chironomidae + Oligocheata	**<0.001**	0.973
%ETOC	**<0.001**	0.553
%EPT	**0.001**	0.915
Evenness	0.513	0.931
Shannon	**0.009**	0.813

4. Discussion

In this study, we assessed aquatic macroinvertebrate communities in relation to water and sediment quality within the Khakhea–Bray Transboundary Aquifer pan region in South Africa. Aquatic macroinvertebrates have been effectively used to assess and monitor the health of aquatic ecosystems (Dalu et al., 2017a, 2017b). Different macroinvertebrate taxa have contrasting ranges of environmental variable characteristics, meaning that minor changes in their environmental preference ranges can result in variation/changes in community structure (Rasifudi et al., 2018). The results indicate that seasonality was a major driver of macroinvertebrate community structure. Seasonality had predictable implications on water quality dynamics, given temperature and solute concentration dynamics. Various environmental variables (i.e., conductivity, salinity, water temperature, pH, dissolved oxygen, sulphur, and sodium) exerted an influence on macroinvertebrate community. We found that macroinvertebrate diversity was low during winter and high in summer, thus supporting our hypothesis.

The results showed that there was a relationship between metal concentration distribution and measured physico–chemical variables (i.e., water temperature, pH, ammonium, conductivity, and phosphates). Water temperature during the present study was observed to be low in winter and high during the summer season which could have affected the structuring of macroinvertebrates causing variations in taxa diversity. These variations in water temperature were due to seasonal climatic variations and in particular, atmospheric temperature changes (Bai et al., 2016). The pH was found to be alkaline, an observation that was expected given the dolomitic landscape which was dominated by calcite rocks. pH was slightly higher in winter when compared to summer, but generally high throughout all the seasons. An increased pH in the study area may be a by–product of calcite leaching into the water from the dolomitic rock in the area. Metals tend to precipitate in alkaline conditions, as the hydroxide ions present in alkaline solutions react with the metal ions to form insoluble metal hydroxides (Hoy, 2001). One of the main effects of metal precipitation on macroinvertebrates is that it can reduce their abundance and diversity as metals can be toxic to these organisms, causing them to die or leave the affected area. Additionally, the accumulation of metals in sediment can make it more difficult for macroinvertebrates to find suitable habitat and food. Ions and calcite from dolomite rock in water become more concentrated as water level decreases due to evaporation, which ultimately contributes to an increase in water conductivity (Bai et al., 2016). The conductivity values do not show any significant variation across seasons. The relatively high conductivity in winter when compared to summer is likely a result of decreased water volume in the drier months.

Ammonium in water is produced by microbiological degradation of organic nitrogenous matter (Buxton et al., 2020). Surface waters generally have lower ammonium concentrations than benthic waters because these nutrients are released from the decomposing organic matter, with its release being governed by anoxic conditions (Bai et al., 2016). It is evident that ammonium and phosphate concentrations were comparatively higher during winter, which could be attributed to cattle grazing, the dominant agricultural activity in the area. Cattle grazing could have strongly impacted wetlands by increasing nutrient input through urine and faecal deposition (Buxton et al., 2020). Several studies (e.g., Buxton et al., 2020; Bai et al., 2016) have highlighted that nutrient concentrations in the wetland pans can increase due to agricultural activities such as cattle grazing. Moreover, the area is characterised by a high densities of subsistence farming with many of the rural inhabitants living below the poverty line. This leads to higher densities of sheep, goats, and cattle regularly utilising these pan systems for water. The effect of livestock on these pan systems is more

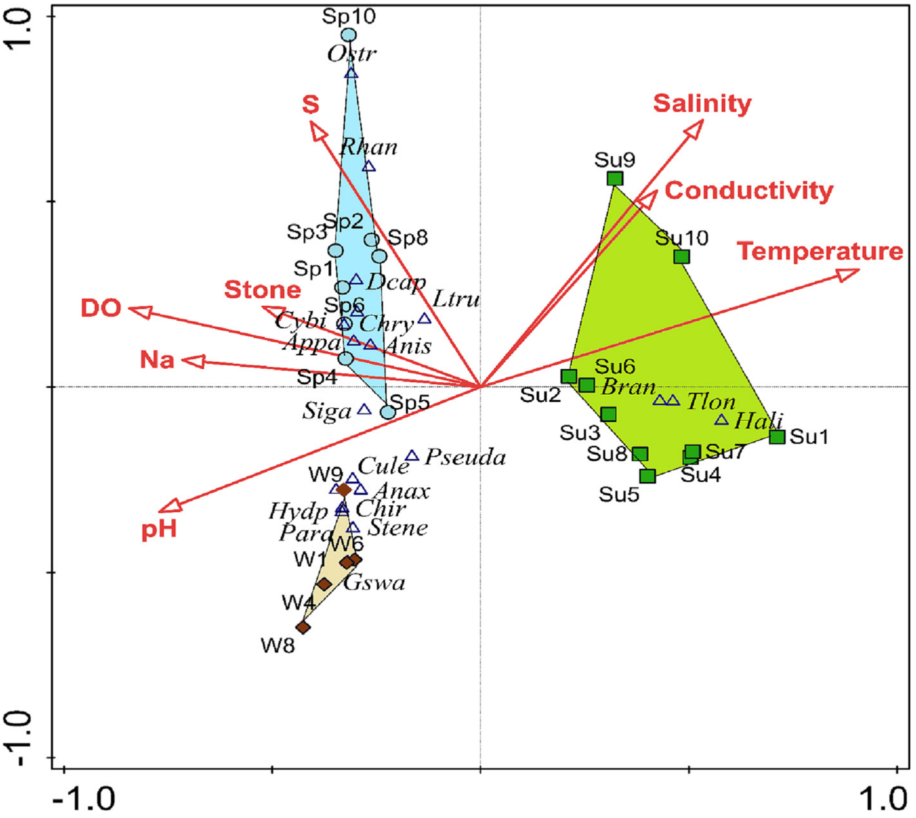

Fig. 4. CCA ordination triplot diagram showing the relationship between measured significant environmental variables with macroinvertebrate taxa across sampling sites. Abbreviations: Su – summer season, W – winter season, Sp – autumn season; the numbers next to seasons indicate pan names/numbers. Ostr – Ostracoda, Rhan – *Rhantus* sp., Dcap – *Demoreptus capensis*, Cybi – *Cybister* sp., Chry – *Chrysops* sp., Appa – *Appasus* sp., Anis – *Anisops* sp., Siga – *Sigara* sp., Pseuda – *Pseudagrion* sp., Cule – *Culex* sp., Anax – *Anax* sp., Chir – Chironominae, Hydp – Hydroporinae, Para – *Paraplea* sp., Gswa – *Gerris swakopensis*, Bran – Streptocephalidae, Tlon – *Triops granarius*, Hali – *Haliplus* sp., Stene – *Stenelmis* sp.

pronounced in winter (drier months) because of the reduced availability of freestanding water.

Metal concentrations in sediments were mostly found to be within background levels for the catchment, and similar to that found in other parts of South Africa and the surrounding regions (Dalu et al., 2022). Sediment quality was acceptable based on the sediment quality guidelines (MacDonald et al., 2000). Metal concentrations in dolomite landscape sediments varied between the seasons, with K being the dominant metal. We speculated that the high K concentration are likely the result of weathering dolomite rock in the study area. The low metal concentrations observed during summer season were similar to metal concentrations observed by Shibambu (2016) in the Wetland Downstream of Makhado Pond (South Africa). This is likely due to metals being re–suspended with sediment and floating further downstream in the water column resulting in low metal collection in the sediment. This is supported by the slight decrease in pH, which was noted during summer, which affected the solubility of trace metals in water (Hoy, 2001). Moreover, high metal concentrations recorded during winter and autumn were similar to metal concentrations observed by Zhang et al. (2016) along a wetland–forming chronosequence. Significantly higher metal concentrations in winter and autumn could be attributed to increased evaporation and decreased rainfall, leading to decreased water levels (Zhang et al., 2016).

The metals recorded in this study include P, Ca, Mn, K, and Fe, which was expected high levels of mineral dolomite in the area. This is supported by Al–Khashman (2013) study which found Ca and Mn to be prominent within a dolomitic landscape. In this study, significantly high values of Ca and Mn were observed across seasons due to the release of calcium from the dolomitic rocks (Al–Khashman, 2006). Calcium often comes from carbonate minerals such as calcite and dolomite, commonly occurring in sediment surrounding the study area (Al–Khashman, 2006). This was supported by the PCA and hierarchical cluster analysis. Among all the tested metals, the correlation analysis of mean concentrations showed that all metals had a strong correlation with each other. In this study, there was a strong correlation between the mean concentrations of all tested metals and the strong correlation may be an indication of the common contamination sources from nearby villages and mutual dependence (Dalu and Chauke, 2020). Thus, it is likely that most of the metals within the Khakhea–Bray sediments originated from a similar source, except for P, which likely entered the system through waste products from cattle.

Anthropogenic activities, such as agriculture, contribute significantly to the enhancement of metal concentrations in most wetlands systems (Bai et al., 2016). In contrast to our second hypothesis however, anthropogenic activities (DtK, DtH and DtL) had little influence on sediment metals, in this study. However, the significant relationship between DtK and metals, Ca and Mg, shows that Ca and Mg concentrations were elevated in close proximity to Kraals. Sheep, goats, and cattle are a source of Mg in these systems as they export excess Mg into the water via urination and defecation. Similar observations were made by Nyangababo et al. (2005) along the Nyando Wetlands of Lake Victoria basin. While conducting the study, it was observed that human–induced pressure such as water withdrawal, sewage discharge, and infrastructure degradation, are likely having a direct impact on the water quality in pans systems as metal concentrations were higher in pans proximate to households and pit latrines/toilets. This is supported by the significant correlation observed for DtH and DtL vs metals (P, Mn K, Cu, Zn, Mg and B).

Overall, the pollution load index (PLI) indicated that the sediment within the pans was good quality within the study area. In contrast to our findings, Shibambu (2016) found that wetland environments can reduce pollution by absorbing pollutants. Furthermore Helson (2012) indicated that wetlands could act as natural water purification systems through filtering and absorbing pollutants in surface water. What this observation mean is that although we did observe a low PLI, it meant that they were relatively low pollutant levels coming from the nearby households.

Macroinvertebrate diversity and abundance in the study varied among the three seasons (winter, summer and autumn), being generally higher in autumn. This was driven by the presence of Ephemeroptera taxa, which indicate good water quality (Dickens and Graham, 2002). Hence, high macroinvertebrate taxa richness was observed in autumn when minimum impacts occurred within the pans. Anthropogenic pressure, such as agriculture, contributes significantly to exportation of pollutants into wetlands systems (Bai et al., 2016). While conducting the study, livestock grazing, infrastructure degradation, littering and water withdrawal were observed. These activities are likely to strongly impact the water quality in pan ecosystems during winter when pans are drying out due to low rainfall.

The high Shannon–Wiener diversity observed in autumn, was likely the product of the increased overall ecosystem quality and health during the season, resulting in the proliferation of sensitive macroinvertebrate taxa. Whereas the low EPT values in aquatic systems indicate a stressful condition (low water quality), high values indicate less stressful conditions (high water quality). Comparisons of EPT diversity and abundance revealed seasonal variation with high EPT values in Autumn and these findings are similar to Nhiwatiwa et al. (2017) found.

Significantly, the order Diptera was dominated by Chironomidae (Chironominae) and Culicidae (*Culex* sp.) families, and these families are known for their high tolerance to water pollution. Thus, these families were found across all seasons, but a high abundance was observed during winter. Arimoro and Ikomi (2008) in his study, observed that aquatic macroinvertebrate communities found within constructed polluted wetlands generally dominated by polluted tolerant macroinvertebrates. There were changes in macroinvertebrate community between winter, summer and autumn, from being dominated by Diptera taxa in winter to being dominated by Ephemeroptera taxa in autumn. These changes are the result of macroinvertebrate taxa responding differently to environmental variables (Ferreira et al., 2014). The differences in species composition and abundance across seasons indicated that aquatic macroinvertebrates may be strongly influenced by environmental parameters (Aschalew and Moog, 2015).

Salinity, conductivity, temperature, stones, pH, sodium, dissolved oxygen, and sulphur were significant variables affecting macroinvertebrate community structure and abundance, as indicated by the CCA analysis. An increase in pH levels was predominantly associated with macroinvertebrate taxa such as *Culex* sp., and Chironominae, as these taxa are adapted to living in slightly acidic to alkaline waters, with a pH range of around 6.0 to 8.0. Whereas an increase in salinity, conductivity, and temperature during summer was strongly associated with crustacean taxa such as Streptocephalidae and *Triops granarius*, which show a tolerance to these conditions. Moreover, the CCA analysis revealed that family taxa such as Ostracoda, *Rhantus* sp., *Demoreptus capensis*, *Cybister* sp., *Chrysops* sp., *Appasus* sp., *Anisops* sp., and *Sigara* sp., were positively associated with high dissolved oxygen, sulphur, and sodium. These species are found within Diptera and Ephemeroptera which are known to show a high infinity to dissolved oxygen (Coetzee et al., 2014).

A high abundance of Mollusca and Crustacea are typical of many temporary wetland ecosystems (Bird et al., 2019) and this was similar to our current study. Both taxa produce diapause life–stages and are, thus, capable of persisting in the sediment through dry periods. *Lymnaea truncatula* was the most abundant Mollusca across all the study pans and seasons. This is a hardy species with a broad tolerance for a wide suite of environmental conditions, allowing it to exploit the conditions associated with these pans (Kouamé et al., 2011). The crustaceans were dominated by large branchiopods, a group synonymous with temporary wetland ecosystems in the region (Bird et al., 2019).

5. Conclusion

The findings of the study have indicated that selected water quality variables (e.g., salinity, pH, temperature) and metal (e.g., Na) concentrations in Khakhea–Bray pan systems influenced macroinvertebrate community structuring. Anthropogenic activity such as cattle grazing in the pans and defecating could have resulted in increased nutrient concentrations, which likely had a negative effect on pan water quality particularly during the winter months when water levels were low. Moreover, the different stages of hydroperiod observed could have influenced the distributions of metal concentrations, as high metal concentrations were recorded in winter (low water level) compared to summer (high water level). Although the proximity of the nearest households, kraal and latrines to the pans were found not to be significant, future studies need to further investigate how anthropogenic activities such as animal grazing/defecating in pans, infrastructure developments, water withdrawal and littering could result in poor water quality within temporary pans, which might strongly influence macroinvertebrate diversity and distribution. Therefore, the knowledge of the relationships between macroinvertebrates and their environment is crucial in understanding how the ecosystem functions particularly within temporary wetland systems.

CRediT authorship contribution statement

FMM: Formal analysis, Methodology, Data curation, Formal analysis, Writing – original draft, review and editing; RJW: Methodology, Visualisation, Investigation, Supervision, Writing – original draft, review and editing; NW: Methodology, Visualisation, Writing – original draft, review and editing; CPM: Investigation, Methodology, Writing – original draft, review and editing; FD: Methodology, Investigation, Visualisation, Investigation, Formal analysis, Funding acquisition, Writing – review and editing; CK: Visualisation, Methodology, Investigation, Writing – review and editing; PS: Methodology, Visualisation, Writing – original draft, review and editing; TD: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, review and editing.

Data availability

All the data collected during this research is presented in the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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