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Multi-metric biomonitoring of a seasonally flooded Baraila wetland using benthic macroinvertebrates as indicator organisms in the middle Ganga River Basin (Vaishali), India

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ABSTRACT

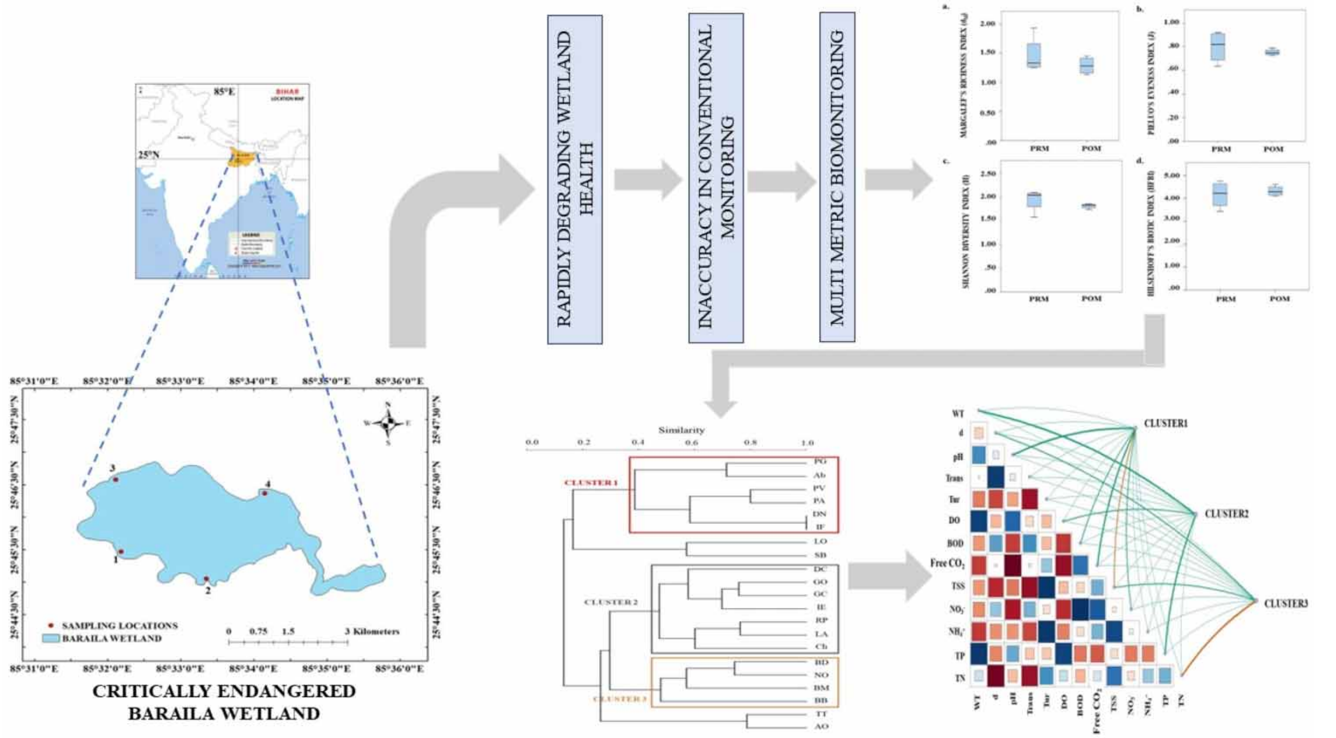
Human encroachment, urban and agricultural expansion, frequent droughts, eutrophication, infestation of weeds, and overexploitation of resources has predominantly impacted the water quality and biodiversity of the Baraila wetland. The present seasonal study has aimed to explore an under-researched subject of analyzing the water quality of the wetland through multi-metric biomonitoring approaches in 2022. A total of 24 water and benthic samples were collected from the four sampling locations. All the water parameters were within a suitable range to support a diverse range of flora and fauna, except total suspended solids (TSS) and nutrients, indicating toward the impact of agricultural expansion in the wetland's area. Of the 13 identified families of benthic macroinvertebrates, the semi-tolerant families of Viviparidae and Bithyniidae have dominated the wetland, with a relative abundance of 27.04 and 37.77%, respectively, in the comparative seasons. Moreover, the indices used in the study have categorized the wetland under moderately polluted condition. The Hierarchical Cluster Analysis (HCA) has revealed the sensitivity toward organic pollution and habitat preferences has played a major role in the species-specific assemblage of benthic community. The Mantel's correlogram further validates that the moderately polluted status of wetland has favored semi-tolerant to tolerant fauna in the wetland.

Key words: Baraila wetland, benthic macroinvertebrates, biomonitoring, Hierarchical Cluster Analysis, Mantel's correlogram

HIGHLIGHTS

- The study uses biomonitoring to document the water quality of Baraila Wetland.
- It was marked by the dominance of semi-tolerant to tolerant benthic species.
- The TSS and nutrients mainly degrade the water quality of the wetland.
- The diversity and biotic indices classified the wetland as moderately polluted.
- The HCA and Mantel's correlogram have explained the assemblage of benthic fauna.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Wetlands are arguably the most productive freshwater ecosystems on earth, serving as an essential reservoir that supports a diverse array of flora, fauna, and microbial life (Dorche *et al.* 2019; Singh & Sharma 2020; Sripanya *et al.* 2023). Despite their ecological significance, a global decline in wetlands has been reported owing to multiple anthropogenic stressors, such as land use changes, overexploitation, destruction or degradation of habitat, agricultural expansion, and water pollution (Best & Darby 2020; Yang *et al.* 2022). Wetland depletion has been documented worldwide, ranging from the rapid disappearance of the Florida Everglades in the United States to the drastic deterioration of the Sundarbans in Bangladesh and India (Sklar *et al.* 2019; Sahana *et al.* 2022). Nearly 91.12% of the wetland degradation has been reported as a negative impact of human development activities on the ecological stability of wetlands in the Jiangsu province in China (Wang *et al.* 2023). Similarly, Dar *et al.* (2021) found nutrient input from the agricultural runoffs and other sources of organic wastes have led the wetland to a hyper-eutrophic state.

Moreover, poor wetland management and monitoring framework are two of the potential reasons behind the rapid decline in global wetlands. To build a sustainable conservation and management plan it is crucial to understand the hydrodynamics of the wetland ecosystem. The interrelationship between aquatic health, and human activity, the source of impairment, and the impact of disturbances on biological entities cannot be explained by the traditionally followed physicochemical method (Stein *et al.* 2008; Johnson & Ringler 2014; Khatri *et al.* 2021; Mezgebu 2022). Biomonitoring, on the other hand, can explain the biological response of aquatic fauna toward stressors indicating a change in water quality (Mehari *et al.* 2014). According to Carter *et al.* (2006), biomonitoring provides a moving picture of the past and present conditions, whereas physicochemical approaches rather a snapshot, primarily reflecting the water quality condition of the time water has been sampled. Among various groups of bio-indicators, macroinvertebrates are the preferred organism for the biomonitoring program by researchers across the globe (Duran & Suicmez 2007; Morse *et al.* 2007; Orwa *et al.* 2014; Lakew & Moog 2015; Neetu *et al.* 2019; Shabani *et al.* 2019; Akyildiz & Duran 2021; Dirisu & El Surtasi 2021; Balisco *et al.* 2022; Sripanya *et al.* 2023). Benthic macroinvertebrates are used as preferable organisms over other aquatic species due to their high diversity, sedentary nature, long life cycles, easy and economical sampling efforts, and a strong and broad variety of responses toward environmental stressors (Patang *et al.* 2018). Several indices based on the biomonitoring data have been developed to assess the quality of the freshwater ecosystem (Nguyen *et al.* 2014; Tampo *et al.* 2021; Wang *et al.* 2023). However, relying on the result of a single index usually fails to reflect the accurate status of aquatic water bodies, exposed to various kinds of human-induced disturbances. Biomonitoring approaches using a multi-metric index overcome these limitations, and strengthen the findings of the biomonitoring dataset (Carter *et al.* 2006). In agreement with the above discussions, Johnson & Ringler (2014) found inclusion of biological data in ecological studies can provide a better understanding of the factors that affect the biological organisms, and water quality of a wetland. Dorche *et al.* (2019) working in the Choghakhor wetland of Iran reported an effective application of biotic indices, as it shows reasonable consistency with the water quality classification.

Like many regions across the globe, India has witnessed a decline of 70% in wetland areas from 1990 till today, due to failure to manage water pollution, usage of water resources, and human negligence (Singh *et al.* 2020). Wetland comprises 4.7% of the total geographical area in India, and shares 6.53% of global wetland biodiversity. Therefore, the degradation and loss of wetlands have been reportedly linked with the loss of biodiversity, and other ecological services in many regions of the Gangetic plains. The Baraila wetland has been considered a potential contender to be categorized under the Ramsar site, after Kabar Tal, in 2020 (Comptroller and Auditor General (CAG) of India 2017). In addition to supporting a population size of 49,819 individuals, and native bird species, it hosts the largest international bird communities in India, from countries like Siberia, Mongolia, Africa, Eurasia, and Japan (Aslam *et al.* 2021; Vaishali 2022). The macroinvertebrates of the wetlands constitute a significant part of the food chain for many of these waterbirds (Meyer *et al.* 2015). The occurrence, distribution, and habitat selection of these migratory birds have been previously reported to be linked with the assemblages of the aquatic benthic macroinvertebrates (Rooney & Bayley 2012). Unfortunately, the biodiversity and water dynamics of the Baraila wetland have been reportedly under multiple anthropogenic pressures, which mainly include encroachment, overexploitation, frequent droughts, eutrophication, habitat degradation, etc. Several studies have been conducted assessing the water quality, geochemistry, irrigation suitability, and mineralogy of the Baraila wetland (Aslam *et al.* 2021, 2023a, 2023b), and have outlined a conservation plan for the wetland (SAPCC 2015; CAG 2017). No comprehensive evaluation using biomonitoring approaches has been conducted in the wetland, emphasizing the critical need to fill this research gap. Therefore, the study aims to (1) assess the water quality of the Baraila wetland using a multi-metric biomonitoring approach and to (2) investigate the relationship between the water quality parameters and community assemblage of benthic macroinvertebrates at the Baraila wetland. This research will not only assess the Baraila wetland's ecological state but also contribute valuable insights into the authorities concerned in developing a conservation strategy, and further encourage similar approaches to various fragile habitats worldwide.

2. MATERIALS AND METHODS

2.1. Study area and site selection

The study was carried out at the Baraila wetland, which is a seasonally flooded wetland in the Vaishali District of North Bihar. It covers a substantial area of 12.7 km^2 expanding from 25°45′58″ & 25°45′37″ N to 85°31′48″ & 85°34′50″ E. The wetland is underlain with a quaternary alluvial floodplain created by the consolidation of the River Ganga, and its associated tributaries, including the Gandak, Noon, and Baya rivers (Galy *et al.* 2008; CGWB 2013; Kumar *et al.* 2021). It lies in the middle Ganga River basin, in which deposits of clay minerals and monotonous flat topography are prevalent (Kumar *et al.* 2022; Sulaiman *et al.* 2023). The climatic conditions of the study area fall within the sub-tropical to sub-humid type, with temperatures varying from approximately 26 to 40 °C during summer, and 4–15 °C in winter (Aslam *et al.* 2023a, 2023b). The district experiences an average annual rainfall of 1,170 mm, with substantial precipitation in July and August. The wetland has been identified as an eco-sensitive zone and is given protected status under the official gazette notification of the government (WII 2017). In addition, the Baraila wetland has been recognized as a wetland of national significance through the National Wetland Conservation and Management Programme (NWCMP), and could potentially be added to the Ramsar database in the future. According to WII (2017), the wetland holds great significance as it serves as one of the main destinations for a wide variety of migratory bird species around the world.

The site selection was done after visually analyzing the habitat and anthropogenic stressors at the Baraila wetland. In this study, a Land Use Land Cover (LULC) map for the Baraila wetland was generated utilizing 30-m resolution data for the year 2021 from ESRI and processing it through the ArcGIS version 10.5 software. The research adopted an unsupervised classification method, identifying and categorizing spectral signatures of various land cover types into distinct classes. The maximum area of the wetland was categorized as rangeland (66.97%), followed by flooded vegetation (29.40%) in the year 2021. Additionally, elevation and delineation maps were produced using digital elevation models (DEMs) satellite data from USGS. The wetland was noted with streamflow mainly in the southern regions, with only 37.05% of the region having an elevation beyond 48 m. Therefore, the accessibility of collecting both water and benthic samples from the same location has mainly governed the site selection process. The names of the selected sampling sites were as follows: site 1 – Loma, site 2 – Dulwar, site 3 – Chakaiya, and site 4 – Kawai Baraila (Figure 1).

2.2. Collection and physicochemical analysis of water samples

Water samples were collected during the pre-monsoon period of March and April, and the post-monsoon period of September and October 2022. A total of 24 water samples, three from each site in both seasons, were collected in a 2.5-L polythene bottle at a depth of about 10 cm below the surface water. The water samples were then transported to the laboratory and kept in a cool place for further analysis. Altogether 13 physicochemical parameters were assessed, where the water temperature (WT), depth (d), transparency, pH, free CO_2, and dissolved oxygen (DO) were estimated *in situ* using a water analysis kit. The remaining parameters were analyzed following the standard methods of APHA (2017), and Trivedy & Goel (1984). The turbidity of the samples was analyzed using a Turbidimeter (Thermo Scientific Eutech. TN-100), TSS, and Biological Oxygen Demand (BOD_5) has been analyzed using Gravimetric and Winkler's Iodometric methods (APHA 2017). The nitrogen parameters such as ammonia (NH_4^-), nitrate (NO_3^-), total nitrogen (TN), and total phosphorus (TP) were estimated within 48 h after collection. High-quality analytical-grade reagents were utilized during the analytical procedures, and the respective equipment underwent calibration before analysis (Sulaiman & Kamari 2024).

2.3. Collection, storage, and identification of benthic macroinvertebrate samples

For the qualitative and quantitative assessments of the benthic macroinvertebrates, samples were collected following the grab sampling method, with the help of an Eckman dredge of size 15.2 cm × 15.2 cm at each location, for the mentioned study periods. Subsequently, the dredged material was sieved through a 425-μm metallic sieve, and the benthic samples were carefully

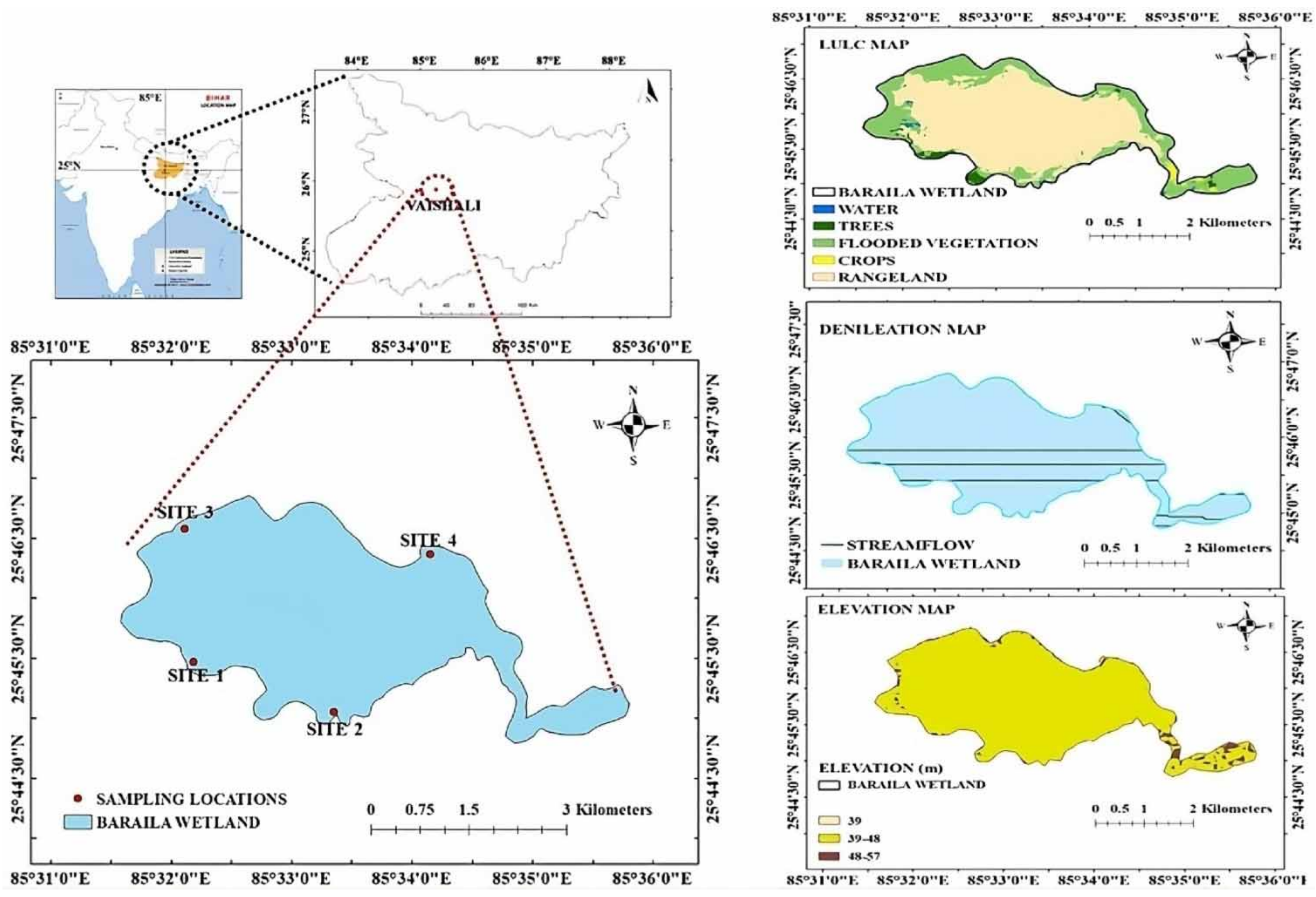


Figure 1 | Study area map of the Baraila wetland.

transferred from the sieve to a white enamel tray. The benthic macroinvertebrates with hard shells were manually handpicked with forceps, while the soft body organisms were collected with the help of a brush followed by their preservation in 10% formalin in a plastic jar and glass vial, respectively. Furthermore, the macroinvertebrates were meticulously sorted and identified up to the species level using appropriate taxonomic keys and manuals (Subba Rao 1989; Dey 2007; Nesemann 2007).

2.4. Ecological metrics

The macroinvertebrate's numeric features such as taxa richness, abundance, and tolerance scores are used to assess the water quality of the wetland as a function of different community-level metrics. The use of biotic indices is effective in assessing the responses of biota to organic pollution, though they may fall short in detecting various other types of pollution. The diversity indices, on the other hand, offer a broader perspective by gauging environmental stress, with higher species richness typically associated with unpolluted environments (Mason 2002). However, it becomes difficult to interpret whether the sensitive species or the tolerant species have contributed to the higher diversity index value in a wetland. Hence, an integrated approach employing both biotic and diversity indices holds promise for a more comprehensive evaluation of wetland water quality. The metrics used, their classification, and their corresponding interpretations that reflect the water quality of the wetland have been shown in Table 1. Here, the study incorporates a slight modification in the BMWP score given

Table 1 | Different indices were used to classify the water quality of the study area in the year 2022

Indices	Formula	Value	Interpretation	References
Margalef Richness Index (d_m)	$d_m = (S - 1)/\ln N$	R < 2.5	Low species richness	Guajardo (2015)
		2.5 > R < 4	Medium species richness	
		R > 4	High species richness	
Pileou's evenness Index (J)	$J = H/\ln S$	0	Uneven distribution between individual species	Guajardo (2015)
		1	Even distribution between individual species	
Shannon-Weiner diversity index (H')	$H' = -\sum p_i \times \ln p_i$	>3	Good	Mason (2002)
		1.0–3.0	Moderate	
		<1	Poor	
Hilsenhoff Family-level Biotic Index (HFBI)	$HFBI = \sum B_i \times S_i$	0.00–3.75	Excellent	Hilsenhoff (1988)
		3.76–4.25	Very good	
		4.26–5.00	Good	
		5.01–5.75	Moderate	
		5.76–6.50	Fairly poor	
		6.51–7.25	Poor	
		7.26–10.00	Very poor	
Biological Monitoring Working Party Baraila Lake (BMWP-BL)	$BMWP = \sum B_i \times W_i$	>100	Very Good	Mason (2002)
		71–100	Good	
		41–70	Moderate	
		Nov-40	Poor	
		0–10	Very poor	
Average Score Per Taxon (ASPT)	$ASPT = BMWP/S$	>7	Very good	Mason (2002)
		6.0–6.9	Good	
		5.0–5.9	Moderate	
		4.0–4.9	Poor	
		<3.9	Very poor	

S = Taxa richness, N = Total abundance, H′ = Shannon diversity index, B = Biotic score, W = weighted score.

by Mason (2002) called the BMWP-Baraila lake (BMWP-BL) score. The widely used approach in biomonitoring freshwater ecosystems, known as the Biological Monitoring Working Party (BMWP) score, is a method that measures organic pollution based on the presence and absence of species belonging to a specific family. The highest BMWP scores are assigned to the most sensitive families of macroinvertebrates, and the lowest scores to those most tolerant. These family scores are then combined to assess the overall BMWP score. The given BMWP method evaluates the water quality based on the presence and absence of species belonging to a family, that are given a particular score. However, a family given a high score might not be relatively abundant, than those given a lower score. In such cases, the assessment might deviate from the actual conditions of the wetland. The study incorporates the weighted score to the previously given formula of the BMWP score method. The weighted score was given based on the %relative abundance (%RA) of the taxa, given 1, 2, 3, 4, 5 when %RA was between 0 and 20, 21 and 40, 41 and 60, 61 and 80, 81 and 100, respectively, and is calculated using the formula given in Table 1.

2.5. Statistical analysis and graphical procedures

The statistical range, mean, and standard deviation of the physicochemical parameters of water, and the values of different ecological metrics used in the study were calculated in Microsoft Excel (2021). The resulting data of the ecological metrics were subjected to Nested ANOVA to assess variations at a 5% significance level, between the studied seasons, and the different sites, respectively. The seasonal difference in the RA of the benthic families was depicted as bar graphs using Microsoft Excel (2021), whereas the comparative differences between seasons for the ecological metrics were shown as a box plot, carried out in SPSS (version 22.0) software. Furthermore, the dendrogram showing preferential assemblages of benthic fauna was plotted using the paired group (UPGMA) method with the Bray Curtis similarity index in PAST software (version 4.03). The software R studio (version 4.3.1), with a vegan package, was used to construct Mantel Corrplot, between physicochemical variables and clusters of benthic macroinvertebrates.

3. RESULTS

3.1. Analytical results of water quality parameters

The statistical results of water quality parameters at the Baraila wetland during the comparative sampling periods in the year 2022 have been shown in Table 2. WT ranged from 28 to 32 °C, with an average of 30 ± 1.41 °C in the pre-monsoon, which has slightly increased to an average of 31.5 ± 1.11 °C in the post-monsoon season. The pH values observed in the study consistently fell within an alkaline range in both the sampling periods. The water pH ranges from 7.5 to 9.2 in the study area, which usually favors a significant number of Gastropoda, and other species of benthic fauna (Pennak 1978). The mean concentration of DO ranges from 7.32 and 8.26 mg/L in the pre-monsoon season and post-monsoon season of 2022, respectively.

Table 2 | Statistical summary of various physicochemical parameters analyzed during the study period of 2022

Parameters	Unit	Pre-monsoon		Post-monsoon		t-test between season
		Range	Mean ± Std	Range	Mean ± Std	*p*-value
Water temperature (WT)	°C	28–32	30 ± 1.41	30–33	31.5 ± 1.11	0.181
Depth (*d*)	ft	2–4	3.25 ± 0.82	2.5–5	3.875 ± 1.13	0.079
pH	–	7.7–9.2	8.38 ± 0.7	7.5–8.5	7.85 ± 0.38	0.205
Free CO_2	mg/L	9.68–20.53	15.10 ± 5.43	7.04–17.6	11.73 ± 4.39	0.857
Transparency	cm	17–34.5	26.16 ± 7.16	16–27	22 ± 4.54	0.161
Turbidity	NTU	28.16–44.7	37.59 ± 7.11	26.4–331.5	128.9 ± 119	0.267
Total Suspended Solid (TSS)	mg/L	19.33–35	28.16 ± 5.83	16.5–457.5	151.12 ± 178.16	0.308
Dissolved Oxygen (DO)	mg/L	2.42–11.92	7.32 ± 3.43	6.54–11.71	8.26 ± 2.05	0.443
Biological Oxygen Demand (BOD_5)	mg/L	3.03–67.20	20 ± 27.27	1.62–5.29	2.76 ± 1.47	0.332
Nitrate (NO_3^-)	mg/L	0.056–0.12	0.09 ± 0.02	0.18–0.35	0.24 ± 0.06	0.124
Ammonia (NH_4^-)	mg/L	0.126–0.211	0.17 ± 0.03	0.05–0.1	0.07 ± 0.019	0.035
Total Phosphorus (TP)	mg/L	0.037–0.09	0.07 ± 0.021	0.15–0.34	0.20 ± 0.07	0.068
Total Nitrogen (TN)	mg/L	1.50–2.4	1.90 ± 0.39	1.13–2.62	1.94 ± 0.55	0.794

The increased DO in the post-monsoon season might be due to stable water conditions and increased photosynthetic activity in warmer temperatures (Gupta *et al.* 2021; Singh *et al.* 2022). The TSS and nutrient parameters are among some other important factors that mainly influence the benthic fauna (Tampo *et al.* 2021; Singh *et al.* 2022). TSS showed the highest concentration in the post-monsoon season 457.5 mg/L and the lowest value of 35.0 mg/L was observed in the pre-monsoon season of 2022. The TN and TP in the wetland were noted with a mean value of 1.90 $\pm$ 0.39 mg/L and 0.07 $\pm$ 0.021 mg/L in the pre-monsoon, and 1.94 $\pm$ 0.55 mg/L and 0.20 $\pm$ 0.07 mg/L in the post-monsoon season. The values of NO_3^-, NH_4^-, and TP have varied greatly among seasons, where NH_4^- was observed with a p-value < 0.05. An increase in the value of TP and NO_3^- in the wetland during the post-monsoon season suggests the influence of rainfall, agricultural run-off, natural vegetation decay, and channelization (Sun *et al.* 2013). The concentration of NH_4^- can be toxic when it exceeds a threshold value. The observed value of NH_4^- in the wetland has varied from 0.05 to 0.211 mg/L, in the sampling year of 2022. A decrease in the value of NH_4^-, with an increase in the value of NO_3^- in the post-monsoon season, suggests the warmer temperature and oxidizing environment might have favored the nitrifying bacteria. Moreover, it seems that among the physicochemical variables TSS and the nutrient parameters are the determining parameters controlling the water chemistry of the wetland.

3.2. Community structure of benthic macroinvertebrates at the Baraila wetland

The community structure of benthic macroinvertebrates encompasses 21 species, belonging to 13 families, 7 orders, 4 classes, and 3 phyla has been identified at the Baraila Wetland, during the study period (Table 3). The wetland has been marked with a total abundance of 395 and 548 organisms of benthic macroinvertebrates in the pre-monsoon and post-monsoon seasons, respectively. Despite seasonal variations, site 4 has been observed with the highest taxa richness of 17 species in pre-monsoon and 13 species in the post-monsoon season. The %RA of all the identified families in the study period at Baraila wetland is shown in Figure 2. The Bithyniidae and Planorbidae have shown their dominance at all sites except at site 4, which is noted with a greater number of individuals from the Viviparidae and Thiaridae family. Species from nearly every family have been encountered at site 4, signifying a suitable habitat condition to support a diverse range of benthic fauna. A greater percentage of tolerant to semi-tolerant families of Chironomidae, Bithyniidae, and Planorbidae at sites 2 and 3 indicates comparatively deteriorated wetland conditions at these sites. Overall, the pre-monsoon season was dominated by the highest %RA of the Viviparidae family (27.04), followed by Bithyniidae (21.68), Planorbidae (19.38), Thiaridae (11.98), and Lymnaeidae (11.98). In contrast, species belonging to the Bithyniidae family have dominated in the post-monsoon season with 37.77% of RA. The result also noted the absence of benthic macroinvertebrates from families such as Gomphidae, Libellulidae, and Physidae, and the presence of *Salfia biharensis* (leeches), from the family Hirudidae in the post-monsoon season at sites 3 and 4. Although the total abundance of macroinvertebrates has experienced a steep increase in the post-monsoon, it has noted a 15% decrease in the taxa richness, indicating the role of monsoon in governing the species composition of the study area (Table 3).

3.3. Water quality assessment based on ecological metrics

The statistical results of various ecological metrics used in the study to assess the water quality of Baraila wetlands have been summarized in Table 4. None of the studied sites has noted a Margalef's richness index value exceeding 2.5, indicating low species richness in the study area. The Pielou's evenness index ranged between 0.64 and 0.92 in the pre-monsoon and ranged between 0.73 and 0.79 in the post-monsoon. A value of the evenness index nearing 1 suggests the absolute even distribution of species encountered at the study sites. Site 3 of Baraila wetland, having the lowest taxa richness among all the studied sites, is more evenly distributed than others. The value of the Shannon diversity index was recorded highest at site 4 (2.1) in the pre-monsoon season, whereas it ranges from 1.74 to 1.86 in the post-monsoon. It falls between 1 and 3 at all the studied sites, which indicates moderately polluted water conditions in the study area. Assessing water quality based on diversity indices somehow fails to incorporate.

The HFBI value has ranged from 3.43 to 4.75 in pre-monsoon and 4.09–4.60 in the post-monsoon season. An increase in the HFBI was noted at sites 1 and 3, whereas a marginal decline was recorded at sites 2 and 4. However, no significant variation was noticed between the seasons as the observed p-value was > 0.05. The BMWP and ASPT values are two of the most widely used biotic indices, which categorize the water quality based on the sensitivity of biotic species toward organic pollution. The highest and the lowest BMWP scores were noted at site 4 (80) and site 2 (28) during the pre-monsoon season. Meanwhile, the ASPT value has classified the study area from poor to moderately polluted wetland. The ASPT value has been observed with a marginal increase at every site (Table 4). Among all the studied sites site 3 was noted with the

Table 3 | Benthic macroinvertebrates, their code, and classification during the comparative seasons in the study area

S. No.	Names of benthic macroinvertebrates	Code	Family	Order	Class	Phylum	Pre-monsoon				Post-monsoon			
							Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4
1	*Bellamya bengalensis*	BB	Viviparidae	Mesogastropoda	Gastropoda	Mollusca	+	+		+	+			+
2	*Bellamya dissimilis*	BD	Viviparidae				+	+		+		+		+
3	*Bellamya micron*	BM	Viviparidae				+			+	+			+
4	*Angulyagra oxytropis*	AO	Viviparidae				+		+	+	+		+	+
5	*Thiara tuberculata*	TT	Thiaridae					+	+	+	+	+		+
6	*Digoniostoma cerameopoma*	DC	Bithyniidae					+	+		+	+	+	+
7	*Gabbia orcula var. producta*	GO	Bithyniidae				+	+		+	+	+	+	+
8	*Pila globosa*	PG	Ampullariidae					+			+	+		+
9	*Pila virens*	PV	Ampullariidae					+		+				
10	*Radix persica*	RP	Lymnaeidae	Basommatophora			+	+	+	+	+	+	+	
11	*Lymnaea accuminata*	LA	Lymnaeidae				+	+	+	+	+	+	+	+
12	*Lymnaea luteola*	LL	Lymnaeidae						+		+		+	
13	*Indoplanorbis exustus*	IE	Planorbidae				+	+	+	+	+	+	+	+
14	*Gyralus convexiculus*	GC	Planorbidae				+	+	+	+	+		+	
15	*Physa acuta*	PA	Physidae							+				
16	*Salfia biharensis*	SB	Hirudidae	Arhynchobdellida	Clitellata	Annelida							+	+
17	*Alboglossiphonia sp.*	Ab	Glossiphoniidae	Rhynchobdellida				+		+		+		
18	*Nepthys oligobranchia*	NO	Nephthyidae	Phyllodocida	Polychaeta					+				+
19	*Coenagrion puella*	CP	Libellulidae	Odonata	Insecta	Arthropoda				+				
20	*Ictinogomphus ferox*	IF	Gomphidae							+				
21	*Chironomus plumosus*	Ch	Chironomidae	Diptera			+		+	+		+	+	+

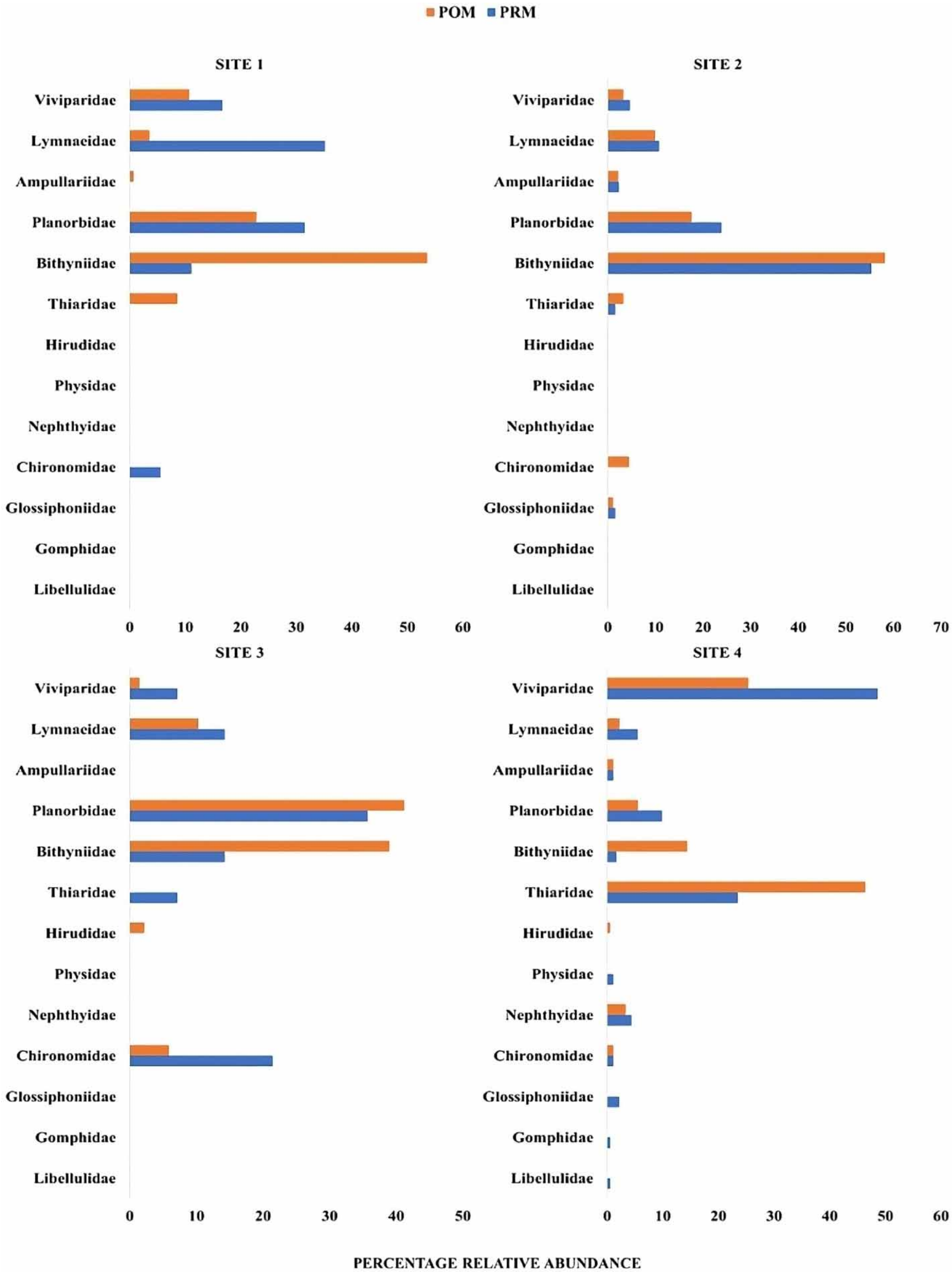


Figure 2 | Percentage of relative abundance of the families encountered at the Baraila wetland.

Table 4 | Seasonal difference in the various indices used in the study

	P_{rm}				P_{om}				Nested ANOVA *p*-value	
	Site 1	Site 2	Site 3	Site 4	Site 1	Site 2	Site 3	Site 4	Seasons	Sites
Margalef's richness index (d_M)	1.27	1.39	1.25	1.93	1.37	1.19	1.13	1.45	0.134	0.186
Pielou's evenness index (J)	0.89	0.64	0.92	0.74	0.74	0.76	0.79	0.73	0.917	0.331
Shannon diversity index (H')	2.07	1.58	2.02	2.1	1.84	1.74	1.82	1.86	0.276	0.258
Hilsenhoff's Biotic Index (HFBI)	3.93	4.51	3.43	4.75	4.6	4.37	4.09	4.19	0.981	0.367
BMWP	47	61	28	80	62	53	46	64	0.863	0.115
ASPT	4.7	5.08	3.11	4.71	5.71	5.3	4.6	4.92	0.422	0.292

lowest ASPT value of 3.11 in and 4.60 in the comparative seasons. The seasonal variations in the studied metrics have been shown as a box plot in Figure 3. As shown in the diagram the richness has declined in the post-monsoon, whereas the abundance of benthic macroinvertebrates remained relatively high in the post-monsoon. The wide inter-quartile range (IQR) in the pre-monsoon indicates higher variation in the observed number of individuals than in post-monsoon. Consecutively, the Shannon diversity index, Margalef's richness index, and Pielou's evenness index have also experienced a decline in their respective values during the post-monsoon season. The box plot of biotic indices showed slight variation in the observed results. The HFBI has been noted with a slight decrease, whereas the BMWP and the ASPT values have relatively lower values in the post-monsoon season. The multi-metric index evaluation through benthic fauna has revealed a more deteriorating wetland condition during the post-monsoon season.

3.4. Assemblage of benthic macroinvertebrates

Hierarchical Cluster Analysis (HCA) is an unsupervised machine learning technique that classifies and quantifies the similarity among distinct variables and aids in identifying patterns, relationships, and trends within a dataset (Shahbazy *et al.* 2020). In the study, the paired group (UPGMA) method with Bray Curtis similarity index was employed to construct an agglomerative cluster that assesses the assemblage pattern of the benthic macroinvertebrate species in the form of a dendrogram (Ogasawara & Kon 2021). The assemblages of benthic macroinvertebrates are grouped into three broad clusters (Figure 4). Cluster 1 comprises two sub-clusters, where the species highly sensitive to organic pollution including *Coenagrion puella*, *Ictinogomphus ferox*, are grouped, whereas *Alboglossiphonia*, *Physa acuta,* and the species of *Pila* are clustered together. The second cluster has marked the assemblage of species that are semi-tolerant (species of family Lymnaeidae, and Planorbidae), to tolerant to organic pollution (*Chironomus plumosus*). Cluster 3 separates the semi-sensitive group of species such as *Bellamya bengalensis*, *Bellamya micron*, *Bellamya dissimilis,* and *Nepthys oligobranchia*. Some species that are either found in greater numbers at every sampling location (*Thiara tuberculata* and *Angulyagra oxytropis*) or are relatively less abundant (*Lymnea luteola and S. biharensis*) have been separately clustered.

3.5. Relation of benthic macroinvertebrates with environmental variables

Furthermore, the study constructed a Mantel correlogram to analyze the relationship between the spatial distributions of clusters obtained in the dendrogram, with the environmental variables, and to see how they correlate at different distance intervals (Figure 5). The relation between environmental variables is interpreted as Pearson's *r*, which ranges from $+1$ (a strong positive correlation) to -1 (a negative correlation), whereas the result of the Mantel test is interpreted as Mantel's *p* and Mantel's *r*. The Mantel's *p*-value $\geq$0.05 is demarcated as green color, which indicates the relation of a variable of interest is non-significant with the other variables, while between 0.01 and 0.05 suggests the presence of a significant association. The Mantel's *r* value corroborates with Pearson's *r*, which means it measures the correlation between the biotic and environmental variables. A Mantel's *r* value $\geq$0.4 is demarcated as a thick line between the clusters and the environmental variables (Figure 4). The result revealed a significant association of cluster 1 with TSS, whereas cluster 3 shows a significant association with TN. A correlation between 0.2 and 0.4 was observed between pH and free CO_2 with cluster 1, whereas cluster 2 showed a moderate correlation with WT, DO, and TP. Cluster 3 which has grouped mainly species of *Bellamya* has been observed to be correlated with the depth and TSS of the wetland.

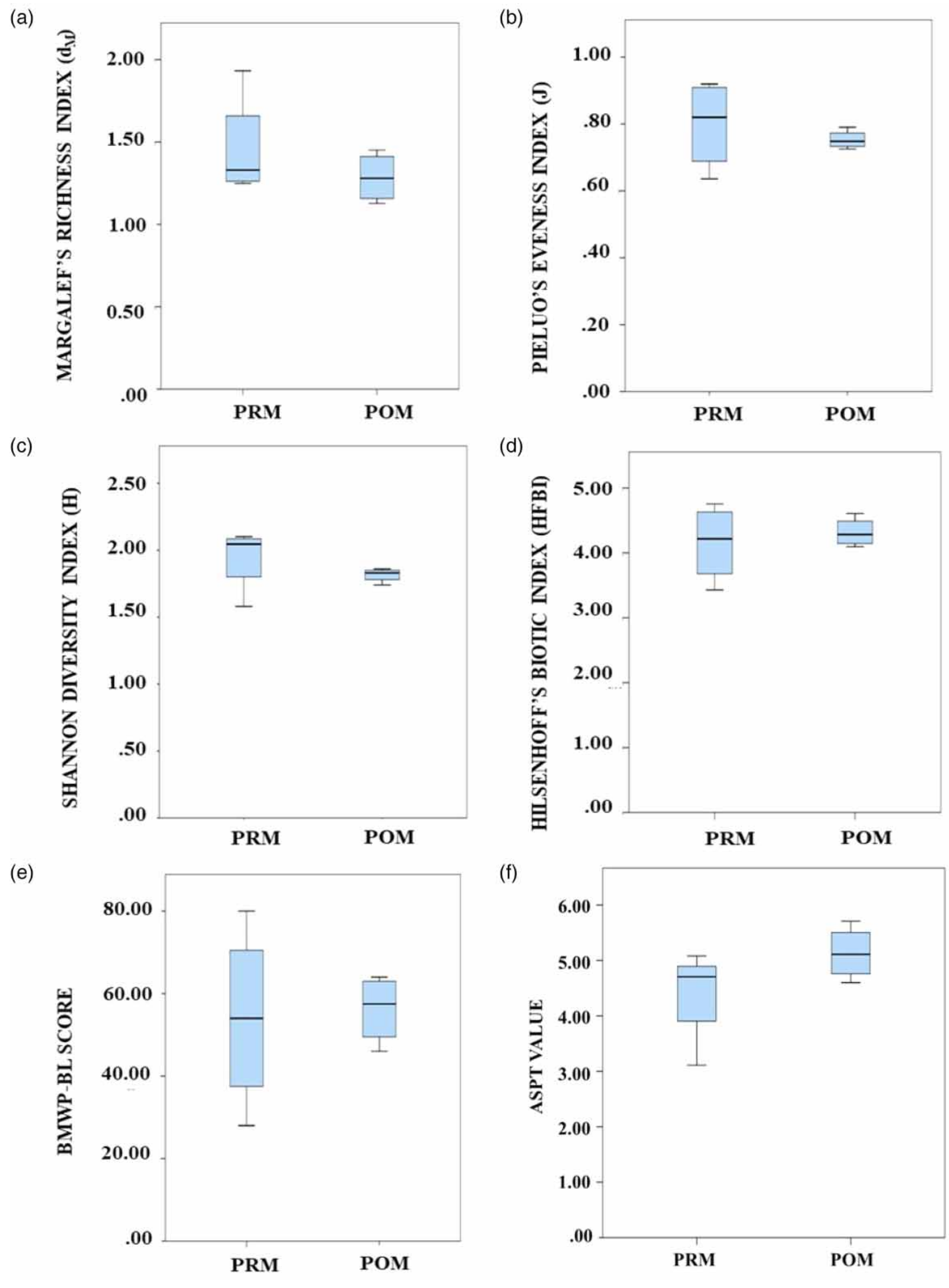


Figure 3 | Box plot showing seasonal differences in the ecological metrics used in the study.

4. DISCUSSIONS

The severity of wetland degradation is often misunderstood by conventional physicochemical monitoring programs. Biomonitoring, especially through sedentary organisms like benthic macroinvertebrates, has notably given better insights into the degree of degradation in a wetland (Miserendino *et al.* 2008; Mehari *et al.* 2014; Banda *et al.* 2023; Sripanya *et al.* 2023). The abundance and diversity of benthic fauna are the function of the physical and chemical characteristics of habitat conditions, as it quickly responds to any change in water quality (Neetu *et al.* 2019). Therefore, the study uses a multi-metric index based on macroinvertebrate community to evaluate the water quality and ecological conditions at the Baraila Wetland.

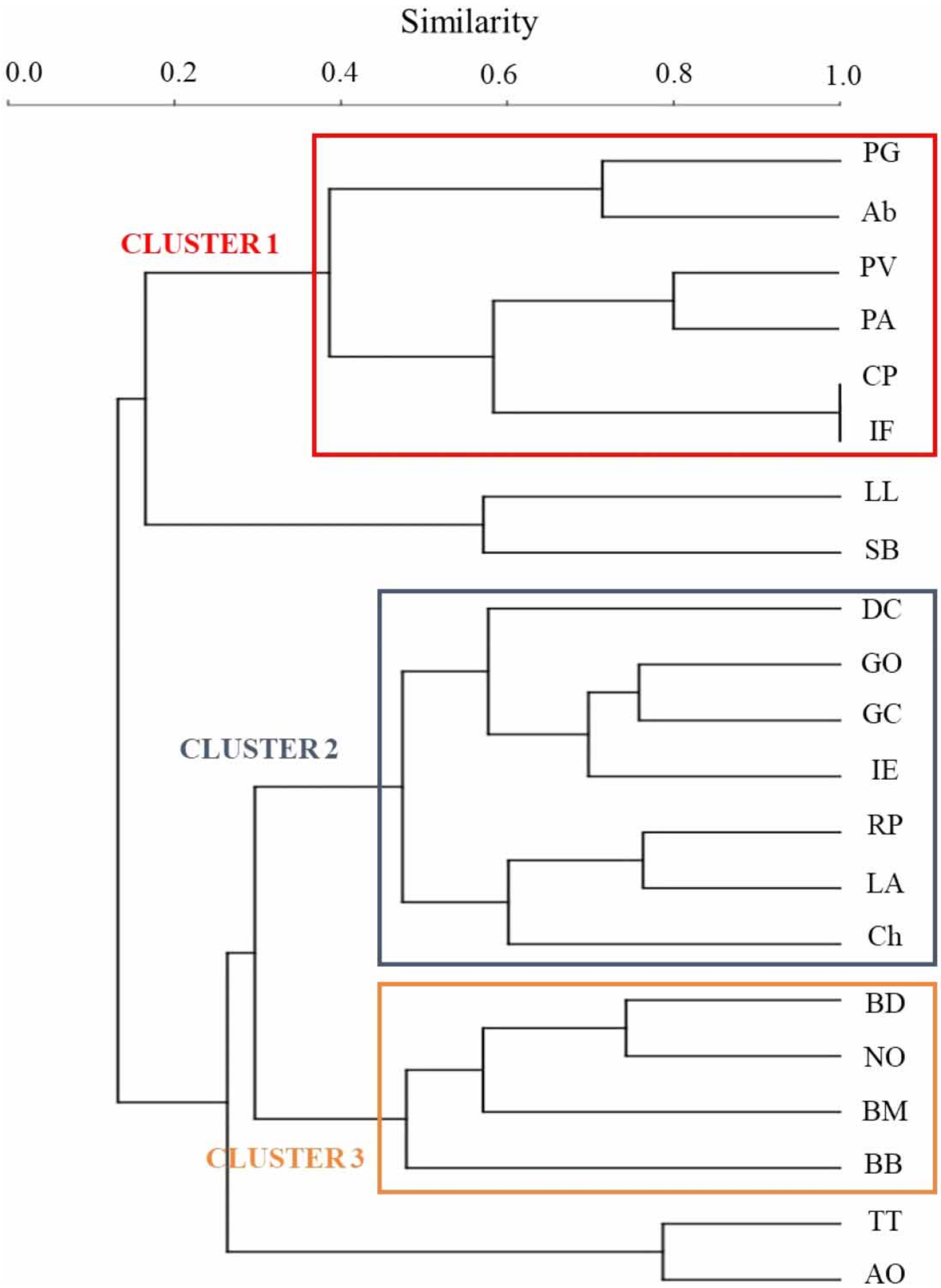


Figure 4 | Dendrogram showing assemblage of benthic macroinvertebrates at the Baraila wetland.

The study area lies in a semi-urban region predominated by agricultural activities near the watershed area of the wetland. Besides excessive nutrient input from agricultural fertilizer, the region is being encroached upon with human settlement, where overexploitation and habitat degradation are some most notable anthropogenic stressors (Aslam *et al.* 2023b). Moreover, the decline in the mean annual rainfall, the infiltration of weeds, heavy siltation, the channeling of river water through canals, clogging up the water resource from the river into wetland, and the local dispute among the inhabitants have caused water scarcity in the Baraila wetland (Aslam *et al.* 2021, 2023b).

Like many other seasonally flooded wetlands of the world the dilution effect of various chemical constituents in the water is largely dependent on the timing, duration, and magnitude of flooding by nearby rivers (Dube *et al.* 2017). The abiotic environment of an ecosystem plays a vital role in a structuring biotic community. The abiotic factors that mainly govern the

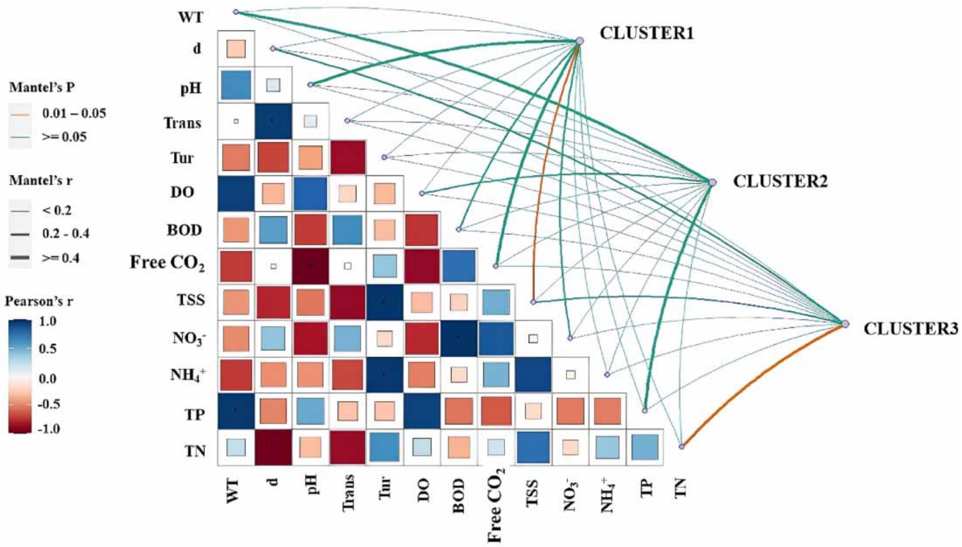


Figure 5 | Mantel's correlogram shows an association between clusters and the environmental variables at the Baraila wetland.

composition and assemblage of benthic macroinvertebrates include WT, depth, DO, nutrient level, TSS in water, the vegetation, and substrate composition of a wetland. It has been well established that water bodies with high DO favor the proliferation of a diverse range of benthic macroinvertebrates (Mehari *et al.* 2014; Mezgebu *et al.* 2019), where rapid fluctuation in DO can lead to the elimination of many species of insects and bivalves from the aquatic ecosystem (Tripathy & Mukhopadhayay 2015). Mishra *et al.* (2015) reported alkaline conditions, and temperatures up to 30 °C favor many species of molluscs which coincides with the findings of the observed results. A relatively higher temperature accelerates the egg hatching, larval development, and survival of various benthic fauna (Baaloudj *et al.* 2022). The absence of many species of insect family has been mainly because of the low water depth throughout the studied region (Basu *et al.* 2013). However, most of the physicochemical parameters in the study area were found to be within the optimal range to flourish the macroinvertebrate community except for the low water depth, turbidity, remarkably higher nutrient load, and increased TSS concentration during the post-monsoon season.

As revealed by the results, the studied region was predominated by the families of Bithyniidae, Viviparidae, Planorbidae, Lymnaeidae, and Thiaridae over the others. The low abundance of Ephemeropteran and Trichopteran, with a relatively higher diversity of semi-tolerant to tolerant species of benthic macroinvertebrates, indicates a moderate level of organic pollution. Furthermore, the absence of bivalves strengthens the discussion of moderate organic pollution in the wetland, as it usually prefers clean and well-oxygenated water conditions (Singh *et al.* 2019). Many previous studies have noted that the sensitive taxa are replaced by semi-tolerant to tolerant fauna in response to organic pollution in a wetland (Miserendino *et al.* 2008; Keddy 2010; Barkia *et al.* 2014). The dominance of benthic fauna of class gastropods in the wetland implies the significance of vegetation and shallow water in structuring their community at the Baraila wetland. In addition, the degree of organic pollution in the wetland was also validated by various indices used in the study, which classified the wetland under the moderately polluted category. There is an insignificant impact of season on structuring the benthic community at the study area as $p > 0.05$ between seasons. However, a decline in taxa richness during the post-monsoon season might be due to an increase in turbidity, TSS, and nutrient levels in the wetland (Velásquez Guarniz 2018). The higher taxonomic richness of benthic macroinvertebrates in pre-monsoon than in post-monsoon agrees with the findings of Molina *et al.* (2014), González *et al.* (2013), and Coayla-Peñaloza *et al.* (2023). Coayla-Peñaloza *et al.* (2023) also found disturbance by flooding after monsoon increases the flow, turbidity, and nutrient load to the lentic ecosystem, which negatively affects the development of benthic fauna.

The results of HCA have grouped benthic fauna into three clusters, where it appears that the sensitivity toward organic pollution and habitat preferences has mainly governed the assemblage of benthic macroinvertebrate community in the study area. The *Alboglossiphonia* species have been observed to remain associated with the leafy vegetation, or are found on the shell of gastropods including species of *Pila*, which eventually validates the results. The clustering of the species of Lymnaeidae, with

Chironomidae and Hirudidae explains their tolerance toward pollution (Singh *et al.* 2020). A study by Mezgebu *et al.* (2019) also spotted the species of Lymnaeidae, Chironomidae, Psychodidae, and Oligochaeta at highly impacted sites. The preference for habitat in shaping benthic community is very much evident in clusters 2 and 3, where species such as *A. oxytropis*, *Gabbia orcula*, *Gyralus convexiculus*, and *Digoniostoma cerameopoma* favoring stagnant water, and dense submerged vegetation are grouped (Tripathy & Mukhopadhayay 2015). The assemblage of *B. bengalensis*, *B. micron*, *B. dissimilis,* and *N. oligobranchia* is because all of them favor silty habitat conditions with relatively sparse vegetation. Similar observations have also been reported by the studies of Leitner *et al.* (2015), Gething *et al.* (2020), and Sripanya *et al.* (2023).

Contrary to the findings of other studies, that have noted a negative correlation of TSS with the benthic community, the significant association of TSS with benthic macroinvertebrates suggests the suspended particles in wetlands might primarily consist of organic matter that serves as food for benthic fauna (Rahman *et al.* 2021). Several studies have suggested that dead phytoplankton and other organic components contribute to the increased level of TSS in a wetland (Hernández *et al.* 2014; Jyväsjärvi *et al.* 2014; Tampo *et al.* 2021). The semi-tolerant to tolerant families of Lymnaeidae, Planorbidae, Hirudidae, and Chironomidae have been noted to thrive in the conditions with enriched nitrogen and ammonia concentration in the water. However, it has to be mentioned here that the level of ammonia in water has ranged from 0.07 to 0.17 mg/L, which has not exceeded the limit to cause acute toxicity even in the tolerant species (Nutile & Solan 2019). Moreover, the findings concur with the observations of Yang *et al.* (2020), who found a significant correlation of benthic macroinvertebrates with TP and TN. Shabani *et al.* (2019) working on a plain wetland in North-East China found TN, NH_4-N, NO_3^-N, and turbidity are among the significant variables that have impacted the distribution of filter-feeding benthos. The species of *Lymnea*, *Gyralus*, *Tubifex*, *Chironomus*, and *Limnodrilus* have been reported from disturbed and organically contaminated sites, which is largely influenced by high TP and biological oxygen demand.

5. CONCLUSION

The study offers a comprehensive understanding of the wetland's true condition, surpassing conventional water quality assessment approaches. This study notably reveals the wetland's moderate pollution status, predominantly attributed to agricultural expansion and human encroachment. The overexploitation, habitat degradation, and inadequate wetland management have resulted in diminished species richness across the wetland, despite suitable water quality conditions. The survival of species, resilient to moderate pollution, with the absence of sensitive fauna, shedding light on the adverse impact of multiple anthropogenic stressors. In addition, the cluster analysis, followed by Mantel's correlogram approach in biomonitoring, has well-documented the role of water quality parameters in shaping macroinvertebrate communities. However, a holistic study on the impact of anthropogenic stressors and the habitat conditions remains an imperative limitation of the research work, which can be acknowledged for further exploration. The research underscores the urgent necessity for proactive conservation measures and informed management strategies to safeguard this potential Ramsar wetland in the near future. Furthermore, the global significance of this study resonates strongly with similar threatened wetlands facing water scarcity, and encroaching urbanization, emphasizing the relevance of these findings on a broader scale.

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AUTHORS' CONTRIBUTION

D. conceptualized, investigated, did formal analysis, did data curation, wrote the original draft, and did software analysis. A.K. conceptualized and supervised the study, provided resources, wrote, reviewed, and edited the article.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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