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Benthic Macroinvertebrates as Bioindicators of Stream Health Within the Central African Copperbelt

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Abstract Globally, stream ecosystem health in mining regions, including the Central African Copperbelt (CACB), is threatened by increased aquatic metal pollution. Stream biomonitoring and bioassessment offers an eco-friendly nature-based approach to determine the aquatic ecological conditions and inform management interventions in mining landscapes. This study conducted monthly between May 2022 and April 2023, explored the taxonomic and functional responses of macroinvertebrates as bioindicators of the ecological condition of four streams in the north-western Zambian Copperbelt of the CACB. 252 sediment samples were collected and analysed for metals. 144 macroinvertebrate samples were collected to determine their taxonomic and functional traits. ANOVA (analysis-of-variance), SIMPER (similarity-percentage), ANOSIM (analysis-of-similarities), CCA (canonical correspondence analysis) and multidimensional biotic index analyses were conducted to correlate environmental and biotic variables. Metal concentrations (mg/kg) in sediments followed the order As (5.27) < Pb (5.75) < Zn (44.6) < Ni (89.3) < Cu (187.5). From ANOVA, the macroinvertebrate community was significantly dominated by Insecta families, mostly Diptera (23.3%) and Coleoptera (16.7%). Trophically, predators significantly dominated in the order Odonata (34.7%) > Hemiptera (31.8%) > Diptera (18.9%). SIMPER depicted a strong influence of Cu, EC, velocity, sediment texture and organic matter on macroinvertebrate diversity and

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spatial distribution. ANOSIM indicated the highest community dissimilarity at the most impacted stream (KYABR). Habitat assessment metrics, including ZISS-1, ASPT, and EPT/Chironomidae ratio, registered a “good ecological status” with the presence of pollution-sensitive taxa (Helodidae and Perlidae), in concordance with environmental variables, confirming the suitability of macroinvertebrates as proxy bioindicators of stream health in mining landscapes. The study confirms the potential of macroinvertebrates, as potential bioindicators of stream health in mining landscapes. Their diversity and distribution is strongly influenced by Cu, EC, velocity, and sediment characteristics while habitat assessment metrics indicate a "good ecological status" despite moderate metal contamination.

Keywords Aquatic metal pollution · Bioassessment · Central African Copperbelt · Benthic macroinvertebrates · Mining · Sustainable development goal 6

1 Introduction

The availability and sustainable management of freshwater resources are pivotal to the attainment of the UN 2030 Sustainable Development Goal 6 and a precursor to the sustenance of the biosphere (Gigliotti et al., 2019). However, freshwater ecosystems are increasingly under threat from anthropogenic pollution from agro-industrial and municipal sources (Xiong et al., 2019), further exacerbated by the impacts of climate change (IPCC, 2023; Woodward et al., 2010). Consequently, the impairment of services and reduction in the provision of goods by freshwater ecosystems is an imminent threat to global sustainability that needs urgent interventions (Cantonati et al., 2020).

Biomonitoring is a nature-based, eco-friendly and less capital-intensive approach used globally to evaluate the ecological consequences and the magnitude of perturbations to freshwater ecosystems (Eriksen et al., 2021; Resh, 2007). Bioindicators, such as microbial communities, macroinvertebrates, macrophytes and vertebrates, exhibit fundamental merits for biomonitoring, including ubiquitous and resident nature, high sensitivity, potential to provide long-term evidence of environmental changes, and suitability as a “clean” nature-based solution (NBS) for environmental management (Zhou et al., 2008). Regarding macroinvertebrates, the fundamental benefits of use are the ease of collection, identification and enumeration, coupled with the existence of extensively tested, well-established, customisable and universal or region-specific multidimensional population and community metrics for biomonitoring (Bongard et al., 2018; Dallas et al., 2018; Dickens & Graham, 2002; Magurran, 2004; Palmer & Taylor, 2004). As such, changes in the abundance and community structure of benthic macroinvertebrates have been employed as proxy indicators of environmental changes from aquatic metal(loid) pollution in river basins globally and regionally (Bere et al., 2016; Byrne et al., 2012; Makalane et al., 2020; Mir et al., 2022; Nnoli et al., 2019; Serrano Balderas et al., 2016). Furthermore, the significant correlation between the conventional physicochemical and macroinvertebrate-based methods in the ecological assessment of stream ecosystems and their complementarity is continuously being established (Edegbene et al., 2023; Jiang et al., 2021). In several studies, the taxonomic and functional attributes of macroinvertebrates have been largely utilised for bioassessment, proven reliable and even preferred (Mangadze et al., 2019; Agboola et al., 2019; Espinoza-Toledo et al., 2021; G. Liu et al., 2024).

Biomonitoring of lotic environments in sub-Saharan Africa using macrozoobenthos incorporates the well-grounded and customisable taxonomic attributes, functional metrics and region-specific rapid bioassessment frameworks (RBFs) (Dallas et al., 2018; Kaaya et al., 2015; Mangadze et al., 2019). The multidimensional concept offers the benefit of comprehensive evaluation while utilising regionally or locally sensitive methods for stream health assessment with minimal ecological impacts (Mangadze et al., 2019). For instance, in southern Africa, region-specific RBFs have been effectively utilised for stream health assessments in Zambia (ZISS; Dallas et al., 2018), South Africa (SASS- 5; Dickens & Graham, 2002), Namibia (NASS; Palmer & Taylor, 2004), and the Okavango Delta (OKAS; Dallas, 2009).

The Central African Copperbelt (CACB), comprising the Congolese and Zambian Copperbelts, with a rich network of stream ecosystems, is a mineral-rich region (Zientek et al., 2014) characterised by intensified mining, industrial expansion and rapid population growth (Larmer, 2021). Hence, the lotic systems of CACB are threatened by anthropogenic disturbances (e.g. mining) causing water pollution (Ouma et al., 2022), reduction of ecosystem goods and services and changes in biodiversity (Peša, 2022) that warrant appropriate actions. Therefore, there is need to explore nature-based eco-friendly assessment approaches, such as biomonitoring using macroinvertebrates, to inform management interventions on the CACB. Despite the multiple benefits previously mentioned, few studies on the CACB (e.g., Banda et al., 2023; Dallas et al., 2018; Ngera et al., 2019; Patrick, 2015) have incorporated benthic macroinvertebrates as biomonitors of stream ecosystem health. Nevertheless, these studies did not focus on the ecological and functional responses of macroinvertebrates to metal(loid) contaminants in streams. For instance, on the Zambian Copperbelt, Banda et al. (2023) only investigated the impact of non-metallic ionic contaminants including Ca, Mg, Na, HCO_3, SO_4, NH_4 and PO_4, alongside other biogeochemical properties of streams in the Barotse floodplain on benthic macroinvertebrate community structure and function. Similarly, on the Congolese Copperbelt, Patrick (2015) and Ngera et al. (2019) correlated the responses of macroinvertebrate assemblages to nitrate, phosphate and sulphates concentrations in the urban Kinshasa's Funa stream and the Neira stream of Kahuzi-Biege National Park in western Democratic Republic of the Congo, respectively. All the authors above, however, unanimously recommended the conjunctive application of traditional physicochemical techniques with biomonitoring methods for optimal assessment of water quality. In another study, Dallas et al. (2018) developed the Zambian Invertebrate Scoring System (ZISS) protocol for rapid bioassessment of southern African tropical river systems. During the ZISS development, while organic and non-metallic contaminants were incorporated, the potential influence of metal(loids) on benthic macroinvertebrates was largely omitted and only briefly and indirectly inferred from previous studies (e.g. Pettersson & Ingri, 2001). To fill this knowledge gap, the present study therefore, integrated macroinvertebrate communities as the biomonitoring complement to conventional aquatic metal(loid) pollution monitoring on the CACB. The study examined the correlation between the taxonomic and functional responses of benthic macroinvertebrates, and environmental changes from metal(loid) pollution of stream ecosystems in the region. The study employed (1) conventional physicochemical assessments of water and sediment quality; (2) taxonomic and functional responses of macroinvertebrates as bioindicators of environmental changes using multidimensional multivariate taxonomic and functional indices and community metrics; and (3) the correlation between the taxonomic and functional responses of macroinvertebrate communities and physicochemical environmental fluxes from metal(loid) pollution in evaluating stream health condition on the CACB. Ultimately, the study aimed at establishing the effectiveness of integrating and utilising benthic macroinvertebrates as an eco-friendly NBS to comprehensively establish the ecological condition of stream ecosystems for sustainable management of water resources in mining landscapes.

2 Materials and Methods

2.1 Study Area and Sampling Sites

Zambia's 125,826 km^2 North-western Province (NWP) extends 10—18° S to 22—33° E within the CACB at 872—1737 m above sea level (ZSA, 2022). Angola borders the NWP on the west, the Democratic Republic of Congo on the northern border, the Copperbelt Province south-eastwards, the Central Province southwards, and the Eastern and Western Provinces on the northeast. The NWP altitude gently decreases west to east from the DRC border and plateaus towards the Zambezi depression with characteristic uplifted surfaces. The Province lies in the tropical savanna zone, dominated by humid subtropical and traces of tropical savanna and subtropical highland oceanic climates with mean annual temperatures between 18 and 26 °C. The annual trimodal season is marked by wet-rainy (November–April), cold-dry (May–July) and hot-dry (August-October) cycles. The rainy season peaks in December-January, with mean rainfall between 1200—1500 mm. Approximately 76.4% of NWP (ca. 96,240 km^2) lies within Agro-ecological Zone (AEZ) III; smaller miombo-dominated forests, interspersed with savanna grasslands, extend to AEZ IIa (ca. 3,150 km^2) and AEZ IIb (ca. 1,020 km^2).

The NWP comprises 11 districts, with Solwezi (study area) as the administrative capital. Three large copper mines, Kansanshi, Lumwana and Kalumbila, operate within the NWP with a projected collective copper production of 640,000 Mt by 2030 (Besa, 2023). Solwezi District covers approximately 3335.8 km^2 and lies 1297–1422 m ASL (ZSA, 2022). The District's human population is ca. 332,623 (2022 census), with an 8% annual growth rate (ZSA, 2022). The Kansanshi sub-catchment (KSC) within Solwezi District is located between 26° 20'—26° 35' E and 12° 0'—12° 12' S (Fig. 1).

The KSC is a significant water tower for Solwezi municipality and surrounding settlements. A rich dendritic network of headwater streams drains into the Solwezi, Kifubwa, and Mutanda Rivers, which converge into the Lunga River, a tributary of the Kafue River. The strategic location of the KSC within a rapidly expanding mining, urbanising and moderately rural agricultural region renders its streams vulnerable to anthropogenic pollution (Hasimuna et al., 2021; Xavier et al., 2022). Minerals in the KSC lie within the Katanga Supergroup sediments of the Zambian CACB. Cu primarily occurs on sulphide complexes (chalcopyrite and bornite) and mixed oxides (chrysocolla-malachite), while Au deposits are associated with copper mineralisation (FQM, 2020). Metal contamination of the surface sediments of four headwater streams within the KSC was assessed (Fig. 1). The geo-location, stream hydromorphology, riparian zone and adjacent land-use characteristics were described per stream site (Table 1).

2.2 Sampling and Laboratory Analysis

On-site measurements, sampling and laboratory analyses were conducted monthly between May 2022 and April 2023, on 252 and 144 sediment and

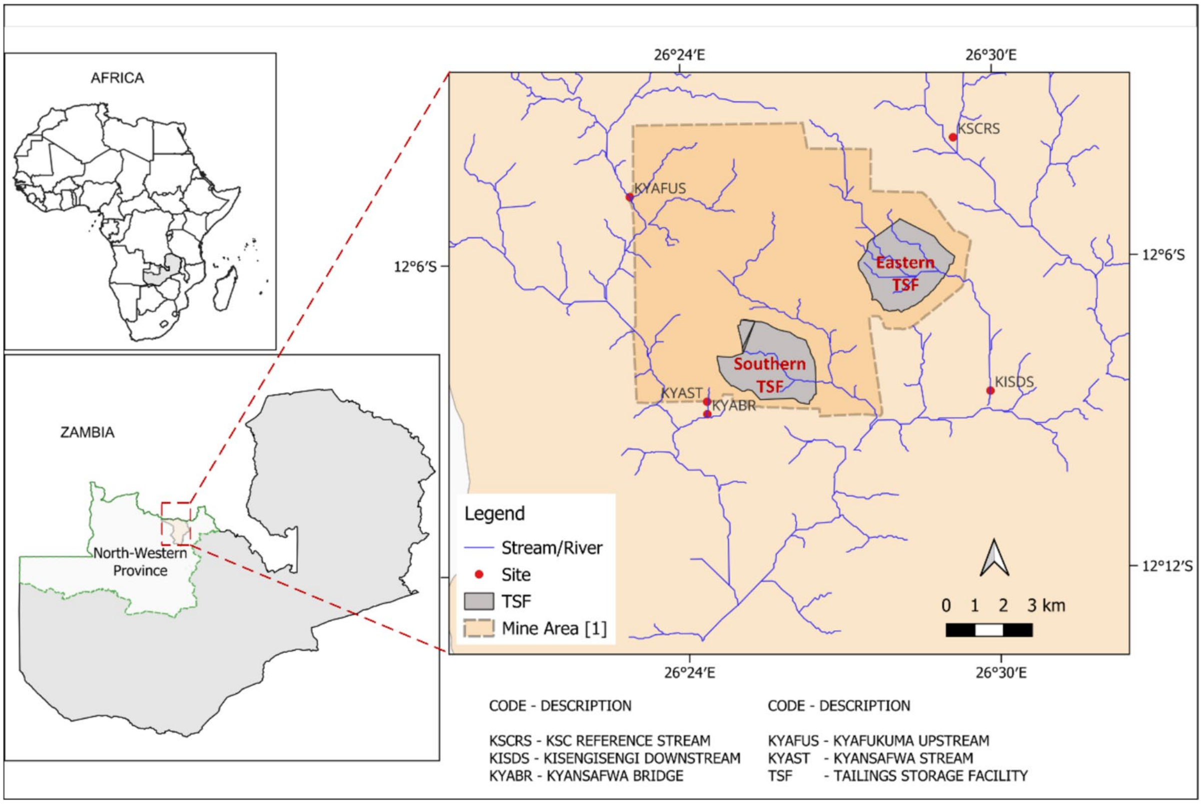

Fig. 1 Geo-location of the study area and sampling sites (inset) in the Kansanshi sub-catchment of Zambia's north-western Copperbelt

Table 1 Geo-location and environmental characteristics of the macroinvertebrate sampling sites in the KSC stream network

#	Stream and site ID	GPS location and elevation (m a.s.l)	Mean channel width (W) and depth (D) (m)	Mean velocity (m/s)	Bed-sediment	Riparian zone features	Adjacent land use
1	Kisengisengi downstream (KISDS)	12° 6′ 43.1"S 26° 29′ 53.6″ E 1329 m	W: 3–5 D:0.5–1.0 Wadable	0.28–0.81	Sandy	Emergent macrophyte; trees; shrubs; 5% canopy	Water abstraction, farming;
2	Kyansafwa stream (KYAST)	12° 8′ 41.9"S 26° 24′ 26.7″ E 1366 m	W:3–4 D:0.5 – 1.0 Wadable	0.21–0.64	Loamy-sand	Grass and shrubs; 60% canopy	Restricted area; reservoir outflow
3	Kyansafwa Bridge (KYABR)	12° 8′ 55.0"S 26° 24′ 24.6″ E 1350 m	W:1.5 D:0.5 Wadable	0.26–0.30	Loamy-sand	Emergent macrophytes; grass; open	Water abstraction; public access
4	Kyafukuma upstream (KYAFUS)	12° 4′ 33.7"S 26° 22′ 59.7"E 1358 m	W:3–5 D:0.5 Wadable	0.50–0.59	Sandy	Emergent macrophytes; grasses; open	Restricted area; drains into wetland from forest reserve

macroinvertebrate samples, respectively, from the four streams in the KSC (Table 1).

2.3 Streamwater Physicochemical Measurements

In-situ water pH, redox-potential (Eh, mV), electrical conductivity (EC, µS/cm), temperature (Temp, °C), and dissolved oxygen (DO, mg/L) were determined in triplicate using a portable multi-probe meter (WTW, 3220, Germany). Water velocity (Vel, m/s) was measured at three-point intervals across the stream channel width using a portable flowmeter (SonTek® FlowTracker2, Xylem Analytics, UK).

2.4 Sediment Sampling, Processing and Metal Analysis

Triplicate 200 g sediment samples were collected at each site over the cold-dry, hot-dry and cold-wet seasons using a precleaned plastic scoop at 0–5 cm depth across the stream channel width. Similarly, reference sediments from the KSCRS stream (26° 29′ 14.6″ E, 012° 03′ 41.0″ S), a protected non-mining area within KSC, were also sampled. The sediments were stored at 4 °C in pre-rinsed, labelled polyethene bottles. In the laboratory, the samples were air-dried at 25–27 °C for 3 days and then oven-dried at 70 °C for 24 h. The samples were then homogenised and sieved through a series of 1-mm and 250-µm stainless steel sieves (BS 410, Endecotts Ltd., London, UK) and stored in 150 g transparent labelled zip-lock polyethene bags. The percentage sediment organic matter (SOM) and clay-silt ratio (CSR) were determined from the dried 2-mm-sieved samples using the "wet-reduction" and "wet-sedimentation" methods (Day, 1965; Walkley & Black, 1934).

Multi-element concentrations in the sediment samples were measured using a portable Niton XL3t GOLDD+ (pXRF) analyser (GOLDD, Thermo Scientific, Waltham, MA, USA) fitted with a 50 kV anode X-ray tube sample excitation system, an 80 MHz signal processor and a "Geometrically Optimised Large Area Drift Detector". Before analysis, the equipment performs an internal calibration and only allows measurements upon verification of operational parameters (Rouillon et al., 2017). The GOLDD detects and quantifies the element intensities, which are internally converted to concentrations. The following instrument parameters were used: start-up-to-warm-up time 3–5 min; reading time 60 s; reading mode: all-geo; unit ppm or %; SE margin ± 2 sd; and detection limits (ppm) (As—4; Cr—10; Cu—10; Ni—25; Pb—5: Zn—7). Six replicates were analysed per sample. For quality control and method validation, manufacturer-supplied reference materials (Thermo Fisher Scientific, Inc.) and reference site samples (KSCRS) were analysed. The analytical accuracy of the instrument was determined by calculating recoveries for As, Ni, Pb, Cu and Zn. The concentrations were interpreted using Thermo Scientific NDT software (ver. 8.5.1).

2.5 Macroinvertebrate Sampling, Processing and Analysis

A multihabitat sampling of macrophytic vegetation, leaf litter, stones-in-current, gravel-sand-mud, riffles and runs, and pools, was conducted using a 500-µm square frame kick-net (30 by 30 cm) with a 20- to 60-s sampling effort per sample proportional to the occurrence of a biotope category in a site (Dallas et al., 2018; Feeley et al., 2012). The Kyafukuma upstream site (KYAFUS), draining from a protected forest area and located away from nearby industrial mining activities, was used for reference (Table 1). Sampling was conducted in triplicates per site over three distinct hydroperiods (cold-dry, hot-dry and cold-wet seasons). Each aquatic biotope present, including macrophytic vegetation, leaf litter, stones, and gravel-sand-mud, was disturbed by kicking or moving vegetation or stones to dislodge the macroinvertebrates into the kick-net placed downstream (Dallas et al., 2018; Dickens & Graham, 2002). Stones with attached macroinvertebrates were gently scrubbed with a soft brush and rinsed with distilled water to wash off the specimen into the kick-net. Moveable large stones/boulders were gently rolled and the substrate was disturbed ("kicked") with the kick-net placed downstream to trap the dislodged macroinvertebrates. Macroinvertebrates attached to the underside of the boulders were gently scrubbed with a soft brush into the kick-net. Then net was carefully but swiftly lifted from the water, and the samples were washed off into trays while removing debris. The integrated samples for each site were transferred into 500-ml plastic containers under 70% ethanol and stored at 4 °C for laboratory processing.

Using Gerber and Gabriel (2002a, 2002b) and Lowe (2012) taxonomic guides, the macroinvertebrates were identified at the family level, considered optimal (Dallas et al., 2018), using a stereomicroscope (OPTIKA SZM-D, Italy; ×10 and ×40 magnification). Depending on the sample density, the whole sample or approximately one-tenth, for families with counts exceeding 50–100 individuals (Demars et al., 2012) was sorted, counted then up-scaled to obtain the taxa and total abundances and additional community metrics (Bongard et al., 2018; Dalu et al., 2017).

Additionally, the macroinvertebrate taxa were classified according to the five dominant functional feeding groups (FFG) (collector-gatherers (CG), collector-filterers (CF), predators (P), scrapper-collectors (SC) and shredders (SH) (Akamagwuna & Odume, 2020; Dalu et al., 2017; Merritt et al., 2019) and further verified from the "Benthic Macroinvertebrate Master Taxa List" online database (BioNet, 2024). Taxa sensitivity weightings of stream pollution level were established following the Dickens and Graham (2002) South African Scoring System version 5 (SASS- 5) and Dallas et al. (2018) Zambian Invertebrate Scoring System version 1 (ZISS- 1) rapid bioassessment protocols into: (a) low sensitivity/high tolerance 1–5; (b) moderate sensitivity/tolerance 6–10; (c) and high sensitivity/low tolerance 11–15. The ZISS- 1 was used to evaluate the stream site health based on the total sensitivity weightings for the families present. For each site, the ZISS- 1 score was divided by the taxa present to obtain the average score per taxon (ASPT). Both metrics were then used determine the stream condition as (Dallas et al., 2018): (i) "Excellent" (ZISS- 1 > 100; ASPT > 7), (ii) "Good" (ZISS- 1 80–100; ASPT 5–7), (iii) "Fair" (ZISS- 1 60–80; ASPT 3–5), (iv) "Poor" (ZISS- 1 40–60; ASPT 2–3), and (v) "Very Poor" (ZISS- 1 < 40; ASPT < 2). The EPT relative abundance (%) and EPT/Chironomidae ratio were also used for stream health condition assessment. Higher EPT relative abundance (%) indicates "higher" stream health status, while a lower EPT/Chironomidae ratio depicts "increased" environmental stress.

Following Magurran (2004, 2021), macroinvertebrate community metrics, including Margalef's richness (M), Simpson's diversity (D), Pielou's evenness (J) and Shannon–Wiener's diversity index (H'), were evaluated to characterise their spatiotemporal responses to environmental perturbations. Moreover, the H'-index score was used to classify the site pollution status based on the macroinvertebrate community diversity ranking as follows: (a) $H' < 1$ "Low" (highly polluted); (b) $1 \leq H' < 2$ "Low-to-moderate" (moderately polluted); (c) $2 \leq H' < 3$ "Moderate-to-high" (slightly polluted); (d) $3 \leq H < 4$ "High" (less polluted) and $H' > 4$ "Pristine" (no pollution) (Magurran, 2004; Shannon, 1948).

2.6 Data Analysis

All statistical analyses (at $p < 0.05$ significance level) and graphical visualisations were conducted in R software (R Core Team, 2023). The data were tested for normality using the Shapiro–Wilk multivariate normality test and $\log_{10}$ $(x + 1)$-transformed due to non-normal distribution. Since the data remained non-normal after transformation, the 1-way ANOVA Kruskal–Wallis (K) non-parametric test was conducted on the original homogenous data, given the absence of extreme outliers, followed by *post-hoc* Dunn's pairwise multiple comparison tests with Bonferroni-correction for significant differences to establish the differences in environmental variables, biological attributes and biotic indices among the KSC streams.

Different functions pulled from the R package "vegan" were used in subsequent multivariate analyses and ordination tests to establish the associations between environmental variables and biotic attributes among the streams. One-factor ANOSIM (analysis of similarities), with Bonferroni p-adjusted Dunn's *post-hoc* test, was used to spatially compare the dissimilarity in the structure of the macroinvertebrate communities based on the Bray–Curtis percentage dissimilarity index. In addition, the square root of the Bray–Curtis dissimilarity SIMPER (similarity percentage) test was used to determine the average contribution of each macroinvertebrate taxa, the most influential taxa, and taxa that significantly contributed to average between-site dissimilarity among the streams. Finally, the association among the macroinvertebrate taxa, environmental variables and biotic indices were examined using the canonical correspondence analysis (CCA) on a χ^2-transformed data matrix with 999 unrestricted Monte-Carlo permutations. A constrained CCA with the "ordistep" function on automatic bidirectional variable selection was used to select the best models that displayed the set of environmental variables and biotic indices, respectively, with a joint significant influence on macroinvertebrate distribution in the different streams. The independent contributions of environmental variables to macroinvertebrate taxa composition and distribution were determined using hierarchical partitioning for CCA coordinates using the "rdacca.hp" package. The significance of the associations between environmental and biological attributes was tested using the "anova.cca" function.

3 Results

3.1 Characterisation of Environmental Variables

From Table 2, all the environmental parameters varied significantly in at least one of the streams (22.6

Table 2 Spatial variability of environmental parameters and sediment metal concentrations (mean ± SD) in the four KSC streams

Parameter	KISDS (n = 60)	KYAST (n = 60)	KYABR (n = 60)	KYAFUS (n = 60)	Pooled (n = 240)	$K\chi^2_{df=3}$	*p*
pH	7.76 ± 0.79^{a}	6.81 ± 0.37^{b}	7.24 ± 0.34^{c}	7.56 ± 0.58^{ad}	7.34 ± 0.66	83.97	< 0.01*
DO (mg/L)	3.74 ± 0.64^{a}	3.69 ± 0.82^{a}	4.29 ± 0.84^{bc}	3.56 ± 1.64^{ad}	3.82 ± 1.09	197.74	< 0.01*
Temp (° C)	23.80 ± 1.11^{a}	22.42 ± 1.73^{b}	21.91 ± 1.51^{bc}	22.17 ± 2.04^{bcd}	22.58 ± 1.78	39.35	< 0.01*
Eh (mV)	-38.05 ± 39.33^{a}	21.86 ± 32.74b	-11.47 ± 34.39^{cd}	-33.39 ± 33.37^{ad}	-15.26 ± 42.14	32.02	< 0.01*
EC (µS/cm)	1370.40 ± 35.24^{a}	315.39 ± 113.35^{b}	264.94 ± 75.52^{c}	443.97 ± 38.69^{d}	598.67 ± 457.04	67.67	< 0.01*
Vel (m/s)	0.62 ± 0.44^{a}	0.28 ± 0.05^{bd}	0.18 ± 0.03^{ce}	0.55 ± 0.22^{af}	0.41 ± 0.31	152.1	< 0.01*
SOM (%)	1.04 ± 0.24^{a}	1.11 ± 0.88^{b}	2.33 ± 1.21^{c}	0.72 ± 0.24^{bd}	1.30 ± 0.98	84.12	< 0.01*
CSR	3.33 ± 0.82^{a}	3.10 ± 2.40^{ab}	10.20 ± 13.75^{abc}	4.73 ± 2.67^{ad}	5.34 ± 7.64	22.59	< 0.01*
As (mg/kg)	4.39 ± 2.89^{a}	5.72 ± 2.85^{ab}	6.76 ± 2.50^{bd}	4.22 ± 2.29^{ace}	5.27 ± 2.83	37.64	< 0.01*
Cu (mg/kg)	49.98 ± 39.12^{a}	81.16 ± 27.37^{b}	128.05 ± 39.31^{c}	490.88 ± 154.63^{d}	187.52 ± 196.02	187.40	< 0.01*
Ni (mg/kg)	69.41 ± 19.59^{a}	93.38 ± 31.77^{b}	110.27 ± 32.32^{c}	84.27 ± 19.28^{bd}	89.33 ± 30.23	56.77	< 0.01*
Zn (mg/kg)	38.54 ± 13.69^{a}	52.64 ± 15.58^{b}	50.59 ± 16.94^{bc}	36.72 ± 12.37^{ade}	44.62 ± 16.27	59.84	< 0.01*
Pb (mg/kg)	5.41 ± 3.52^{a}	7.82 ± 2.74^{b}	6.40 ± 2.71^{ab}	3.37 ± 1.85^{cde}	5.75 ± 3.20	74.00	< 0.01*

p-values with an asterisk (*) indicate significant differences at $p < 0.05$. Different lowercase superscript letters connote significant variability between sites at $p < 0.05$ using post-hoc Dunn's test. SOM – sediment organic matter, CSR – %clay:%silt ratio, and $K\chi^2$ denotes Kruskal–Wallis χ^2 statistic, df = degrees of freedom

$< K\ \chi^2_{df=3} < 197.7$, all $p < 0.01$). Dunn's *post-hoc* test with Bonferroni p-value adjustment indicated pairwise site-specific differences for the different variables ($0.001 \leq p \leq 0.05$). The water pH was fairly neutral across the streams except at KYAST (6.81 ±0.37), where it was significantly lower. DO ranged from 3.36 ±1.04 mg/L at KYAFUS to 4.29 ±0.84 mg/L at KYABR, which reported a significantly higher concentration than the other streams. Water Temp at KISDS (23.8 ±1.11 °C) was significantly higher compared to other streams. The EC at KISDS (1370.4 ±35.2 µS/cm) was generally 3-to-fivefold compared to the rest of the streams and also significantly higher. Water Vel was lowest at KYABR (0.18 ±0.03 m/s) and highest at KISDS (0.62 ±0.44 m/s). Vel at KISDS was also 22% higher compared to the catchment pooled Vel (0.41 ±0.31 m/s).

From sediment analysis, KYABR recorded significantly higher SOM (2.33 ±1.21%) and CSR (10.21 ±13.8), respectively. The mean As at the KYABR (6.76 ±2.5 mg/kg) and KYAST (5.72 ±2.85 mg/kg) were significantly higher than the remaining streams. Cu in sediment was highest at the KYAFUS (490.9 ±154.6 mg/kg), although all the streams sampled varied significantly from each other for Cu concentrations. Furthermore, pooled Cu concentration was 62% below the highest value at KYAFUS but 32–74% above the other three streams sampled. The highest Ni concentration recorded at KYABR (110.3 ±32.2 mg/kg) was 109% above the pooled mean Ni (89.33 ±30.23 mg/kg). All the paired stream comparisons for Ni were significantly different except for KYAST (93.38 ±31.77 mg/kg) versus KYAFUS (84.27 ±19.28 mg/kg). The mean Zn in sediment ranged from 36.72 ±12.37 mg/kg at KYAFUS to significantly higher concentrations (52.64 ±15.58 mg/kg) at KYAST. Pb in sediments was significantly higher at KYAST (7.82 ±2.74 mg/kg) and KYABR (6.40 ±2.71 mg/kg). Overall, the metal content in stream sediments increased in the order As < Pb < Zn < Cu < Ni at KISDS and KYAST, respectively. Similarly, metal concentrations followed the order Pb < As < Zn < Ni < Cu at KYABR and KYAFUS, respectively.

4 Biological Diversity and Ecological Quality

4.1 Macroinvertebrate Composition and Distribution

A total of 18118 individuals were sampled and sorted into three classes (Insecta, Crustacea and Gastropoda), 10 orders and 30 families (Online Resource 1). Insecta, Gastropoda and Crustacea comprised 70, 20 and 10% of the orders, respectively. Considering the family level distribution of class Insecta comprising 80% of recorded families, 23.3% were Diptera (Dipt), 16.7% each were Coleoptera (Cole) and Hemiptera (Hemi), 10% comprised Odonata (Odon), and 3.3% each for Trichoptera (Tric) and Plecoptera (Plec).

From Table 3, the spatial abundance ranged from 3514 (19.4%) at KISGS to 5179 (28.6%) individuals at KYABR and varied significantly ($K\chi^2_{df(3)} = 27.81$, $p < 0.05$). The abundance at KYAFUS was significantly lower than at KYAST and KYABR but higher compared to KISDS (Dunn's test, $p < 0.05$). Furthermore, the relative abundance of Diptera was highest at KISDS (26.2 ±3.9%) and KYABR (22.1 ±8.6%), while Hemiptera (30.6 ±11.9%) and Odonata (23.8 ±3.9%) were highest at KYAST and KYAFUS respectively. The pooled relative abundances were lowest for Stylommatophora (0.4 ±0.7%) and highest for Hemiptera (21.4 ±9.7%) and Diptera (21.1 ±10.4%), respectively.

Considering mean abundance at the family level (Online Resource 1), Atyidae (51.7 ±44.3 ind.) and Caenidae (33.3 ±9.1 ind.) were significantly higher at KISDS ($p = 0.045$) and KYAFUS ($p = 0.029$). Furthermore, Lymnaeidae (114.7 ±88.1 ind.) and Nepidae (44.0 ±48.9 ind.) were significantly higher at KYABR ($p = 0.048$) and KYAST ($p = 0.045$) respectively. Mean Simuliidae abundance (107.9 ±37.6 ind.) was significantly higher at KYAFUS ($p = 0.048$). Two gastropod families, Neritidae (108 ±36.4 ind.) and Planorbidae (96.7 ±70.5) had a significantly higher abundance at KISDS ($p = 0.045$).

The overall functional feeding group (FFG) categorisation at the family level ranked the macroinvertebrate into predators (P, 57.9%), collector-gatherers

Table 3 Structure and trophic characteristics of benthic macroinvertebrate orders in KSC streams

Parameter	KISDS ($n = 12$)	KYAST ($n = 12$)	KYABR ($n = 12$)	KYAFUS ($n = 12$)	Pooled ($n = 36$)	$K\chi^2_{df=3}$	p
Abundance (ind. and %)	3514 (19.40)^{ae}	5020 (27.71)^{ac}	5179 (28.59)^{a}	4405 (24.31)^{bdf}	18,118 (100)	27.81	< 0.00*
Rel. abundance (%)							
Coleoptera (Cole)	14.05 ± 3.90	18.64 ± 8.05	11.78 ± 4.2	14.15 ± 12.53	14.66 ± 7.29	0.64	0.89
Decapoda (Deca)	2.23 ± 3.87	1.05 ± 1.81	1.48 ± 0.95	3.69 ± 4.06	2.11 ± 2.75	1.13	0.77
Diptera (Dipt)	26.24 ± 15.49	17.51 ± 10.44	22.13 ± 8.57	18.45 ± 10.08	21.08 ± 10.40	2.28	0.52
Ephemeroptera (Ephe)	9.44 ± 6.34	11.93 ± 6.14	5.87 ± 4.17	12.16 ± 11.48	9.85 ± 6.95	1.92	0.59
Hemiptera (Hemi)	15.87 ± 12.03	30.59 ± 11.89	20.90 ± 2.86	18.38 ± 6.28	21.44 ± 9.73	3.31	0.35
Neotaenioglossa (Neot)	6.17 ± 10.69	n/a	4.45 ± 7.71	4.18 ± 7.24	3.70 ± 6.84	1.28	0.73
Odonata (Odon)	18.62 ± 1.26	15.80 ± 2.06	15.23 ± 5.25	23.81 ± 3.86	18.36 ± 4.62	5.67	0.13
Plecoptera (Plec)	0.21 ± 0.37	1.55 ± 2.68	3.01 ± 3.06	3.78 ± 3.99	2.14 ± 2.82	2.06	0.56
Stylommatophora (Styl)	0.77 ± 1.34	0.43 ± 0.75	0.34 ± 0.32	0.13 ± 0.23	0.42 ± 0.72	0.41	0.94
Trichoptera (Tric)	6.38 ± 7.27	2.50 ± 2.29	14.82 ± 18.9	1.26 ± 1.15	6.24 ± 10.32	2.52	0.47
Trophic guilds							
% CF	5.58 ± 2.86	0 ± 0	26.84 ± 19.43	9.21 ± 4.05	10.41 ± 13.53	7.41	0.06
% CG	13.72 ± 11.38	20.47 ± 1.75	5.61 ± 2.03	17.57 ± 3.61	14.34 ± 7.82	4.74	0.19
% P	55.43 ± 13.22	68.89 ± 2.89	51.33 ± 27.12	60.38 ± 6.92	58.96 ± 14.88	2.59	0.46
% SH	8.00 ± 2.01	0.0 ± 0	14.88 ± 8.74	12.84 ± 1.85	8.93 ± 7.15	7.72	0.05
% SC	17.28 ± 2.82^{a}	10.84 ± 3.42^{a}	1.34 ± 0.89^{a}	0.0 ± 0.0^{b}	7.36 ± 7.65	10.53	0.02*

p-values with an asterisk (*) indicate the overall significant difference in at least one site at $p < 0.05$. Different lowercase superscript letters connote significant variability between sites at $p < 0.05$. *CF* collector-filterers, *CG* collector-gatherers, *P* predators, *SH* shredders, *SC* scrapper-collectors, and ind. = individuals

(CG, 14.8%), collector-filterers (CF, 10.5%), shredders (SH, 10%) and scrapper-collectors (6.8%) (Online Resource 1). The P guild comprised five orders ranked as Odonata (34.7%) > Hemiptera (31.8%) > Diptera (18.9%) > Coleoptera (7.5%) > Plecoptera (7.1%). Within the CG, Ephemeroptera (67.5%) was most abundant above Coleoptera (22%) and Decapoda (10.4%). The CF consisted of 58.9 and 41.1% Trichoptera and Diptera, respectively, while the SH were uniquely Diptera. In the SC, two

Table 4 Macroinvertebrate order-level indices (mean ± SD) of the biotic integrity of streams in north-western Zambian Copperbelt ($n = 12$)

Index	KISDS	KYAST	KYABR	KYAFUS	$K\chi^2_{df=3}$	p
Taxa Richness (*TR*)	6^{a}	5^{a}	7^{b}	7^{b}	11.0	0.01*
Margalef's (*M*)	2.25 ± 0.08^{a}	1.38 ± 0.05^{b}	2.06 ± 0.07^{a}	1.82^{b} ± 0.04	10.33	0.02*
Rel. dominance (R_D)	24.03 ± 2.98^{a}	33.15 ± 7.67^{a}	40.44 ± 1.81^{b}	33.83 ± 1.65^{a}	8.13	0.03*
Pielou's (*J*)	0.9 ± 0^{a}	0.7 ± 0^{b}	0.85 ± 0^{a}	0.85^{a} ± 0	11.0	0.01*
Simpson's (*D*)	0.18 ± 0.02^{a}	0.23 ± 0.02^{a}	0.26 ± 0.02^{b}	0.22 ± 0.01^{a}	8.74	0.03*
Shannon–Wiener (*H*)	2.64 ± 0.11^{a}	2.23 ± 0.07^{a}	2.26 ± 0.11^{a}	2.43 ± 0.08^{a}	8.28	0.04*

p-values with an asterisk (*) indicate overall significant differences in at least one site at $p < 0.05$. Different lowercase superscript letters in a row indicate significant site-specific variability using *post-hoc* Dunn's test at $p < 0.05$

gastropod orders, Stylommatophora (69.3%) and Neotaenioglossa (3.7%), and one Insecta (Coleoptera, 27%) characterised the FFG.

In Table 3, a similar ranking of the pooled relative abundance at the order level for FFGs where P (59 ± 14.9%) > CG (14.3 ± 7.8%) > CF (10.4 ± 13.5%) > SH (8.9 ± 7.2%) > SC (7.4 ± 7.7%) was observed. Spatially, CF (26.8 ± 19.4%) and SH (14.9 ± 8.7%) were highest at KYABR, CG (20.5 ± 1.8%) and P (68.9 ± 2.9%) at KYAST but with no significant variability. However, SC (17.3 ± 2.8%) were significantly higher at KISDS ($p = 0.013$).

4.2 Indices of Biotic Integrity

In Table 4, taxa richness (TR) was lowest at KYAST (5) and significantly higher at KYABR and KYAFUS (7), respectively ($p = 0.03$). The Margalef's richness index (M) was, however, significantly higher at KISDS compared to KYAST ($p = 0.01$). The relative dominance, R_D (%) among the macroinvertebrate orders ranged from 24 ± 3% (KISDS) to 40.4 ± 1.8% (KYABR) and was significantly higher at KYABR ($p = 0.03$). Pielou's evenness index (J) at KISDS (0.9) was, however, significantly higher ($p = 0.005$). The Simpson diversity index (D) varied from 0.18 ± 0.02 at KISDS to a significantly higher value (0.26 ± 0.02) at KYABR ($p = 0.02$). In contrast, the Shannon–Wiener diversity index (H) was highest at KISDS (2.64 ± 0.11), followed by KYAFUS (2.43 ± 0.08) and lowest at KYAST (2.23 ± 0.07). Nevertheless, the *post-hoc* Dunn's test did not identify significant site-specific variability in macroinvertebrate diversity at the Order level from the H-index.

5 Macroinvertebrate Sensitivity Distribution Profiles as Bioindicators of Stream Health

The ZISS- 1 score, ASPT, EPT relative abundance, EPT/Chironomidae ratio, pollution tolerance index (TI) and the macroinvertebrate diversity ranking H-index were determined to evaluate the stream health condition (Table 5). The ZISS-1 score ranged from 36.3 ± 2.9 ("Very poor") at KYABR to 83.3 ± 4.6 ("Good") at KISDS. Consequently, KYABR score was significantly lower than KISDS ($p = 0.008$). The pooled average ZISS- 1 score (63.8 ± 19.5) indicated a "fairly" healthy stream network. The ASPT at KYABR (5.2 ± 0.4, "Good") was also significantly lower than KYAST (13.3 ± 0.9, "Excellent"). The overall ASPT (9.7 ± 3.2) reflected an "excellent" stream health condition. The EPT relative abundance ranged from 8.4 ± 3.1% ("Low") at KISDS to 25.2 ± 4.2% ("Moderate") at KYAFUS, while the EPT/Chironomidae ratio was 126 ± 62.5 ("Moderate") and 322 ± 106.5 ("High") in the respective streams and a generally "good" biotic condition.

Table 6 Distribution of tolerances among macroinvertebrate families in the KSC streams

Tolerance	Rating	KISDS	KYABR	KYAST	KYAFUS
High	1—5	10	6	10	8
Moderate	6–10	4	1	2	4
Low	11 – 15	1	0	1	1
Total		15	7	13	13

Table 5 Macroinvertebrate order-level assessment multimetric indicators (mean ± SD) of stream health in KSC, north-western Zambian Copperbelt ($n = 12$)

Metric	KISDS	KYAST	KYABR	KYAFUS	Pooled ($n = 36$)	$K\chi^2_{df=3}$	p
ZISS- 1 score	88.33 ± 4.62^{a}	66.67 ± 4.62^{a}	36.33 ± 2.89^{ab}	64.00 ± 0.00^{a}	63.83 ± 19.53	10.25	0.017*
ASPT	11.04 ± 0.58^{a}	13.33 ± 0.92^{a}	5.19 ± 0.41^{ab}	9.14 ± 0.00^{a}	9.68 ± 3.16	10.65	0.014*
H-index (HI)	2.64 ± 0.11^{a}	2.23 ± 0.07^{a}	2.26 ± 0.11^{a}	2.43 ± 0.08^{a}	2.39 ± 0.19	8.28	0.041*
Tolerance index (TI)	5.11 ± 0.45^{a}	5.13 ± 0.23^{a}	3.90 ± 0.19^{a}	4.88 ± 0.13^{a}	4.75 ± 0.57	7.05	0.071
%EPT rel. abundance	8.36 ± 3.12^{a}	19.66 ± 1.34^{a}	23.99 ± 16.23^{a}	25.51 ± 4.21^{b}	18.98 ± 10.53	7.00	0.072
EPT:Chironomidae ratio	125.99 ± 62.50^{a}	238.97 ± 39.81^{a}	n/a	322.09 ± 106.54^{a}	229.02 ± 107.13	5.07	0.079

p-values with an asterisk (*) indicate overall significant differences in at least one site at $p < 0.05$. Different lowercase superscript letters in a row connote significant site-specific variability using *post-hoc* Dunn's test at $p < 0.05$

The TI was lowest at KYABR (3.9 ±0.2, "High-tolerance") and highest at KISDS (5.1 ±0.1, "Moderate-tolerance"). At the same time, the pooled index (4.8 ±0.6) reflected a generally "highly tolerant macroinvertebrate community in the KSC. The spatial and pooled H'-index score was generally "moderate-to-high" and varied from 2.2 ±0.1 at KYAST to 2.6 ±0.1 at KISDS, and 2.4 ±0.2 overall indicating a "slightly polluted" stream network.

In Table 6, the distribution of macroinvertebrate families in response to pollution tolerance indicated generally higher proportions of highly-tolerant taxa in the four streams. KISDS and KYAST had 66.7 and 76.9% highly-tolerant and 26.7 and 30.8% moderately-tolerant families, respectively. At the same time, Perlidae (Plecoptera) (collected at KISDS and KYAFUS) and Helodidae (Coleoptera) (from KYAST) were the only highly pollution-sensitive families reported in the KSC (Online Resource 1).

5.1 Spatial Variability in Macroinvertebrate Community Structure

5.1.1 SIMPER Analysis

SIMPER analysis established the most influential orders and families pairwise across the four streams that contributed to at least 70% of the differences based on the Bray–Curtis dissimilarity index (Online Resource 2). At the order level, Odonata and Diptera were the highest contributors, accounting for 18.5–23.3% and 15.6–18.9% between-stream dissimilarities. Pairwise contributions were as follows: between KISDS and KYAST (Odonata 23.3%; Diptera, 14.0%), KISDS and KYABR (Neotaenioglossa 17.6%; Hemiptera 16.1%), KISDS and KYAFUS (Diptera 18.9%; Odonata 16.9%), KYAST and KYABR (Odonata, Coleoptera, Diptera, 44.1%), KYAST and KYAFUS (Odonata 22.4%; Diptera 19.3%), and KYABR versus KYAFUS (Diptera 15.6%; Hemiptera 14.5%). Considering the families, the pollution-tolerant generalist scrapper-collector (SC) Hydropsychidae and pollution-sensitive predatory Perlidae were the two top contributors overall, with 8.7–11.6% and 8.4–10.6%, respectively, to stream dissimilarities in macroinvertebrate community structure. The two top family contributors were as follows: KISDS versus KYAST (Hydropsychidae 8.7%; Perlidae 8.3%), KISDS versus KYABR (Perlidae 8.4%; Thiaridae 7.6%), KISDS versus KYAFUS (Perlidae 10.3%; Hydropsychidae 9%), and KYABR versus KYAFUS (Perlidae 10.6%; Coenagrionidae 7.6%).

Furthermore, the SIMPER test determined the significance of the contribution of each taxon at the order and family level to the overall pairwise dissimilarities (Online Resource 2). Ephemeroptera significantly influenced the overall differences in macroinvertebrate distribution between KISDS and KYAST ($p = 0.037$), while Decapoda was a significant contributor to the dissimilarities between KISDS and KYAFUS ($p = 0.046$) and KYAST versus KYAFUS ($p = 0.045$). Considering the number of families contributing to the significant dissimilarity between sites, KYAST versus KYABR recorded the highest number of taxa (11) under orders Coleoptera (Hydrophilidae, Helodidae, Dytiscidae, Gyrinidae), Diptera (Culicidae, Tabanidae), Hemiptera (Nepidae), Odonata (Coenagrionidae, Libellulidae), and Stylommatophora (Lymnaeidae) and Trichoptera (Hydropsychidae) ($p =$ 0.001–0.038). The lowest number of significantly contributing taxa (3) to pairwise dissimilarity was between KISDS and KYAFUS, comprising Neritidae, Simuliidae and Muscidae (p =0.032–0.039). In Table 7, the cumulative average between-site dissimilarity for orders and families ranged from 41.97% and 51.83% (KISDS versus KYAFUS) to 44.04% and 74.16% (KYAST versus KYABR), respectively, with more taxa and higher dissimilarity ratios at the family level.

Table 7 Average between-site percentage dissimilarity of macroinvertebrate taxa in KSC streams

Order				Family			
Stream	KISDS	KYAST	KYABR	**Stream**	KISDS	KYAST	KYABR
KYAST	43.11			KYAST	70.98		
KYABR	40.57	44.04		KYABR	72.96	74.16	
KYAFUS	41.97	42.49	43.36	KYAFUS	51.83	63.69	62.47

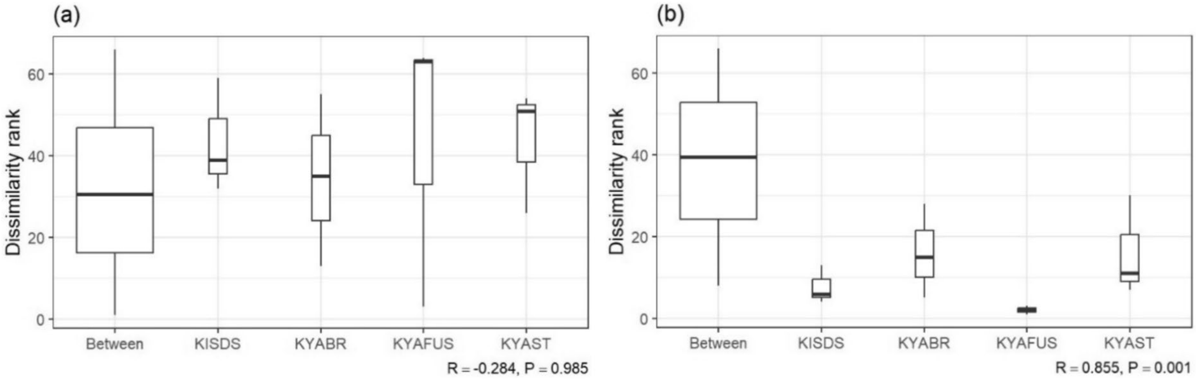

Fig. 2 Bray–Curtis dissimilarity for macroinvertebrate (**a**) orders and (**b**) families between and within the four streams in the KSC

5.1.2 ANOSIM Tests

ANOSIM test statistically compared the average dissimilarities in the distribution of macroinvertebrate orders and families between and within the four streams (Fig. 2). Little dissimilarity was observed for the distribution of macroinvertebrate orders between the four streams ($R = -0.284$, $p = 0.985$). However, at the family level, highly significant between-site differences were noted ($R = 0.855$, $p = 0.001$). Dunn's pairwise *post-hoc* comparison tests for significant dissimilarities across sites at the family level similarly indicated highly significant differences between KYAFUS and KISDS ($p = 0.00003$), KYABR ($p = 0.002$) and KYAST ($p = 0.000$). Furthermore, the order level dissimilarity rank at the 50 th percentile between streams was 30.5 and within streams from 35 (KYABR), 39 (KISDS), 51 (KYAST) to 63 (KYAFUS). At the family level, between-stream differences rank was 39.5 while dissimilarity within streams increased from 2 (KYAFUS), 6 (KISDS), 11 (KYAST) to 15 (KYABR).

5.2 Canonical Correspondence Analysis and Ordination

From Fig. 3, most taxa, both at the order and family level, were clumped around the centre of the CCA biplots. For instance, Orders Diptera, Hemiptera and Ephemeroptera and Families Potamonautidae, Aeshnidae, Chironomidae, Muscidae and Elmidae in respective biplots. Additionally, at the family level, KYAFUS (S4 C, S4 W, S4D) was located around the biplot centre.

At the order level, along the CCA1 axis, the distribution of Decapoda, Neotaenioglossa and Plecoptera was positively associated with As, Ni, DO and CSR in KISDS (S1 C), KYABR (S3 C) and KYAFUS (S4 C) (Fig. 3a). In addition, Coleoptera and Ephemeroptera positively correlated with Eh and Temp at KISDS (S1D), KYAST (S2 W) and KYAFUS (S4 W). On the CCA2 axis, the distribution of Odonata and Hemiptera in KYABR (S3 W) and KYAFUS (S4 W) was influenced by Cu concentration, while Vel and EC correlated with Trichoptera distribution. The hierarchical partitioning of the average independent contribution of each environmental variable ranged from 3.8% (pH) to 23.3% (Zn).

Regarding the families KISDS (S1 C-S1 W-S1D), the influence of pH, Temp and Vel was associated with the distribution of Atyidae, Thiaridae, Athericidae, and Perlidae (Fig. 3b). At the KYAST (S2 C-S2D-S2 W), Eh was associated with the distribution of Nepidae, while in KYABR (S3 C-S3D-S3 W), SOM and CSR correlated with Lymnaeidae, Dytiscidae abundances. The average independent contribution of individual environmental variables ranged from 1.29% (As) to 30% (EC), where Cu, Vel and EC significantly influenced the spatial distribution of macroinvertebrate families ($R^2_{adj} = 0.388$, $p = 0.005$).

Figure 4 illustrates the associations between biotic indices and the distribution of macroinvertebrate orders and families in the different stream sites, with most taxa at both levels clustered around the centre of the respective CCA biplots. In Fig. 4a, the ASPT, ZISS- 1 and tolerance index (tol_index) correlated positively with order Coleoptera in KYABR (S3 W), KYAST (S2 C, S2 W) and KYAFUS (S4

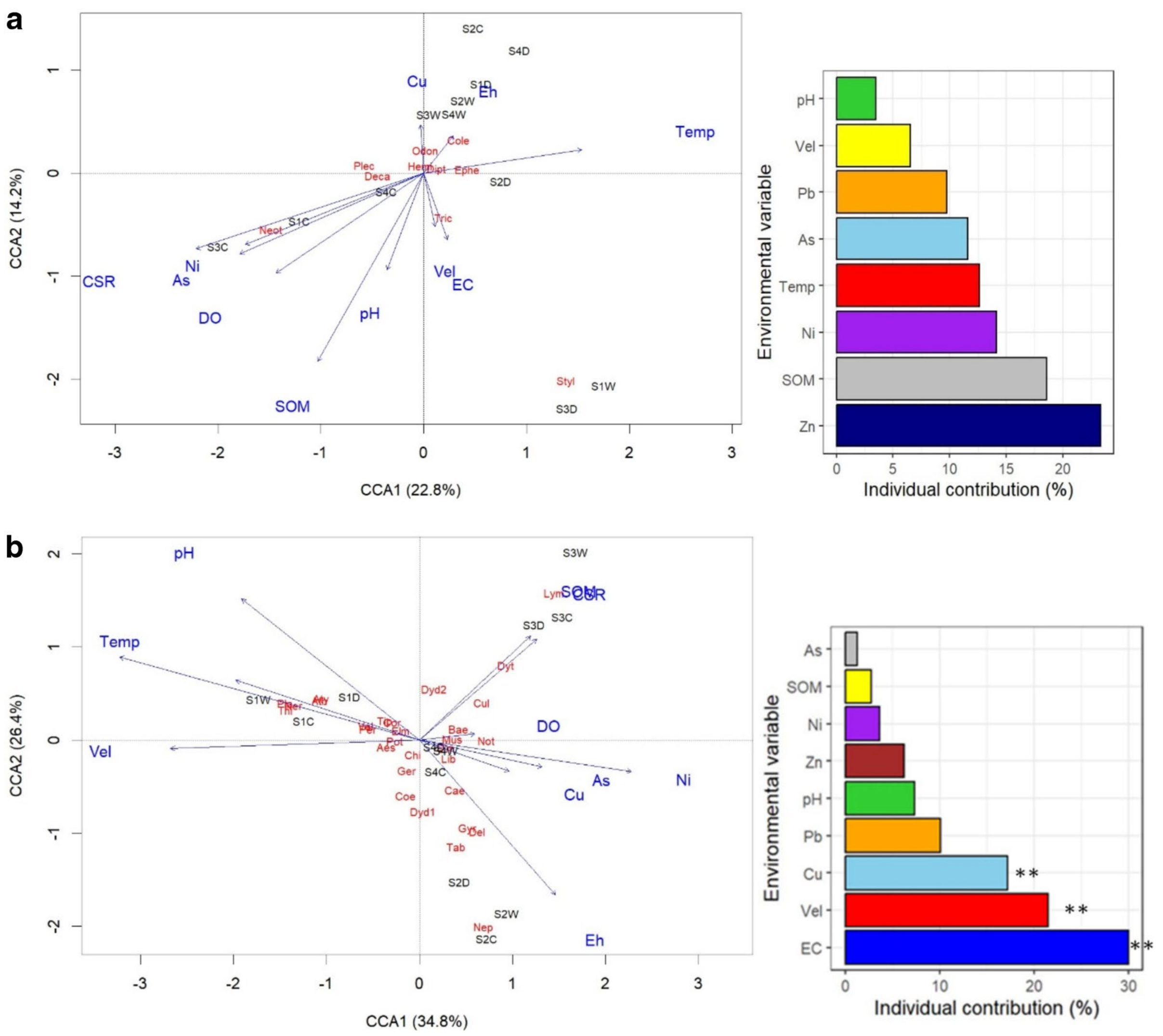

Fig. 3 Canonical correspondence analysis biplots of the influence of environmental variables on the spatial distribution of macroinvertebrate (a) orders and (b) families in the KSC streams. Independent contribution of environmental variables and significant contributors (** $=p \leq 0.01$) for macroinvertebrate orders ($R^2_{adj} = 0.712$) and families ($R^2_{adj} = 0.388$) are also depicted. Order codes (Table 1). Family codes (Online Resource 1)

W, S4D). The EPT relative abundance (% ept.r.ab) was moderately associated with Ephemeroptera in KYAST (S2D). The Shannon index strongly correlated with Trichoptera and Ephemeroptera in KYAST (S2D). The taxa dominance and Simpson index were strongly associated with Neotaenioglossa in KYABR (S3C). Individual index contributions ranged from Simpson (0.7%) to EPT relative abundance (45.1%) and accounted for 30.7% of the variation in the distribution of macroinvertebrate orders in the different streams.

Among the various families in Fig. 4b, taxa richness correlated with Hydropsychidae, Culicidae, Dytiscidae and Lymnaeidae in KYABR (S3 C, S3 W, 3D). Shannon index associated with Atyidae, Athericidae, Perlidae, and Thiaridae in KISDS (S1 W, S1 C, S1D). The individual index contribution to the distribution of families ranged from Simpson (6.5%) to ZISS- 1 (17.1%) with significant associations from Pielou's and ZISS- 1 ($p = 0.005$) and taxa richness ($p = 0.01$) while explaining 32.7% of the total variation in the distribution and composition of macroinvertebrate families in the

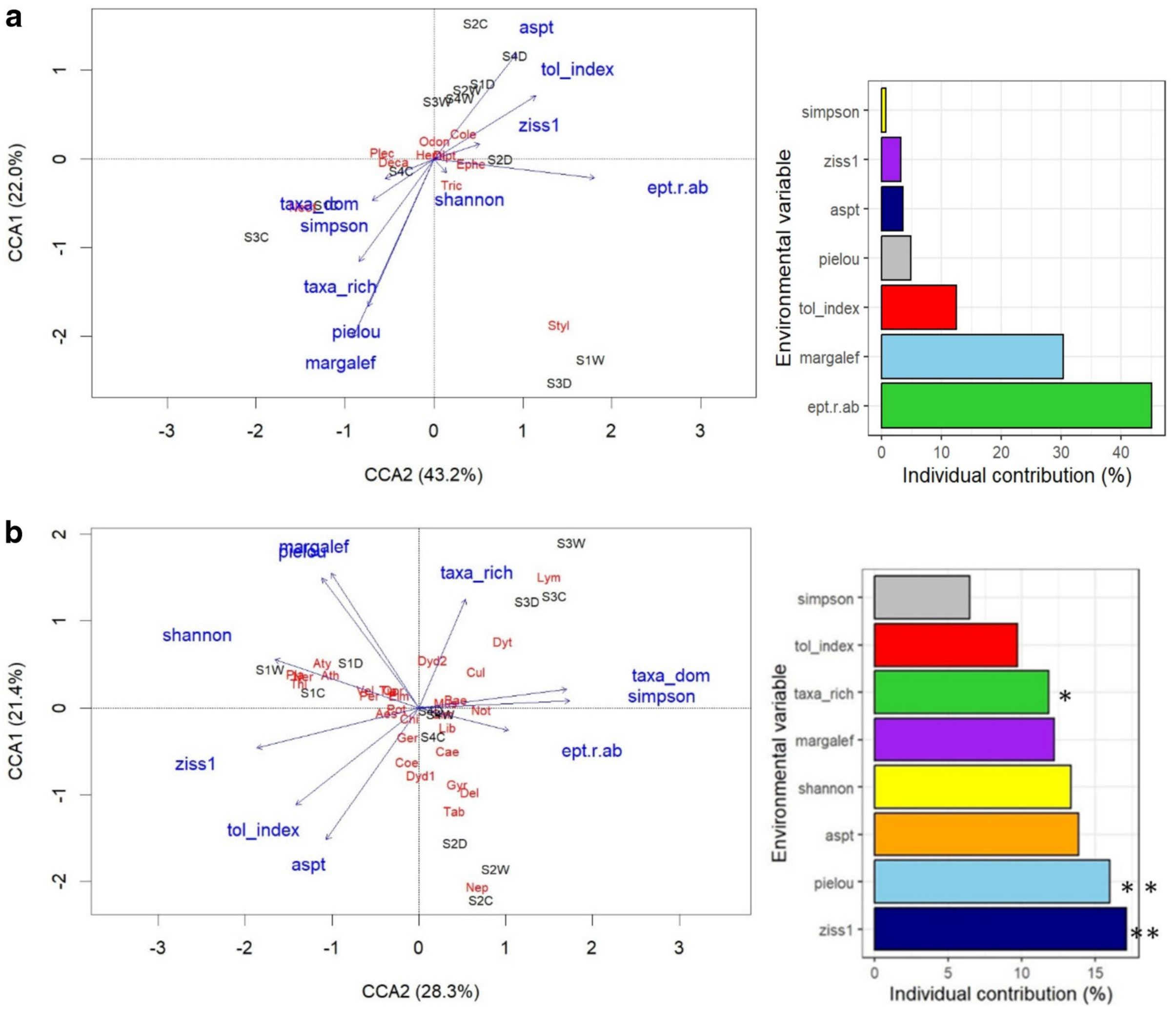

Fig. 4 Canonical correspondence analysis biplots of the influence of spatial distribution of macroinvertebrate (a) orders and (b) families on biotic indices in the KSC streams. Independent contributions of individual biotic indices and significant contributors (* $=p \leq 0.05$; ** $=p \leq 0.01$) influenced by macroinvertebrate orders ($R^2_{adj} = 0.307$) and families ($R.^2_{adj} = 0.327$) are also indicated. Order codes (Table 1). Family codes (Online Resource 2)

streams of the KSC. Furthermore, the most impacted KYABR (S3D, S3 C, S3 W) was negatively associated with ZISS- 1, ASPT and TI.

5.3 Macroinvertebrate Compositional Patterns and Responses to Environmental Variability

The order level constrained ordination analysis (CCA) did not yield significant influences for both environmental and biological metrics. However, at the family level, the selected environmental variables were marginally related to the distribution of macroinvertebrate families at the different streams along the first CCA1 ($\chi^2 = 0.341$, pseudo-$F_{3,8} = 2.551$, $p = 0.001$). Furthermore, Vel ($p = 0.035$), EC ($p = 0.005$), and Cu ($p = 0.03$) had significant associations with macroinvertebrate family composition in the respective sites. Considering the multivariate biotic indices, the selected metrics marginally reflected the biological attributes of the macroinvertebrate family composition at the different streams along CCA1 ($\chi^2 = 0.766$, pseudo-$F_{3,8} = 3.867$, $p = 0.001$). Correspondingly, taxa richness ($p = 0.01$), Pielou's evenness index ($p = 0.005$) and ZISS- 1 score ($p = 0.005$) significantly described the biological characteristics of macroinvertebrate families across the four streams.

6 Discussion

6.1 Spatial Variability in Environmental Characteristics

Spatiotemporal dynamics of environmental variables in lotic ecosystems, including physicochemistry and hydrology, variably influence their biotic components (Junqueira et al., 2016; Kennen et al., 2010). The interrelationship between streamwater physicochemistry and metal toxicity depends on (i) the speciation, fate and bioavailability of metals in the aquatic environment, and (ii) the internal biochemistry, physiology or behavioural responses of stream biota (Pinheiro et al., 2021).

Water velocity is a major determinant of energy transfer, material flows and physicochemical changes in lotic systems (Rolls et al., 2012). For instance, the significantly higher SOM and CSR in sediments at KYABR corresponded to the lowest stream velocity since this allowed the settling of particulate matter as water flows through the *Typha domingensis*-dominated riverine wetland. The variability in stream velocity also significantly influences the distribution, composition and functional traits of aquatic macroinvertebrates (Rolls et al., 2012). Such flow-related differences have been observed in the Kiiminkijoki river system, in northern Finland, where the structure and FFG composition of macroinvertebrate assemblages differed significantly with variable stream velocities (Heino et al., 2004).

Among other parameters, pH remained relatively stable and circumneutral across the four KSC streams. Other studies in the Zambian Copperbelt have also reported the high buffering capacity causing a neutral-alkaline pH of its lotic ecosystems (Pettersson & Ingri, 2001; von der Heyden & New, 2004). The pH stability in the KSC may be attributed to catchment alkaline geologic formations characterised by high carbonate-bicarbonate buffering and surface runoff generation (Kříbek et al., 2023; Pinheiro et al., 2021). Water pH is a critical driver of water chemistry and biochemical processes in aquatic environments that, in turn, influence the solubility, bioavailability and toxicity of trace elements. Additionally, pH below 4 promotes the release of H^+ ions and consequent secondary mineralisation of metal(loids) from sediment, enhancing their bioavailability and toxicity to benthic biota (Zhang et al., 2014). For instance, in Berezina (2001), pH below 4 and above 9 caused a decrease in species composition and abundance of stream macroinvertebrates due to their differential sensitivities. While molluscs dominated at pH >9, the chironomids abundance decreased and at pH <4, there was low richness and abundance of the EPT taxa. Furthermore, Fornaroli et al. (2018) confirmed the negative effects of acidic pH and high metal concentrations on the EPT abundance and richness in US headwater streams.

The moderate DO (3.8 ± 1.1 mg/L) and water temperature (22.6 ± 1.8 °C) range in the KSC streams provided a suitable environment for the survival of macroinvertebrate communities (Peng et al., 2020; Zhang et al., 2014). In the aquatic environment, both parameters essentially influence physicochemical and biochemical processes that consequently impact on composition and distribution of biota (Eady et al., 2013; Skoulikidis et al., 2009). In this regard, Peng et al. (2020) noted increasing abundance and diversity of pollution-sensitive scrapper, shredder and collector-filterer macroinvertebrates with increasing DO (4.4–6.86 mg/L) in a sub-tropical south China river system. Similarly, Jiang et al., (2021) reported even higher DO levels (9.17–9.34 mg/L) with correspondingly higher collector-filterer and shredder abundance and diversity in the Weihe and Hanjiang river basins, in central China. Eady et al., (2013) on the other hand linked the distribution of specialist taxa to sites with greater temperature variability, and generalist taxa to areas with stable temperature regimes and variability in the Keurbooms and the Kowie-Bloukrans Rivers, in South Africa's Cape Province. Macroinvertebrates also exhibit optimal biochemical and physiologically-dependent temperature ranges for survival, growth and development (Bonacina et al., 2023). According to Bonacina et al. (2023), a 0–20° C change in water temperature caused a shift to partial anaerobic metabolism in *Chironomus* sp (Diptera) while shorter development time was noted in *Soyedina* sp (Plecoptera) and *Eukiefferiella* sp (Diptera) at 10 and 14° C respectively.

The Eh indicated a slightly anoxic to mildly oxic water environment considered sufficient to support benthic communities (Brune et al., 2000). Increasing Eh facilitates oxidation of sulphides and breakdown of SOM, hence enhancing the release of adsorbed metals from sediments (Brune et al., 2000). The distinctively high EC at KISDS (1370 µS/cm) could be attributed to the KSC catchment geology, which influences dissolution and transport of mineral elements via subsurface stream flow, which emerges as a spring at the source (Bonacci & Roje-Bonacci, 2023). EC values signify the magnitude of ionic elements in the aqueous environment. Although the pH, Temp, DO, EC, and Eh varied significantly among the streams, all the values were below the ZEMA guidelines (Environmental Council of Zambia; ECZ, 2010).

Aquatic metal concentrations in sediments were safely below the background values (Key et al., 2004) and those from other studies in the north-western Zambian Copperbelt lotic environments (Hasimuna et al., 2021; Key et al., 2001). However, significant variability was noted across the streams, particularly with high Cu content (491 mg/kg) in sediment at KYAFUS with likely potential toxicity of benthic biota (Ambrosino et al., 2023). However, Hasimuna et al. (2021) observed much lower levels of Cu (0.13–10.4 mg/kg) and Zn (0.03–0.2 mg/kg) in sediments of Kifubwa and Solwezi rivers in north-western Zambia possibly influenced by differences in local mineralogical and hydrological peculiarities and the intensity of anthropogenic activities (Mir et al., 2022). Increased bioavailability of metals significantly impacts the abundance and diversity of pollution-sensitive taxa further disrupting the trophic structures (Mor et al., 2022).

Regarding the spatial responses of macroinvertebrates to variability in streamwater physicochemistry, most taxa were generally clumped at the centre of the CCA biplots (Fig. 3), indicating that no specific environmental variable defined their distribution (Niba & Sakwe, 2018). Further, this pattern illustrates that most of the streams exhibited uniform environmental characteristics which supported relatively similar macroinvertebrate communities (Makalane et al., 2020). Nevertheless, selected environmental variables influenced the distribution of certain families. For instance, the Lymnaeidae were positively and strongly influenced by sediment properties (SOM and CSR) at KYSBR (S3) while Eh exhibited the largest influence on Nepidae at KYAST (S2).

6.2 Multimetric Characteristics of Macroinvertebrates as Bioindicators of Stream Health Condition

Two Insecta orders, Diptera and Hemiptera, were the highest contributors to macroinvertebrate abundance in the KSC streams, particularly at the KYABR, which experiences higher anthropogenic disturbances (Table 2). This trend is not surprising due to the high diversity and ubiquity of Class Insecta in tropical lotic environments (Hauer & Lamberti, 2007). The higher abundances possibly reflect the degree of anthropogenic perturbations at KYABR that favoured tolerant dipteran and hemipteran taxa while reducing the sensitive groups (Nnoli et al., 2019). Furthermore, the higher abundance of four highly pollution-tolerant gastropod families (Thiaridae, Neritidae, Planorbidae, Lymnaeidae) also indicated deteriorating water quality at the KISDS. Gastropods are structurally and functionally highly adapted to poor water quality and therefore excellent colonisers multiplying rapidly in “poor” environments (Afwanudin et al., 2019). In contrast, two highly pollution-sensitive families, Perlidae (Plecoptera) and Helodidae (Coleoptera), were reported at the less disturbed KISDS, KYAST and KYAFUS streams (Online Resource 2). While disturbances cause homogenous communities dominated by tolerant taxa to such kind of disturbances, the presence of pollution-sensitive taxa suggests “fair-to-good” stream conditions in the KSC stream network (Hickey & Clements, 1998; Mangadze et al., 2016).

Regarding trophic relationships, in a disturbed stream ecosystem, eurytopic generalists, including collector-filterers (CF) and collector-gatherers (CG), tend to dominate over the specialised feeders such as shredders (SH) and scrapper-collectors (SC), whereas predators (P) thrive, provided the prey supply is sufficient (Akamagwuna & Odume, 2020; Dennis et al., 2011). In the KSC streams, the most dominant taxa comprised the P (58.96%), mainly from Odonata, Hemiptera and Diptera, which further corresponds to their higher abundance (Table 3). The trophic relationships observed in this study concur with Powell et al. (2023), who also reported increased P abundance by 19% compared to non-predatory macroinvertebrate taxa (15%) between 2002 and 2019 in European streams. Additionally, in the KSC streams, the generalist CF and CG taxa were relatively abundant compared to the specialists (SH and SC), implying a slightly disturbed but stable trophic system (Roveri et al., 2020). According to Rawer-Jost et al. (2000), anthropogenic disturbances cause fluctuations in the abundance and diversity of specialist taxa, leading to changes in trophic composition and food availability for higher trophic-level taxa. In the present study, the SC proportion decreased from 17.3% and 10.8% in the less-impacted KISDS and KYAST streams to 1.34% in the most-impacted KYABR stream. Interestingly, the P abundance also declined from 68.96% at KYAST to 51.33% at KYABR. Similarly, Erasmus et al. (2021) observed a decline in the SC from 9.6% to 3.1% at an impacted site, while the SH only occurred at the least impacted site. In contrast, the P taxa increased from 14.6% to 34.9% from the least to the most impacted site since all the P taxa recorded in KSC were pollution-tolerant (Online Resource 1).

Considering trophic balance, a community with higher abundances of P is relatively unsustainable in the long term due to reduced abundance among non-predatory taxa from increased predation pressure unless food availability, such as organic matter, is sufficient to sustain non-predator communities (Rawer-Jost et al., 2000). In the KSC, for instance, the high SOM at KYABR supported higher SH (14.9%) and CF (26.8%) taxa despite the high proportion of P (51.3%). However, according to Mor et al. (2022), predation pressure on non-predator communities in the KSC streams could be lower than anticipated if the present P taxa are particularly pollution-sensitive. This possibly explains the relatively moderate abundance of generalist non-predatory taxa (10.4% CF and 14.3% CG) despite high P abundance (58.96%) in the KSC. Overall, eurytopic and generalist families occurred in almost every stream thus indicating a wide ecological amplitude (Skoulikidis et al., 2009). Besides the taxonomic indicators, macroinvertebrate functional feeding traits also provided additional biological parameters for assessing the causes and consequences of ecological changes in stream conditions (Jiang et al., 2021).

Functional diversity indices are invaluable proxy bioindicators of stream health conditions (Agboola et al., 2019). While taxa richness (TR) indicated significantly higher values for KYABR and KYAFUS against KYAST, Margalef's richness index (M) detected higher taxa richness at KISDS compared to KYAST (Table 4). The disparity could be attributed to the ability of the M-index to account for both taxa richness and abundance, which makes the index a better predictor of taxa richness (Magurran, 2004). However, both streams are moderately impacted by anthropogenic activities, which likely increase the richness of tolerant taxa and generalist feeders. The significantly higher relative dominance at KYABR against KISDS further suggests the abundance of tolerant macroinvertebrate taxa associated with Odonata, Coleoptera and Diptera (Online Resource 1) (Erasmus et al., 2021). In addition, the high Pielou's evenness index (J) at KISDS depicts an increasingly uniform taxa density and, thereby, a less disturbed stream compared to KYABR (Herrmann et al., 2022). Interestingly, Simpson's diversity index picked KYABR as the most diverse ecosystem in the KSC, mostly attributed to the higher intensity of anthropogenic disturbances in agreement with Liu et al. (2022) and Bere et al. (2016), considering its accessibility and vulnerability to the public. In contrast, other studies have noted a decrease in macroinvertebrate community diversity indices with increased anthropogenic perturbations in streams (Ambrosino et al., 2023; Bae et al., 2021).

Macroinvertebrate sensitivity distribution profiles provide an overall scenario of stream health conditions (Kumari & Maiti, 2020). In this study, five metrics (ZISS- 1, ASPT, EPT/Chironomidae ratio, TI and H'-index) were used to evaluate stream conditions in the KSC (Table 5). From the indices, the general indication of stream health ranged from "slightly polluted" (H'-index) to "excellent" (ZISS- 1). In respect to these trends, the KSC streams are minimally impacted by anthropogenic disturbances. Confirmatory findings have also been observed for relatively less impact on Zambian rivers based on ASPT and ZISS- 1 bioassessments (Dallas et al., 2018) and southern Africa lotic systems using the SASS- 5 (Odume et al., 2012) and NASS- 2 (Palmer & Taylor, 2004). Elsewhere, studies have similarly employed macroinvertebrate bioassessment metrics to effectively evaluate the ecological conditions of stream ecosystems (Serrano Balderas et al., 2016; Sun et al., 2024). Furthermore, the high EPT relative abundance and the EPT/Chironomidae ratio in response to environmental variables in the KYAFUS and the pooled ratio depict a “good” stream health condition in KSC. In contrast, Liu et al. (2022) noted higher EPT richness and density at mining-impacted sites along glacier-fed streams in China’s Tianshan mountains. Nevertheless, the EPT taxa have been effectively applied to evaluate the stream condition of Southern Africa lotic systems, including the semi-urban Tsitsa River in South Africa’s Eastern Cape Province and the Swartkops River (Akamagwuna et al., 2021; Odume et al., 2012), and the Manyame River in Zimbabwe (Bere et al., 2016).

In lotic ecosystems, the complex interactions of natural environmental factors and anthropogenic disturbances cause spatial heterogeneity in abundance and distribution among macroinvertebrate communities (Roveri et al., 2020). In the KSC, the orders with the highest pairwise dissimilarity (44.4%) between KYABR and KYAST were mostly influenced by predatory and pollution-tolerant families of Odonata (Coenagrionidae, Libellulidae), Coleoptera (Hydrophilidae, Dytiscidae, Gyrinidae) and Diptera (Culicidae, Tabanidae), and pollution-sensitive Helodidae (Coleoptera) (Online Resource 2) which were higher at KYAST (Table 3, 68.9%). Furthermore, KYAST had higher %CG (20.5) and %SC (10.8), thereby providing a sufficient food base for the dominant P taxa. The pattern aligns with the environmental characterisation of KYAST with less anthropogenic impacts, including lower Eh, EC, As, Cu, and Ni contamination compared to the KYABR stream (Online Resource 2). Moreso, as observed in our findings and in agreement with other studies, aquatic metal pollution and deterioration of water quality in streams negatively impacted the abundance and diversity of pollution-sensitive macroinvertebrate taxa while enhancing the dominance of tolerant taxa (Bae et al., 2021; Bere et al., 2016). Out of the 30 families reported at KSC, 66–77% were highly tolerant, and only 6.7% (Helodidae and Perlidae) were highly sensitive to aquatic pollution (Table 4; Online Resource 1). Iwasaki et al. (2012) observed a reduction in macroinvertebrate abundance and altered community composition at two Zn (> 1 mg/L) polluted stream sites in Western Japan, also marked by high pollution-tolerant Chironomidae abundance. Furthermore, another study in Tokushibetsu River, northern Japan, highlighted a significant reduction in the abundance of pollution-sensitive Heptageniidae mayflies at the most contaminated upstream sites with Cd, Cu, Pb and Zn concentrations 0.8–3.7 times above Japan’s water quality guidelines (Namba et al., 2021).

In general, the clustering of most macroinvertebrate taxa categories at the centres of the CCA biplots reflected a relative similarity in the environmental characteristics among the streams, which displayed minimal spatial influence in taxa segregation in the KSC (Makalane et al., 2020; Niba & Sakwe, 2018). Nevertheless, the distribution and trophic interactions of pollution-sensitive taxa in lotic systems may be determined by fluctuations in environmental conditions (Edegbene et al., 2023; Kumari & Maiti, 2020; Odume et al., 2012; Roveri et al., 2020).

7 Conclusions

In this study, macroinvertebrate taxonomic and functional attributes, including taxa richness, dominance, diversity, tolerance, and trophic relationships (FFG) provided insights into the magnitude of anthropogenic disturbances in the KSC. There was a positive correlation between environmental and biological (taxonomic and functional) indicators of stream health assessment. Both approaches rated the KSC streams as “less impacted” and, therefore, in an ecologically “fair-to-good” status, evidenced by the presence of highly pollution-sensitive Helodidae (Plecoptera) and Perlidae (Coleoptera). The application of multiple abiotic, taxonomic and functional metrics provided a complementary ecological assessment since the differences in sensitivities among the metrics were compensated to increase the overall precision and accuracy of stream health bioassessment. Besides, the macroinvertebrate-based assessment provided a “historical status” eco-friendly approach which can be integrated with conventional methods in the rapid assessment of stream health in mining landscapes.

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Data Availability Primary data used in this manuscript are available as supplementary material. Any additional supplementary data will be made available upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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