

# ASSESSMENT OF RIVER HEALTH USING BENTHIC MACROINVERTEBRATES IN THE GROOT LETABA RIVER, SOUTH AFRICA

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**Abstract.** The Groot Letaba River catchment in South Africa, is known for agricultural activities, such as citrus and avocado farming. These activities are causing degradation of the river, especially near areas of commercial farming and human settlements. Benthic macroinvertebrates are good water quality bioindicators and are used to assess the health of aquatic ecosystems. The current study assessed river health of the river using macroinvertebrate community structure in relation to environmental variables. The macroinvertebrates were distributed among 42 families and 13 orders. The results revealed distinct spatial variation among the sampling sites and their associated macroinvertebrate communities, with the downstream having the least taxa richness and most of the tolerant taxa. The family richness and diversity were higher in the upstream and midstream sites. The changes in macroinvertebrate assemblages were primarily due to changes in water quality as a result of environmental factors, such as total dissolved solids (TDS), conductivity, dissolved oxygen and total nitrogen, and organic matter from agricultural activities.

**Keywords:** *aquatic ecosystems, community structure, nutrients, water quality, anthropogenic activities*

## Introduction

The ecological consequence of land use change on river health is a prominent environmental issue worldwide (Zhao et al., 2017; Krajenbrink et al., 2019). Effluents from human activities such as agriculture, mining and industries that enter freshwater ecosystems affect the water chemistry and consequently affect the aquatic biota (Mwedzi et al., 2016; Mangadze et al., 2016; Addo-Bediako et al., 2021). Many human activities have caused changes in community structure in different aspects of diversity, such as richness (Brasil et al., 2019), abundance of individuals (Paiva et al., 2017), beta diversity (Cunha and Juen, 2017; Brasil et al., 2017), and functional diversity (Addo-Bediako, 2021).

Aquatic macroinvertebrates are predominantly used as bioindicators of freshwater ecosystems, because of their ability to reflect changes in water quality over time. They are key components of aquatic food webs that link organic matter and nutrient resources (e.g. leaf litter, algae and detritus) with higher trophic levels (Li et al., 2010). Aquatic macroinvertebrates consist of different taxa with a wide tolerance range to environmental pollution (Qu et al., 2013; Rasifudi et al., 2018), therefore they provide strong information for interpreting cumulative effects (Kripa et al., 2013).

The use of aquatic macroinvertebrates for assessing environmental conditions has been widely accepted (Zhang et al., 2014; Baker and Greenfield, 2019). They are commonly used to monitor running water ecosystems (Masese and Raburu, 2017;

Chellaiah and Yule, 2018; Krajenbrink et al., 2019). Several macroinvertebrate indices have been developed worldwide to monitor river health. The macroinvertebrate indices primarily recognise the different taxa tolerances to pollution (Zhao et al., 2017; Hamid and Rawi, 2017; Chellaiah and Yule, 2018). Sometimes, a subset of taxa may be selected to become proxies of the whole community (Masese and Raburu, 2017). A proper understanding of how anthropogenic disturbances affect freshwater ecosystems is essential for an effective management and conservation biodiversity and ecosystem services of freshwater ecosystem (Brasil et al., 2019; Wilkinson et al., 2019; Asmamaw et al., 2021).

The Groot Letaba River catchment in South Africa is dominated by agricultural activities, especially commercial citrus farming. Usually, chemical fertilisers, pesticides and fungicides, fortified with specific concentrations of elements are used to enhance the genetic, physical and physiological quality of crops (Sheehy et al., 2015). When these chemicals enter the freshwater ecosystems, they can lead to undesirable effects on the aquatic biota and human health (Kroflíč et al., 2018). Adequate research on the fate, occurrence and impact of land use changes is lacking in the Groot Letaba River. Information on the water quality of the Groot Letaba River between Tzaneen town and the Kruger National Park (KNP) is necessary to provide an indication of the extent to which the agricultural activities have affected the water quality and aquatic biota in the river. The main objective of the present study was to assess the river health using benthic macroinvertebrate communities at the family level in relation to various abiotic factors in the Groot Letaba River.

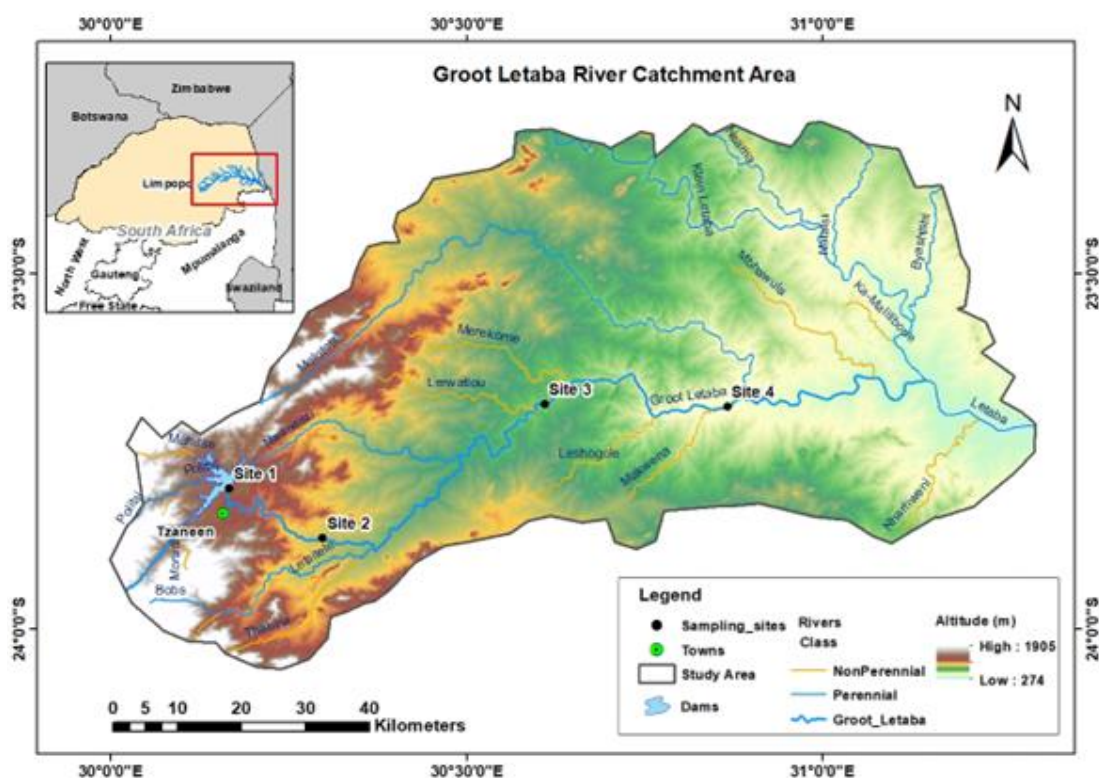
## Materials and methods

### Study area

The Groot Letaba River falls within the Olifants River Basin Water Management Area (WMA). The sub-catchment drains a surface area of about 13,670 km<sup>2</sup> (DWAf, 2006). The river is about 461 km long and the mean width of the study area is between 10 and 12 m. The mean discharge during raining season is about 35.2 m<sup>3</sup> s<sup>-1</sup> (Katambara and Ndiritu, 2009). The Groot Letaba River is a perennial river contributing over 50% of the downstream flow of the Olifants River into the Kruger National Park (KNP). Good management of water flow of the river is of great importance for its sustenance in the park. The Groot Letaba River catchment between Tzaneen Dam and the Kruger National Park (KNP) was selected for the study. The area is between two large impoundments, Tzaneen Dam and the Nondweni Weir, where intensive agricultural activities take place in the catchment and may have impact on the water quality of the river. The area is dominated by commercial farming (citrus, mango, avocado, bananas, cotton, maize and vegetables), of which more than 42% of this agricultural land use is under irrigation (SOR, 2001).

The study sites were selected based on their accessibility (being able to reach easily) and safety (as some parts of the river is infested with crocodiles and hippopotamus); Site 1 (23°48'01"S, 30°10'01"E) in the upstream, Site 2 (23°52'19"S, 30°17'54"E) and Site 3 (23°41'00"S, 30°36'34"E) in the midstream, and Site 4 (23°40'53"S, 30°52'24"E) in the downstream of the river (*Fig. 1*). Site 1 was situated just below Tzaneen Dam and is characterised by riparian and in-stream vegetation. However, the vegetation is mostly invasive plants including lantana, castor-oil plant, bugweed, large cocklebur and peanut butter cassia. The substrate consists mainly of cobbles (20%),

gravel (30%), sand (20%), silt (20%) and clay (10%). Site 2 was near 400 ha citrus farm, called Letaba Estate and it is characterised mainly by rock (10%), cobbles (10%), gravel (20%), sand (30%), silt (20%) and clay (10%). The riparian vegetation included large trees and reeds. Other anthropogenic activities besides citrus farming, include a golf course and human settlements in Nkowankowa Township. Site 3 was surrounded by many small farms. The substrate consists mainly of rocks (5%), cobbles (10%), gravel (20%), sand (30%), silt (25%) and clay (10%). Site 4 was at a weir and near small farms, human settlements and a waterworks facility. The substrate at this site consists mainly of pebbles (10%), sand (30%), silt (40%) and clay (20%). The percentage substrate composition was visually estimated. The size classes of the substrate composition were boulders (>256 mm), cobble (64–256 mm), pebbles (16–64 mm), gravel (2–16 mm), sand (0.06–2 mm) and silt/clay (<0.04–0.06 mm).



**Figure 1.** The selected study sites between Tzaneen Dam and KNP of the Groot Letaba River

### **Sampling of water and macroinvertebrates**

Sampling was carried out at four sampling sites in the Groot Letaba River between Tzaneen and KNP in July and November 2015, and also in June and December, 2016. Water (1000 ml) samples were collected using acid pre-treated sampling bottles. The samples were refrigerated immediately for laboratory analysis. Physicochemical variables such as pH, temperature, dissolved oxygen, salinity, conductivity and total dissolved salts were determined in situ using a YSI 556 Multi Probe system; a handheld multi parameter instrument. Turbidity and nutrients (nitrite, nitrate, ammonium, total nitrogen, phosphate) were determined at the Department of Biodiversity Laboratory (University of Limpopo) within 24 h after sampling using various test cell kits (Merck Pharo 100 Spectroquant).

Aquatic macroinvertebrates were sampled biannually for two years in triplicates at the four selected sites in the river. They were collected using a 30 by 30 cm sampling net with a 500 µm mesh size. Macroinvertebrates were collected from various biotopes: Stones; the substrate was disturbed for a period of 5 min when collecting from stones in current to free macroinvertebrates. The stones were kicked or turned over against each other to dislodge the macroinvertebrates. Vegetation; the net is pushed vigorously into the vegetation by moving backwards and forwards through the same area for a total length of approximately 2 m. Gravel, sand and mud; the substrates were stirred by shuffling or scraping with the feet, whilst continuously sweeping the net over the disturbed area to catch dislodged biota. Each of the samples was then washed down to the bottom of the net and carefully tipped into a tray by inverting the net.

Larger obstructing leaves, twigs and other loose debris and stones were checked for clinging macroinvertebrates, then removed from the tray. Samples were identified to the family level in the field using an Invertebrate Field Guide Manual (Gerber and Gabriel, 2002), with the aid of magnifying glass. However, macroinvertebrates which could not be identified in the field were preserved in 70% ethanol in labelled containers. This was done to prevent the samples from decomposing and predators from preying on other macroinvertebrates. The samples were transported to the laboratory (University of Limpopo's Biodiversity Laboratory) for further identification using a stereomicroscope (Leica EZ4).

### Data analysis

Analysis of variance (ANOVA) was used to compare water variables among river sites after testing for homogeneity of variances (Levene's test,  $p > 0.05$ ) and normality of distribution (Shapiro-Wilk test,  $p > 0.05$ ). The same tests were used to assess for differences in macroinvertebrate abundance among sites using SPSS statistical software version 25. The Shannon–Weiner diversity and Shannon evenness indices were used to compare the macroinvertebrate diversity and evenness at the sites.

The Shannon–Weiner diversity index (Eq. 1) and evenness (Eq. 2) were also calculated to determine diversity within the different sampling sites.

$$H = \sum[(P_i) * \ln(P_i)] \quad (\text{Eq.1})$$

where  $P_i$ =proportion of total sample represented by species.  $i$  – divide number of individuals of species  $i$  by total number of samples.

$$E = \frac{H}{H_{max}} \quad (\text{Eq.2})$$

where  $H_{max} = \ln(S)$ .  $S$ =number of taxa (taxa richness)

Statistical difference of abundance, tolerance to pollution and diversity index among the sites was determined using Kruskal-Wallis test, a non-parametric one-way Analysis of Variance (ANOVA) test. The spatial distribution of macroinvertebrate assemblages along the main environmental gradients was determined using multivariate statistical analysis, Canonical Correspondence Analysis (CCA). It was used to establish the relationship between water quality parameters and macroinvertebrate abundance of macroinvertebrate community at family level. The analysis was performed using CANOCO version 5 (Smilauer and Leps, 2014).

## Results

### Physicochemical parameters

The results of the physicochemical parameters and nutrients of the water are shown in *Table 1*. The pH levels recorded were between 7.7 and 8.3, characterising the water to be slightly alkaline. The average water temperatures were between 20°C and 25°C. The dissolved oxygen was recorded below recommended saturation levels (80–100%) at Site 2 (59%) and Site 4 (53%). Generally, there was an increasing trend of salinity, conductivity, total dissolved salts (TDS) and turbidity from Site 1 to Site 4. There was no significant difference in any of the physicochemical parameters among the sites ( $p>0.05$ ). Similarly, there was no significant difference in the levels of nitrite, nitrate and ammonium among the sites, except for total nitrogen ( $p=0.025$ ).

**Table 1.** Physicochemical parameters and nutrients recorded at the different study sites of the Groot Letaba River

Physicochemical parameters	Site 1		Site 2		Site 3		Site 4		p-value	Standard Guideline values
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
pH <sup>#</sup>	7.8 - 8.3		7.7 - 8.1		8.1 - 8.3		7.9 - 8.1		0.120	6.5-9.0 <sup>c</sup>
Temperature °C	20	±2.0	25	±4	24	±4	26	±3.0	0.307	-
Dissolved oxygen %	82	±22	59	±10	83	±40	53	±41	0.681	80 - 120 <sup>a</sup>
Salinity ‰	0.03	±0.00	0.04	±0.00	0.28	±0.36	0.31	±0.29	0.343	<0.05 <sup>a</sup>
Conductivity m/S	61	±7.0	78	±20	160	±27.0	274	±151	0.041*	-
TDS mg/l	32	±7.0	40	±2	73	±12.0	147	±71	0.008*	-
Turbidity NTU	27	±28	9	±4	8	±5.0	23	±22	0.487	8.0 <sup>b</sup>
Nitrite (NO <sub>2</sub> <sup>-</sup> )	0.02	±0.02	0.01	±0.01	0.01	±0.01	0.03	±0.03	0.572	0.06 <sup>c</sup>
Nitrate (NO <sub>3</sub> <sup>-</sup> )	0.88	±0.19	1.45	±0.35	1.88	±0.39	1.05	±0.23	0.043*	13 <sup>c</sup>
Ammonium (NH <sub>4</sub> <sup>+</sup> )	0.06	±0.04	0.03	±0.02	0.14	±0.11	0.10	±0.10	0.389	0.019 <sup>c</sup>
Total nitrogen*	0.74	±0.43	1.49	±0.37	2.02	±0.42	1.18	±0.22	0.007*	0.5 <sup>a</sup>
Phosphate (PO <sub>4</sub> <sup>3-</sup> )	0.23	±0.31	0.03	±0.02	0.06	±0.02	0.10	±0.09	0.493	-
Phosphorus (P)	0.06	±0.05	0.03	±0.03	0.09	±0.06	0.07	±0.08	0.722	0.005 <sup>a</sup>

### Macroinvertebrate assemblages

The overall macroinvertebrate composition and abundance recorded at the sampling sites are presented in *Table 2*. A total of 8080 individual macroinvertebrates belonging to 42 families were collected. The numbers of families recorded at Site 1, Site 2, Site 3 and Site 4 were 30, 32, 29, and 26, respectively. The dominant orders were Diptera and Trichoptera, comprising 9 and 8 families respectively. Site 1 had the highest number of individuals of 3506 (43.4%), followed by Site 2 with 2197 individuals, accounting for 27.2%, Site 4 with 1367 individuals accounting for 16.9%, and Site 3 had 1,010 (12.5%) individuals. At Site 1, Chironomidae, Daphniidae and Hydropsychidae were the dominant families, at Site 2, the dominant families were Chironomidae, Baitidae and Simuliidae, at Site 3, the dominant families were Elmidae, Hirudinidae and Hydropsychidae, and at Site 4, the dominant families were Planariidae, Lumbricidae, and Elmidae. The most dominant family was the Chironomidae with 2163 individuals (26.8%), most of them were collected at Sites 1 and 2.

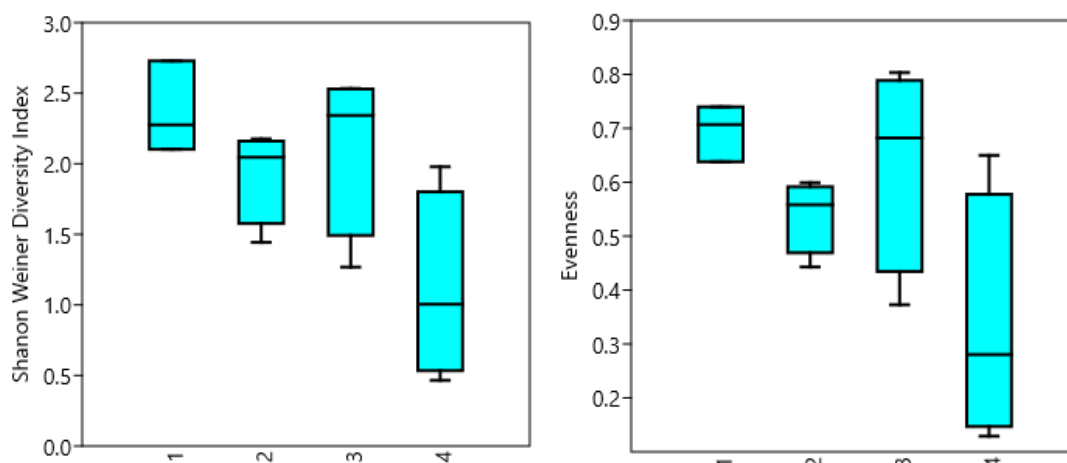
The highest Shannon–Weiner’s diversity index ( $H'$ ) was at Site 3, followed by Site 1, then Site 2 and lowest at Site 4 (*Fig. 2*). However, the Shannon evenness index was highest at Site 1, followed by Site 3, Site 2 and the lowest at Site 4, but the index values

fell below the ideal metric value of 1, which is indicative of similar proportions of all taxa in the system (Fig. 2). Statistically, there was a significant difference in species diversity and evenness among the sites ( $p < 0.05$ ).

**Table 2.** The average (+SD) number of individual macroinvertebrates recorded from sampling sites of the Groot Letaba River

Order	Family	Abbr	Site 1	Site 2	Site 3	Site 4	p-value
Spongillida (Sponge)	Spongillidae	Spon	1.0±0.0	0.0	1.0±0.0	2.0±2	0.19
Tricladida (Flatworms)	Planariidae	Pla	229±202	1.0±1	4.0±4	379±561	0.06
Lumbriculida (Earthworms)	Lumbricidae	Lum	167±44	168±182	19±16	192±196	0.25
Hirudinida (Leeches)	Hirudinidae	Hir	0.0	2.0±3	178±249	23±24	0.04*
	Potamonautidae	Pot	4.0±4	0	0	1.0±0.0	0.23
Cladocera (Daphnia)	Daphniidae	Dap	908±1181	0.0	0.0	0.0	0.04*
Ephemeroptera (Mayflies)	Baetidae	Bae	92±68	190±130	43±14	20±18	0.04*
	Caenidae	Cae	46±73	76±95	42±31	29±38	0.9
	Heptageniidae	Hep	2.0±4	4.0±4	12±12	0	0.14
	Leptophlebiidae	Lep	3.0±4	9.0±15	92±109	19±22	0.42
	Teloganodidae	Tel	0.0	163±127	1.0±0.0	0	0.82
	Tricorythidae	Tri	0.0	2.0±4	56±58	0	0.10
Odonata (Dragonflies & Damselflies)	Calopterygidae	Cal	0.0	8.0±14	0.0	3±3	0.11
	Chlorocyphidae	Chl	8.0±14	0.0	1.0±0.0	2±3	0.32
	Coenagrionidae	Coen	0.0	53±22	3±6	0.0	0.03*
	Libellulidae	Lib	5.0±6	9.0±12	5±5	0.0	0.18
Lepidoptera	Crambidae	Cram	0	17.0±21	0	0	0.01*
Trichoptera	Ecnomidae	Ecn	0.0	2.0±3	1±0.0	1.0±0.0	0.50
	Hydropsychidae	Hyd	566±391	7.0±5	116±105	164±125	0.07
	Philopotamidae	Phil	2.0±2	0.0	0.0	0.0	0.04*
	Barbarochthonidae	Bar	49±43	1.0±2	9.0±14	0.0	0.21
	Glossosomatidae	Glo	0	6.0±12	2.0±4	0.0	0.74
	Hydroptilidae	Hydr	7.0±5	70±27	21±20	75±105	0.37
	Leptoceridae	Lepc	6.0±6	0	2.0±5	0.0	0.05*
	Sericostomatidae	Ser	129±114	3.0±5	13±21	1.0±0.0	0.2
Coleoptera (Beetles)	Elmidae	Elm	2.0±2	14±21	234±243	122±67	0.02*
	Gyrinidae	Gyr	1.0±0.0	0	0.0	2±3	0.71
Diptera (Flies)	Athericidae	Ath	3.0±5	1.0±0.0	0.0	0.0	0.45
	Ceratopogonidae	Cer	1.0±1	6.0±5	0	1.0±0.0	0.06
	Chironomidae	Chir	1057±1052	1106±922	48±31	61±41	0.05*
	Ephydriidae	Eph	0.0	3.0±7	0.0	0.0	0.43
	Muscidae	Mus	1.0±1	14±25	0.0	3.0±7	0.37
	Psychodidae	Psy	1.0±2	1.0±0.0	1.0±0.0	0	0.95
	Simuliidae	Sim	193±156	189±178	5.0±6	20±21	0.04*
	Syrphidae	Syr	0.0	0.0	1.0±0.0	1	0.29
Tabanidae	Tab	1.0±2	0.0	0.0	3.0±1	0.07	
Basommatophora (Snails)	Ancylidae	Anc	7.0±6	6.0±2	30±51	0	0.61
	Lymnaeidae	Lym	0.0	6.0±7	1.0±0.0	51±10	0.27
	Physidae	Phy	1.0±2	24±23	1.0±0.0	62±94	0.14
	Planorbinae	Plan	9.0±12	33±46	5.0±8	13±19	0.34
Venerida (Bivalves)	Corbiculidae	Cor	5.0±5	3.0±3	63±61	98±141	0.09
	Sphaeriidae	Sph	0	0	0	19±37	0.6
Total			3506	2197	1010	1367	

\*Significant difference ( $p < 0.05$ )



**Figure 2.** Comparison of Shannon–Weiner's diversity index and taxa evenness among the sites

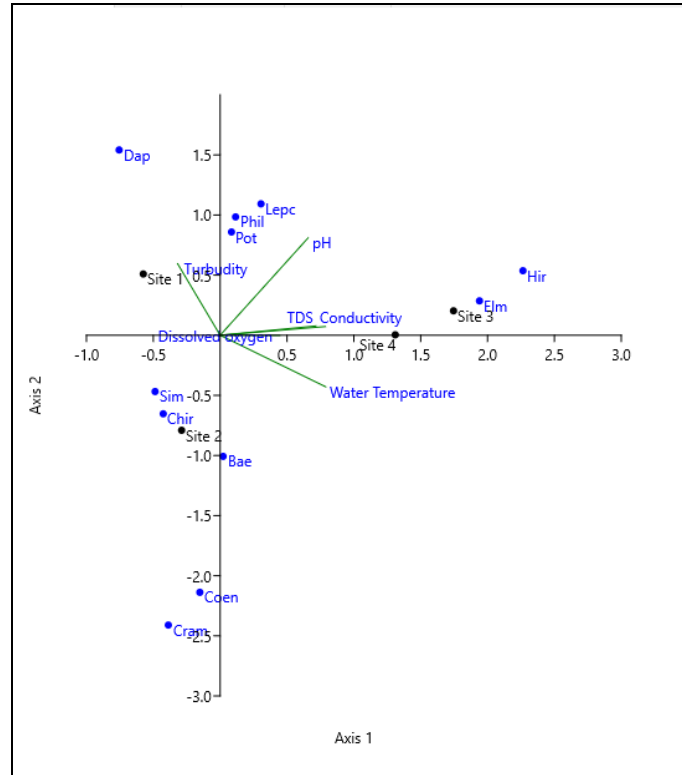
### Canonical correspondence analysis

Although all 42 families were identified (*Table 2*), only families that showed significant difference among the sites were used for the CCA analysis. The CCA ordination revealed relationships between taxa abundances and measured physicochemical variables. The first and second canonical axes accounted for 64% and 30% respectively of the variation in the data set. The positioning of the physicochemical parameters in *Figure 3* shows that the first gradient is positively correlated with temperature, conductivity and TDS. For the CCA ordination plot between the taxa abundance and nutrients, Site 3 and 4 were associated with high ammonia, total nitrogen and nitrate, and Site 1 with phosphate (*Fig. 4*). In the figures, it can be inferred that Elmidae and Hirudinea have their maximum abundance at Site 3, and Simuliidae, Chironomidae and Baetidae at Site 2.

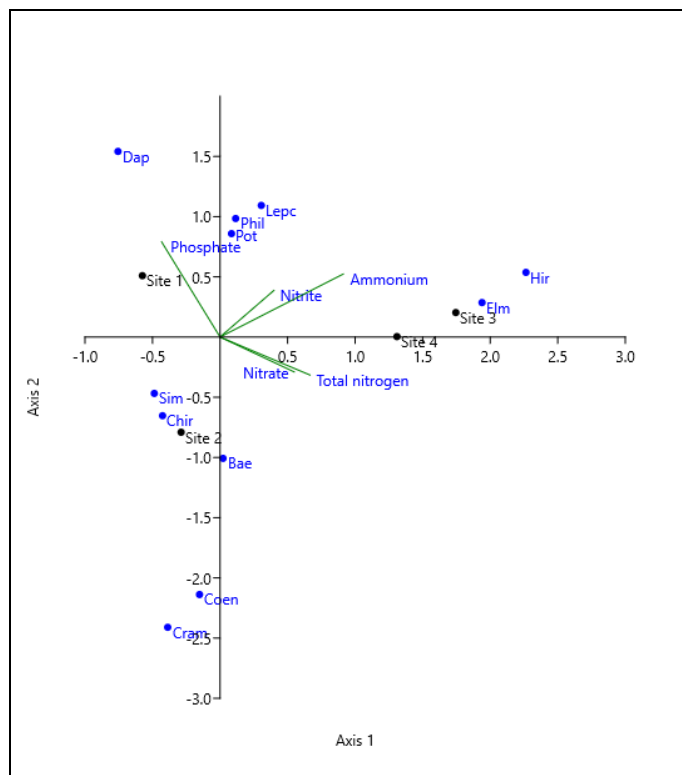
## Discussion

### Physicochemical parameters

South African inland waters have pH range between 6 and 8, and the pH levels recorded in the Groot Letaba River were within the range, but were slightly lower than some rivers within the Limpopo province, such as the Ga-Selati River (Rasifudi et al., 2018) and Steelpoort River (Matlou et al., 2017), with recorded highest pH levels of 9.3 and 9.5 respectively. The temperatures recorded in the Groot Letaba River were within the range of South African inland waters (5 and 30°C), and the levels were similar to other rivers in the province (Matlou et al., 2017; Rasifudi et al., 2018). The dissolved oxygen recorded was below recommended saturation levels (80–120%) at Site 2 (59%) and at Site 4 (53%). This may be due to the presence of weirs at Site 2 and 4, which have reduced the water current and could have reduced oxygen saturation. There was an increasing trend of salinity, conductivity and total dissolved salts (TDS) from Site 1 to Site 4. The highest salinity recorded was at Site 4 with an average of 0.31‰, which was within the levels recorded in other South African waters (Dalu et al., 2017; Rasifudi et al., 2018).



**Figure 3.** Canonical correspondence analysis (CCA) showing the relationship between physicochemical variables and macroinvertebrate families



**Figure 4.** Canonical correspondence analysis (CCA) showing the relationship between nutrient levels and macroinvertebrate families



The total nitrogen was above the target water quality range (TWQR) of 0.5 mg/l at all sites and could have been contributed by both nitrite and ammonium. The concentration of ammonium also exceeded the CCME (2012) recommended guideline of 0.019 mg/l at all sites. The concentration of nitrite recorded at Site 4 (0.03 mg/l) was above the CCME (2012) recommended guideline of 0.02 mg/l. Most of the physical parameters did not show significant differences among the sites, except for conductivity, turbidity, TDS, nitrate and total nitrogen (*Table 1*).

### ***Macroinvertebrates***

The macroinvertebrate taxa assemblages across the four sites were different (*Table 2*). The distribution reflected changes in the water quality along the river, as stream conditions are known to influence macroinvertebrate distribution (Asmamaw et al., 2021). This observed pattern was most likely due to differences in organic nutrient inputs from farmlands, (Wagenhoff et al., 2011; Deborde et al., 2016). The sites with minimal anthropogenic disturbances were found to be with improved water quality, represented by high dissolved oxygen, lower conductivity and TDS levels, coupled with higher richness, and diversity (Fierro et al., 2017; Keke et al., 2017).

Sites 3 and 4 showed lower taxa richness and could be attributed to more anthropogenic activities, including surface run-off from the nearby farms and upstream of the river. The higher macroinvertebrate taxa richness at Sites 1 and 2 could be due to high dissolved oxygen and high streambed heterogeneity respectively. High abundance of sensitive taxa, such as Hydropsychidae at Site 1 and Baetidae and Teloganodidae at Site 2, could be due to the good water quality at these sites, despite the low dissolved oxygen at Site 2. Sensitive taxa are known to have preference to clean running waters (Masele and Raburu, 2017). Furthermore, Site 2, though in the midstream, was characterised by some features of headwater zone biotopes, such as a bedrock with small patches of pools filled with sand on some parts and marginal vegetation. Conversely, the downstream site (Site 4), was particularly dominated by taxa known to survive in areas with low dissolved oxygen (DO) levels, thus, they are tolerant to water pollution, for example, Planariidae and Lumbricidae (Mwedzi et al., 2016). The low taxa richness at Site 4 may be due to unavailability of suitable biotopes for macroinvertebrates preferred habitats. The site also lacks riparian vegetation to provide shade. Shading provided by marginal vegetation is known to increase the richness and diversity of benthic macroinvertebrates (Hamid and Rawi, 2017).

Tolerant families are poor indicators of river health because they can thrive in both poor and good water quality, as exhibited by Chironomidae in this study. Sensitive families on the other hand, can be better indicators because some may not thrive in poor water quality (Dalu et al., 2017; Krajenbrink et al., 2019). The presence of most of the Ephemeroptera and Trichoptera at Sites 1, 2 and 3 indicates that this part of the river has good water quality and possibly suitable microhabitats. However, the low taxa richness at Site 4 suggests that the water quality is deteriorating, though some sensitive families such as Hydropsychidae and Hydroptilidae were present at Site 4, an indication that the site is not highly polluted, despite having the highest conductivity and TDS, and lowest DO. Generally, the absence of Plecoptera in any of the sites is a sign that the water quality throughout the river is deteriorating from organic pollution. Organic pollution has been reported to affect macroinvertebrate assemblages (Friberg et al., 2011; Baker and Greenfield, 2019). Recent studies in South Africa have reported the effect of

organic pollution such as nutrients on macroinvertebrate assemblages (Mangadze et al., 2016; Dalu et al., 2017; Baker and Greenfield, 2019).

The lowest diversity and evenness values were at Site 4, whereas Sites 3 and 1 had the highest diversity and evenness values respectively. The low species diversity and the dominance by few families downstream (Site 4), could be attributed to poor water quality resulting from changes in physicochemical parameters and nutrients from the surrounding farming activities into the river channel. Generally, the low diversity index values which normally range between 0.0 and 5.0, and the evenness index values which were below 1, is an indicative of increasing stress in the river system. The CCA plots indicated Site 2 to be associated with both moderately tolerant and tolerant families, such as Baetidae, Chironomidae and Simuliidae. Similarly, Site 3 was associated with both moderately tolerant and tolerant families, such as Elmidae and Hirudinidae.

The findings of the spatial distribution of benthic macroinvertebrates of the Groot Letaba River are consistent with other studies in the Olifants River Basin which also reported that anthropogenic activities along the river continuum influenced the water quality and benthic macroinvertebrate assemblages (Makgoale et al., 2021; Mmako et al., 2021; Raphahlelo et al., 2022). Thus, the macroinvertebrate assemblages reflect the water quality and health of the river at each sampling site, since they integrate the changes and interactions of the physicochemical environments (Keke et al., 2017; Moog et al., 2018). Site 1 and Site 2 had the highest abundance, taxa richness and diversity. This could be due to diverse and proximate stable refugia like vegetation, large rock, riffle and pool sequences that may lead to rapid recolonization of taxa following spate of disturbances (Bogan et al., 2017; Rosser and Pearson, 2018).

## Conclusion and recommendations

The results of the study show that the Groot Letaba River is being impacted by increased anthropogenic activities especially farming activities and subsequently degrading the water quality and affecting the macroinvertebrate assemblages. However, some of the macroinvertebrate taxa seem to have developed adaptations to changes in environmental conditions. Macroinvertebrate taxa richness and diversity significantly decreased in the downstream with poor conditions. The absence of sensitive Plecoptera families also signifies increasing deterioration of water quality in the river. However, the presence of sensitive taxa at all the sites is an indication that the water is moderately polluted. Nonetheless, the continuous anthropogenic activities such as agriculture would continue to cause an increase in nutrient concentration, which might have a very serious impact on the river and the aquatic biota. There is a need therefore to maintain and protect the ecological integrity of the river by controlling anthropogenic activities in the Groot Letaba River catchment.

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