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# Spatio-temporal variability in macroinvertebrate community structure and ecological health status of a tropical dynamic river-estuarine system, India: An integrated approach of multivariate analysis

Sudhanshu Charan Mohapatra<sup>a</sup>, Vishnu Vardhan Kanuri<sup>a,\*</sup>, Kiran Kumar Vaddem<sup>a</sup>, Sarmistha Saha<sup>a</sup>, Syed Yakub Ali<sup>a</sup>, Ajeet Verma<sup>a</sup>, Mrinal Kanti Biswas<sup>a</sup>, Ajit Kumar Vidyarthi<sup>b</sup>

<sup>a</sup> Central Pollution Control Board, MoEF & CC, Regional Directorate, Kolkata, India

<sup>b</sup> Central Pollution Control Board, MoEF & CC, New Delhi, India

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# ABSTRACT

River-estuarine ecosystems are under severe anthropogenic threat due to resource exploitation, transportation, sewage/industrial discharges, and pollutants from surrounding areas. Monitoring the water quality and biological communities is essential for assessing ecosystem health and sustainability. Present study integrated the ecological community data along with water quality analysis to understand the impact of anthropogenic pressures on benthic macroinvertebrates. Samples were collected from 10 locations (comprising of both rural and urban areas) for Benthic macroinvertebrates, physico-chemical and microbiological parameters along the lower stretch of the Bhagirathi-Hooghly river-estuarine (BHE) system during the post-monsoon seasons of 2020, 2021, and 2022. During the entire study period, a total of 5730 individuals from 54 families in 19 orders of 3 phylum of macroinvertebrate were recorded. Among them Thiaridae (27.1%) and Chironomidae (22.8%) were found to be the most abundant families. Based on the water quality data Cluster analysis and nMDS indicated two distinct groups of locations: Group-I with rural settings and Group-II with urban settings. Alpha diversity metrics showed higher diversity (2.817) and evenness (0.744) in rural locations (Group-I) compared to urban locations (Group-II). The overall saprobic score of the macroinvebrate data revealed Group-I (5.09) to be in good condition, while Group-II (4.95) showed moderately polluted conditions. Redundancy analysis (RDA) highlighted the correlation of pollution-tolerant species (Chironomidae, Culicidae) with high organic loads i.e., biochemical oxygen demand (BOD), chemical oxygen demand (COD) in Group-II. In contrast, Group-I locations exhibited positive correlations with Dissolved Oxygen (DO) and supported less pollution-tolerant organisms (Coenagrionidae, Dytiscidae). The study emphasizes the importance of integrated analysis of ecological community data and water quality parameters to assess the health status of river-estuarine ecosystems.

### 1. Introduction

River-estuarine ecosystems are highly perturbed by severe anthropogenic activities, including resources exploitation, transportation, recreational proposes and the adoption of sewage/industrial discharge. These ecosystems are also vulnerable to harmful substances from the surrounding areas through natural draining systems (Shinn et al., 2009). In view of the deterioration of these environments, several countries have developed monitoring systems to either assess water quality/pollution or evaluate the biological community to understand the health status and maintain the sustainability/surveillance. However, evaluation of these ecosystems considering the contaminants/pollutants/biological health is often inadequate. Integrated analysis of ecological community data, such as biological diversity together with water quality, can provide an auxiliary sign for assessing anthropogenic pressures affecting biological communities (Gernes and Helgen, 2002; Walker et al., 2009; Van Ael et al., 2015; del Valle and Astorkiza, 2018).

Benthic macroinvertebrates are the most consistently emphasized biotic component of the riverine and estuarine ecosystems, as they are the principal organisms that have great significance in ecosystem engineering and function. They play a crucial role in the food web dynamics

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<sup>\*</sup> Corresponding author.Central Pollution Control Board Regional Directorate Kolkata, West Bengal, India. *E-mail address: vishnuchemistry@gmail.com* (V.V. Kanuri).



Fig. 1. Map showing the study area red dots representing the sampling locations.

including nutrient cycling through their burrowing and feeding activities via bioturbation (Hynes, 1978; Hutchings, 1998; Constable, 1999; Pennifold and Davis, 2001; De Roach et al., 2002; Dauvin, 2007; Wildsmith et al., 2009; Mermillod-Blondin, 2011; Stolyarov, 2013). Unswervingly, they noted as suitable ecological health indicators of the immediate environment due to their important characteristics, viz., diversity and tolerance to stress (Morris et al., 2014). They can survive extreme exposure to contaminants/pollutants, have long-life spans in response to the changes occur in sediment and water quality over time, and some species have viable ecological importance (Dauer, 1993; Reiss and Kröncke, 2005; Dauvin, 2007). Additionally, their abundance, ease of sample collection, and small home ranges will facilitate the use of macroinvertebrates for site-specific studies on pollution (Nunes et al., 2008; Díaz-Jaramillo et al., 2010).

Most of the studies in the tropical freshwater ecosystems have shown the impact of anthropogenic activities on macroinvertebrate diversity and their association with the surrounding environment (Baumgartner and Robinson, 2017; Agra et al., 2021; Arimoro and Keke, 2021). Although these ecosystems are often sullied through various stressors (Dudgeon, 2010; Strayer and Dudgeon, 2010; Arimoro and Keke, 2017; Keke et al., 2021; Arimoro and Keke, 2021), their studies are biased towards limited geographic regions (Boyero et al., 2009). Despite the rapid growth in the studies related to the ecological monitoring in the tropical environments during the recent-past decades a significant gap remains in understanding the biodiversity structure (Tonkin et al., 2016; Arimoro and Keke, 2017) of macroinvertebrates when compared with the temperate regions (Tonkin et al., 2016; Keke et al., 2021). Consequently, the studies on the biodiversity affected by unraveled natural and anthropogenic disturbances and their continuous monitoring are very much important at a specific region/site for better conservation and protection of biodiversity in that region. Whereas, macroinvertebrates distribution-related studies were limited to a few regions along the largest riverine system (The River Ganges) of India (Nesemann et al., 2004, 2007, 2011; Kumar et al., 2013; Agrawal et al., 2019; Goel et al., 2021; Sharma and Behera, 2022).

The present study is considered to explore the data in a holistic manner, focusing on the spatio-temporal gradients of benthic macroinvertebrates communities and hydro-chemical characteristics along the lower stretch of the Bhagirathi-Hooghly river-estuarine (BHE) system through systematic illustration and statistical assessment using various uni- and multivariate statistical models along with various metrics i.e., alpha ( $\alpha$ ) metrics associated with richness, diversity of macroinvertebrate assemblages and the effect of anthropogenic activities will be assessed.

### 2. Materials and methods

# 2.1. Study area

The river Ganga is one of the most significant and iconic rivers in India. It originates in the state of Uttarakhand in the Himalayas and travels a distance of about 2000 km before it enters the state of West Bengal at Farakka in Murshidabad district. At Farakka the river bifurcates to two major riverine systems Padma and Bhagirathi-Hooghly. Bhagirathi-Hooghly flows continuous towards south and travels approximately 520 km before it reaches its final destination, the Bay of Bengal, at Ganga Sagar. The Ganges, after entering into the West Bengal, is popularly known as Bhagirathi-Hooghly River (BHR). The lower region of the BHR system, spanning about 295 km from Nabadwip to Ganga Sagar, falls under the tidal/estuarine zone (CIFRI, 2004), and is referred to as the Bhagirathi-Hooghly river-estuarine (BHE) system. This region is mostly characterized by the mixing of fresh and saline water under the influence of tides. Being a transition zone between fresh and saline ecosystems it is highly dynamic and ecologically important zone. The Bhagirathi-Hooghly river-esturaine (BHE) system plays a crucial role in the socio-economic and ecological aspects of the region. The

mid-region of the BHE is of particular significance as it serves as a major source of domestic water supply for the highly urbanized metropolitan twin cities of Kolkata and Howrah, as well as other towns in the area. Additionally, the water from the estuary is utilized for various industrial purposes (KMDA, 2017). As BHE traverse through highly urbanized towns of West Bengal, it is also facing severe anthropogenic threats viz., continuous influx of untreated sewage and industrial discharge, along with other pollutants from densely populated and urbanized areas.

In order to assess the health status of the lower stretch of the BHR, comprehensive sampling has been conducted including benthic macroinvertebrates, physico-chemical parameters, and microbiological parameters during the post-monsoon seasons (lean flow) of 2020, 2021, and 2022. For this a total of 10 locations were selected (Fig. 1). Out of these, 6 locations (4 were selected from densely populated districts, and 2 were from the metropolitan twin cities (Kolkata and Howrah) of West Bengal) that are being under the influence of tide. Additionally, 4 locations were chosen from the semi-urban stretch of the BHR system. The purpose of selecting these diverse locations was to assess the impact on the distribution and fate of macroinvertebrates under the influence of various environmental conditions along the lower stretch of the BHE system.

## 2.2. Sampling analytical methodology adopted

## 2.2.1. Physico-chemical parameters and microbiological analysis

Subsurface water samples were collected from the middle of the river. A shallow water sampler was employed to collect water samples for the analysis of various physicochemical and microbiological parameters like temperature, pH, conductivity, total suspended solids (TSS) total dissolved solids (TDS) dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD) nitrate, ammonia, phosphate, sulphate, calcium, magnesium, total hardness, total alkalinity, fluoride, sodium, and potassium. Insitu temperature was recorded using a Brannan thermometer. About one liter of sample was collected in high-density polyethylene (HDPE) containers for the estimation of general parameters and 2.5 L for BOD. Samples were also collected for the estimation of coliforms in 100 ml pre-sterilized glass bottles. All the samples were ice preserved to maintain lower temperatures until analysis. About 100 ml of sub-samples were collected separately for COD and Alkali metals (Na and K), preserved with conc. H<sub>2</sub>SO<sub>4</sub> and conc. HNO3 respectively. For the estimation of Dissolved Oxygen, samples were fixed insitu with Winkler's reagents. Electrical Conductivity and pH were measured using pre-calibrated conductivity and pH meters respectively. The accuracy of pH analysis was  $\pm 0.007$ . Dissolved oxygen (DO) was measured using the Winkler's titration method and the precision of the analysis was  $\pm 0.07\%$ . Fluoride and nutrient parameters viz., ammonia, nitrate, phosphate and sulphate were estimated following standard spectrophotometric procedures. The total hardness, calcium and magnesium were measured following complexometric titration. Flame photometer is used for the analysis of sodium and potassium. COD analysis was performed following acid digestion in the presence of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> followed by back titration with ferrous ammonium sulphate. All these analyses were performed according to standard methods given in American Public Health Association (APHA, 2017). All the sample analysis was completed within 2 days after sampling, except for BOD. The sample collected for BOD was directly siphoned to five 300 ml BOD bottles. Two bottles were fixed with Winkler's reagent for the initial DO and the rest of the three bottles were incubated at 27  $^\circ C$ temperature in the BOD incubator for 3 days before fixation. Finally, the change in DO among initial and after incubation was measured and represented as BOD (BIS, 1991). The samples collected for the enumeration of total and fecal coliforms was assayed following a three-stage multiple tube fermentation technique prescribed by standard methods of APHA 2017. The results obtained were statistically expressed as MPN index/100 ml.

#### Tables 1

Descriptive statistics of Physico-Chemical and micobiological parameters in the Bhaghirathi-Hooghly river-estuarine system during post-monsoon season of 2020, 2021 and 2022.

	2020	2021	2022	Total
EC	387.7 ± 35.17 (324–459)	433.1 ± 65.25 (318–566)	398.8 ± 25.19 (357–451)	406.54 ± 47.84 (318–566)
pH	$8.3 \pm 0.2$ (7.9–8.5)	$7.8 \pm 0.41$ (7–8.5)	$7.6 \pm 0.3$ (7–7.9)	$7.9 \pm 0.43$ (7–8.5)
DO(mg/L)	$9.05 \pm 1.23$ (7.2–11)	7.43 ± 1.86 (4–9.3)	$6.63 \pm 1.41$ (4.6–8.8)	7.71 ± 1.79 (4–11)
BOD(mg/L)	$3.14 \pm 2.62$ (0.4–7.2)	$3.65 \pm 2.06$ (2.5–9)	$2.7 \pm 1.83$ (1–7)	$3.17 \pm 2.16 \ (0.4-9)$
COD(mg/L)	$12.9 \pm 4.61$ (8–20)	9.6 ± 9.16 (5–35)	$11.2 \pm 2.94$ (7–15)	$11.24 \pm 6.1$ (5–35)
TSS(mg/L)	$25.8 \pm 12.9 \ \text{(5-54)}$	$40.5 \pm 18.12 \; \textbf{(870)}$	$17.5 \pm 17.52$ (1–47)	$27.94 \pm 18.5$ (1–70)
TDS(mg/L)	$220.6 \pm 16.77$ (194–258)	$215.4 \pm 36.97$ (155–280)	$214.7 \pm 19.4 \ \textbf{(187-251)}$	$216.9 \pm 25.21 \; \text{(155-}280\text{)}$
Na(mg/L)	$15.28 \pm 2.14$ (11.8–18.15)	$23.03 \pm 4.83 \ \textbf{(16.1-33.1)}$	$21.4 \pm 1.72$ (18.5–23.5)	$19.91 \pm 4.6 \ (11.8 - 33.1)$
K(mg/L)	$4.19 \pm 0.45 \ (3.36  4.73)$	$4.53 \pm 0.89 \ (3.53 6.6)$	$4.84 \pm 0.91$ (2.9–6)	$4.52 \pm 0.8 \; (2.9  6.6)$
Alkalinity(mg/L)	$161.3 \pm 17.96$ (126–195)	$159.5 \pm 23.02$ (116–204)	$150.2 \pm 9.73$ (132–162)	$157 \pm 17.84$ (116–204)
Hardness(mg/L)	$161 \pm 12.31$ (132–180)	$151.9 \pm 17.72$ (124–180)	$144 \pm 8.06 \ (132 - 160)$	$152.3 \pm 14.65$ (124–180)
Ca(mg/L)	43.4 ± 3.31 (36–47)	$39.49 \pm 6.3$ (28.3–47)	34.6 ± 4.2 (28–41)	$39.17 \pm 5.89$ (28–47)
Mg(mg/L)	$12.75 \pm 1.94$ (10.5–17)	$12.85 \pm 1.99 \ \text{(9-15.8)}$	$13.8 \pm 2.4$ (11–19)	$13.14 \pm 2.1$ (9–19)
Cl-(mg/L)	$18.7 \pm 5.04$ (15–31)	$27 \pm 7.82$ (20–41)	$24.1 \pm 5.33$ (12–30)	$23.27 \pm 6.92$ (12–41)
Fluoride(mg/L)	$0.3 \pm 0.06 \; \textbf{(0.24-0.42)}$	$0.45 \pm 0.12 \; (0.27  0.6)$	$0.4 \pm 0.13 \; (0.3  0.72)$	$0.39 \pm 0.12 \; \textbf{(0.24-0.72)}$
Phosphate(mg/	$0.1 \pm 0.05$ (0.02–0.15)	$0.12 \pm 0.08 \; (0.03  0.26)$	$0.07 \pm 0.05 \; (0.03  0.2)$	$0.1 \pm 0.07$ (0.02–0.26)
L)				
Sulphate(mg/L)	$22.2 \pm 6.07$ (18–39)	$23.8 \pm 1.82$ (20–26)	$21.5 \pm 1.91$ (17–24)	$22.5 \pm 3.82 \ \text{(17-39)}$
Nitrate(mg/L)	$0.25 \pm 0.37$ (0.03–1.23)	$1.01 \pm 0.43$ (0.35–1.74)	$0.28 \pm 0.24 \; \textbf{(0.05-0.69)}$	$0.51 \pm 0.49$ (0.03–1.74)
Ammonia(mg/L)	$0.33 \pm 0.37 \; (0.02  1.03)$	$0.72 \pm 1.35$ (0.02–4)	$0.2\pm0.43\;(0.011.3)$	$0.41 \pm 0.82$ (0.01–4)
TC(MPN/100 ml)	$419873 \pm 565390 \ \text{(}4901600000\text{)}$	$624900 \pm 745589 \text{ (}130002200000\text{)}$	$1250000 \pm 2054205$	$712832 \pm 1157861 \text{ (490-}5400000\text{)}$
			(70000–5400000)	
FC(MPN/100 ml)	84327.5 $\pm$ 71936 (20–160000)	$83053 \pm 85795 \text{ (490-240000)}$	$308983 \pm 634234 \text{ (79001600000)}$	$139960 \pm 319122 \text{ (20}1600000\text{)}$

### 2.2.2. Benthic macroinvertebrates sampling & identification

Macroinvertebrate sampling has been conducted during lowest lowtide from the inter-tidal region at 6 locations from the urban environment, and from the banks to 1 mt depth at 4 locations of semi-urban stretch of BHE system. Selection of intertidal zones for sampling the macroinvertebrates is considered, as substratum formed in these regions are stable after the removal of surface sediments due to the influence of tides, which provides a valuable insights of the existing benthic ecosystem. These kind of environments provides a stable pelophilic species (like gastropods, crustaceans etc.) which are opportunists and rather resistant to and resilient from perturbations (Diaz, 1989 & Diaz, 1994).

Macroinvertebrates were collected at all the locations using a rectangular frame D-net (20  $\times$  30 cm with a mesh size of 0.6 mm) to kick sampling for 10 min (Gabriels et al., 2010). Also the bottom sediments were disturbed with the feet for the effective collection of macroinvertebrates. Apart from this, a set of sediment samples were collected at each location from 1 m square area with a depth of approximately 5-10 cm and sieved the samples using a sieve of mesh size of 0.6 mm. Finally, the samples were sorted for macroinvertebrates in the field, 4% formalin was used to preserve the sorted samples in PET bottles and labelled. Subsequently the samples were identified in the laboratory up to the family level using a stereomicroscope following the identification keys (Wood, 1992; Zwart et al., 1995; Jessup et al., 2003; Akolkar et al., 2017; Bouchard, 2021) and enumerated the individual family count. Based on the available literature, Central Pollution Control Board (CPCB) has adopted Biological Monitoring Working Party (BMWP) tolerance score established in UK (Armitage et al., 1983; Gabriels et al., 2010) was used in the establishment of the multimetric macroinvertebrate index in order to assess the water quality.

# 2.2.3. Statistical analysis of environmental variables and macroinvertebrate data

To emphasize the distribution patterns of macroinvertebrates from the lower stretch of BHR system, biodiversity and richness of the macroinvertebrate families were assessed using classical alpha-diversity metrics including families number S, Shannon–Weiner diversity index (H), Margalef's species richness index (d) and Pielou's evenness (J) were calculated following Eq. (1), Eq. (2) and Eq. (3) respectively.

$$d = (S-1) / (\log_e N) - - - - - - - - - 2$$

(Where  $P_i = n_i/n$  (proportion of the sample belonging to the *i*th family), *S* is the number of families and *N* is total no. of individuals of all the families in a sample)

Also, species turnover, beta similarity based on Bray– Curtis similarity were estimated following Whittaker (1960), 1972; Wilson and Shmida (1984); Koleff et al., (2003), to evaluate the assemblage patterns among the families and their interactions with one another and with the environment.

Detrended correspondence analysis (DCA) was performed using CANOCO 4.5 to evaluate the suitable response model (linear or unimodal) for both the macroinvertebrate and environmental data. The gradient lengths of DCA outcomes has less than two standard deviations. Consequently, redundancy analysis (RDA) was performed on the square toot transformed data to envisage the existing relationship between macroinvertebrate metrics and environmental variables and plotted the ordination diagram (ter Braak and Smilauer, 2002). The Monte Carlo randomization test with 499 permutations was used to test the significance level and to evaluate the best probability for the observed patterns ter Braak and Verdonschot, 1995). All these statistical analysis were performed using statistical software packages viz., SPSS 11, Primer v6, CANOCO v4.5 and Microsoft Excel.

### 3. Results and discussion

### 3.1. Distribution of hydro-chemical and microbiological characteristics

The pH and DO levels in an aquatic ecosystem play a crucial role in determining the fate of the aquatic life, as they exerts a fundamental influences on the overall health, well-being, and survival of the organisms thriving within the ecosystem. The pH values varied from neutral to alkaline (7.0–8.5 with an avg. 7.9  $\pm$  0.43) during the entire study period. Notably, lower pH values were observed during 2021 when compared with the other two years. The mean concentration of DO

Pearsons Corr	elation betwo	een the Physi	co-Chemical	l and micobi	iological pa	rameters.												
Parameters	EC	Ηd	DO	BOD	COD	SST	TDS	$Na^+$	$\mathbf{K}^{+}$	T.Alk.	T.Hard.	$\mathrm{Ca}^{2+}$	$\mathrm{Cl}^-$	$\mathbf{F}^-$	$PO_4^{3-}$	$NO_3^-$	$\rm NH_4^+$	TC
EC	1																	
hd		1																
DO	$610^{**}$	.567**	1															
BOD	.700**		420*	1														
COD	.536**		$416^{*}$	.684**	1													
TSS	.423*					1												
TDS	.767**			.598**	.586**	.385*	1											
$Na^+$	.595**	$592^{**}$	$606^{**}$					1										
$\mathbf{K}^+$	.555**		$592^{**}$	.508**	.435*		.363*	.594**	1									
T.Alk.	.769**			.639**	$.610^{**}$		.654**		.455*	1								
T.Hard.	**609.			.494**	.517**	.427*	.728**			.704**	1							
$Ca^{2+}$	.449*			.372*	.365*		.444*			.640**	.821**	1						
$Mg^{2+}$							.450*											
Cl <sup>-</sup>	.678**		463**	.515**			.422*	$.591^{**}$	.366*				1					
$\mathbf{F}^{-}$								.563**	.427*				.384*	1				
$PO_4^{3-}$	.444*						.528**				.419*	.472**			1			
$SO_4^{2-}$										$.364^{*}$								
$NO_3^-$	.543**			.519**				.477**					.448*	$.480^{**}$		1		
$NH_4^+$	.789**		483**	.754**	.712**		.654**	.529**	$.611^{**}$	.638**	.500**	.400*	.588**		$.391^{*}$	.466*	1	
TC	.443*	506*		$.621^{**}$			.504*		.507*					.453*	.457*		.474*	1
FC				.450*					.426*					.525**	.422*			.906**

ranged from 6.6 to 9.05 mg  $L^{-1}$  (Table 1). The higher values of DO were encountered during 2020 at the upstream locations. Whereas, the lower values were observed during 2022. Furthermore, BOD and COD concentration were found to be high during post-monsoon periods of 2021 and 2022 respectively. The alkalinity concentrations were found to be varied from 116 to 204 mg  $L^{-1}$ . The Pearson's correlation analysis revealed a strong negative correlation (p < 0.01) between DO and BOD as well as COD, indicating that high concentration of organic matter played an important role in depleting oxygen levels during those periods (Table 2). The values obtained in the present study are corroborated with the results described for the study region by Kanuri et al. (2020). Also, the positive correlation between Alkalinity and BOD, COD indicates that the remineralization in the presence of microbial community might be the important source for alkalinity into the environment. Coliforms acts as an indicator species to evaluate the microbial contamination in aquatic environments. The mean TC and FC counts ranged from 419873 to 1250000 and 83327 to 308983 MPN/100 ml respectively. This indicates that system contaminated with coliforms. These observation underscores the significance of increased human activities, including domestic sewage effluents and industrial discharges, following the COVID period, which have impacted the distribution of DO, BOD, COD, TC and FC in the BHE System. The descriptive statistics of the hydro-chemical and biological parameters along the lower stretch of the BHE System during the post-monsoon seasons of 2020, 2021, and 2022 are depicted in Table 1. The mean values of pH and DO observed in the study area were well within the limits of primary water quality criteria for outdoor bathing prescribed by MoEF & CC. However, the concentrations of biochemical oxygen demand (BOD) and fecal coliforms (FC) were found to exceed the standard criteria.

# 3.2. Benthic macro invertebrate family assemblage patterns and their contribution

From the total 30 samples collected during the entire study period (i. e., Post-monsoon season of 2020, 2021 & 2022) a total of 5730 individuals from 54 families in 19 orders of 3 phylum were recorded (Table 3). Arthropoda is the dominating phylum followed by Mollusca and Annelida. Among them Thiaridae (27.1%) and Chironomidae (22.8%) were found to be the most abundant families with significant spatio-temporal variability in their individual count. Also, *k*-dominance plot on overall data of Macroinvertebrate families indicated that the upstream locations having high diversity (Fig. 2). A significant increase in the total no. of animals' observed during post-monsoon 2021 when compared with 2020 (i.e., 2.3 times) and decreased by 0.7 times during post-monsoon 2022 when compared with 2021 (Table 3).

Cluster analysis and nMDS powered with SIMPROF was performed based on the Bray-Curtis similarity over the square root transformed macroinvertebrate family abundance for the entire study period exhibited a two reasonably convincing groups of locations with 43% similarity. Group-I comprises of rural locations (Falgu, Pagla, Berampore, Kolabheria) and Group-II formed with urban locations (Serampore, Kharda, Belghoria, Bally, Howrah and Garden Reach) (Fig. 3a). Similarities (characterizing families) within the groups to percentage contribution of macroinvertebrate families and dissimilarity (discriminating families) between the groups has been assessed through SIMPER analysis. This shows that average intra-group similarities of Group-I and Group-II among the macroinvertebrate families were 50.0% and 62.16% respectively. Whereas, the inter-group average dissimilarity was found to be 58.2%. Also, displayed that the a total of 18 macroinvertebrates families has contributed a cumulative percentage of 91 for Group-I (viz., Thiaridae, Coenagrionidae, Chironomidae, Planorbidae, Palaemonidae, Lymnaeidae, Viviparidae, Atyidae, Dytiscidae, Octochaetidae, Hydrophilidae, Libellulidae, Nepidae, Physidae, Ephemeridae, Unionidae, Bithyniidae & Notonectidae). Whereas, only 12 families were contributed a cumulative percentage of 91 for the Group-II (viz., Chironomidae, Thiaridae, Parathelphusidae, Viviparidae, Nephtyidae, Planorbidae,

### Table 3

Macroinvertebrate taxonomical distribution and abundance in Semi-Urban(Gr-I) and urban (Gr-II) agglomerates along the Bhaghirathi river-estuarine system.

			2020		2021		2022		Total	
Phylum	Order	Family	Gr-I	Gr-II	Gr-I	Gr-II	Gr-I	Gr-II	Gr-I	Gr-II
Arthropoda	Coleoptera	Dytiscidae	42	20	17	11	2	1	61	32
		Hydrophilidae	0	0	12	8	59	6	71	14
		Gyrinidae	0	0	0	0	1	0	1	0
		Noteridae	0	0	0	0	3	0	3	0
		Heliplidae	0	0	0	0	0	4	0	4
	Diptera	Chironomidae	36	120	85	635	158	275	279	1030
	Dipieru	Culicidae	6	10	30	7	100	4	37	30
		Ephydridae	1	6	0	5	0	- -	1	13
		Symbidae	1	0	0	0	0	0	1	0
		Tabanidae	1	0	0	0	0	0	1	0
		Mussidae	1	0	0	0	0	0	1	0
		Developedidoe	0	0	0	0	0	9	0	9
		Psychodidae	0	0	0	0	1	0	1	0
	- 1	Tipulidae	1	0	0	0	0	0	1	0
	Ephemeroptera	Ephemeridae	0	0	24	0	0	0	24	0
		Baetidae	0	0	0	0	8	0	8	0
	Hemiptera	Belostomatidae	0	5	11	2	5	7	16	14
		Hebridae	1	0	0	0	0	0	1	0
		Nepidae	2	0	5	0	5	0	12	0
		Notonectidae	1	0	4	1	6	0	11	1
		Pleidae	1	0	0	2	5	0	6	2
		Mesovelidae	0	0	0	0	1	1	1	1
		Gerridae	0	0	0	0	0	2	0	2
		Corixidae	0	0	0	0	35	0	35	0
	Oddoneta	Aeshnidae	1	1	0	0	0	0	1	1
		Coenagrionidae	30	0	52	4	33	3	115	7
		Cordulegastridae	5	0	0	0	0	0	5	0
		Corduliidae	1	0	0	0	0	0	1	0
		Libellulidae	0	0	24	0	37	2	61	2
		Gomphidae	0	0	0	0	2	0	2	0
		Protoneuridae	2	0	Ő	ů 0	0	0	2	Ő
	Decanoda	Atvidae	39	2	19	5	185	6	243	13
	Decapoda	Hymenocomatidae	0	0	0	3	105	68	245	71
		Palaemonidae	58	0	171	5	0	7	220	12
		Paratholnuae	36	150	1/1	100	0	/	229	12
		Paramelphusidae	0	150	0	102	0	152	0	384
	To a second s	Sesarinidae	0	0	0	0	0	/	0	/
Nr.11	Isopoda	Circianidae	2	1	0	5	3		5	13
Mollusca	Unionoida	Unionidae	2	0	18	3	9	6	29	9
	venerida	Cordiculidae	0	0	0	14	0	3	0	17
	Basommatophora	Lymnaeidae	44	30	29	18	35	3	108	51
		Physidae	0	0	0	0	58	6	58	6
		Planorbidae	15	33	32	23	31	28	78	84
		Ancylidae	0	0	0	0	1	0	1	0
		Stenothyridae	0	0	0	0	0	6	0	6
	Littorinimorpha	Bithyniidae	21	1	13	15	120	11	154	27
	Mesogastropoda	Thiaridae	43	233	138	774	240	127	421	1134
		Viviparidae	23	28	43	124	46	25	112	177
	Cycloneritida	Neritidae	0	0	0	0	0	4	0	4
		Septariidae	0	0	0	0	0	5	0	5
	Neotaenioglossa	Pleuroceridae	0	0	0	0	10	22	10	22
Annelida	Opisthopora	Octochaetidae	9	3	20	0	0	0	29	3
	Hirudinea	Piscicolidae	1	2	13	4	0	0	14	6
	Phyllodocida	Nereididae	3	0	0	0	0	16	3	16
		Nephtyidae	0	87	23	89	0	25	23	201
	Opisthopora	Lumbrioidae	0	0	0	0	25	0	25	0
3	18	54	392	741	783	1859	1125	830	2300	3430
-			072	, 12	,	1007	1120	000	2000	0,00

Lymnaeidae, Culicidae, Bithyniidae, Hydrophilidae, Dytiscidae, and Belostomatidae). This shows that only few common families (7 Nos.) were encountered between the two Groups out of 23 families that involved in the cumulative contribution of 91%. Also, a significant disparity among the macroinvertebrate families was revealed by ANO-SIM (Global R: 0.865 at p = 0.5%) between the two groups identified through nMDS. This reveals that the existence of inconsistency in distribution of the macroinvertebrate families among the groups assembled them as discriminating families. The percentage distribution of individual macroinvertebrate families that contributed >4% for the urban and rural agglomerates for the entire study period are shown in Fig. 4.

The data was analyzed for individual year to evaluate intra-annual variation in the macroinvertebrate community following cluster analysis and nMDS powered with SIMPROF. The results revealed the same

two groups for 2020 dataset whereas, there is an existence of inconsistency in framing the same groups during 2021 and 2022 with few locations (Fig. 3d, c & b). Also, SIMPER analysis considering the same Groups formed with the overall data i.e., Group-I & II revealed that the intra-group similarity for Group-I, the average similarity percentages for the respective years are 48.81, 41.23 and 37.12, indicating an increase in variability within the group between years due to increase in the macroinvertebrate family distribution. Similarly the Group-II also reflecting the same trend with an average similarity percentages for the respective years are 52.45, 50.27 & 42.63.

### 3.3. Benthic macroinvertebrate diversity indices

Community ecology plays a crucial role in establishing a significant



Fig. 2. Cumulative dominance patterns of Macroinvertebrate families along the Bhagirathi-Hooghly River-Estuarine System.

understanding on ecology of the populations, community structures and their interactions with the environment (Jackson and Blois, 2015). Biodiversity and richness of the macroinvertebrate families were assessed using classical alpha-diversity metrics (including Margalef richness (d'), Shannon-Wiener Index (H') & Pielou's evenness (J')) to evaluate the assemblage patterns among the families and their interactions with one another and with the environment. The alpha-diversity metrics for the entire study period are given in Table 4. The ANOVA analysis on entire data for the indices in 2020, 2021 & 2022, as well as the overall data among the groups, showed significant variability (n = 29, P < 0.05); however, no such variation was observed between the years. Irrespective of the year, total number of families encountered was found to be high in the upstream location when compared with downstream, whereas their number of individuals was found to high during 2021 when compared with 2020 and 2022. Similar observations were also reported along the upstream of River Ganga at Patna by Goel et al. (2021).

Also, the richness (Margalef richness (d)), diversity (Shannon-Wiener Index (H') and Pielou's Evenness (J') indices were decreased from Group-I to Group-II (Table 4). High evenness in the upstream (Group-I) may represents the similar type of distribution in the taxonomical composition (similar density), while low evenness indicates the dominance/presence of a single family (Maurer and McGill, 2011) or due to the presence of homogeneous distribution of macroinvertebrate assemblage in the downstream locations (Sánchez-Fernández et al., 2010; He et al., 2019) might be due to anthropogenic interventions. The results are in corroborated with the findings over Mandakini River reported by Rawat et al., 2020. Even the existence of spatio-temporal variability of the alpha metric indices over the macroinvertebrate assemblage influenced by anthropogenic stressors when compared with pristine environments was also reported elsewhere (De Moor, 1992; Cuffney et al., 2000; Cross et al., 2006; Mehler et al., 2015; Aera et al., 2019). Overall, the alteration in the alpha metrics of the macroinvertebrate families from upstream to downstream of the BHE system might be due to the existing dynamics in the environmental factors viz., water quality and sediment characteristics and substrate composition of the benthic habitat. From the above findings, it indicates that the rural locations showed high diversity when compared to urban-influenced locations.

## 3.4. Saprobic score and ecological health status

The existing diversity indices discussed above may not offer a comprehensive perspective when assessing the health and pollution

levels of the BHE system. To establish effective management strategies, it is imperative to develop an integrated biological metric that can assess pollution status and its gradient accurately. One of the earliest applications of biological data in evaluating water quality and ecosystem health is through species tolerance indices (Rosenberg and Resh, 1993; Allan, 1984). These indices encompass various metrics used across different regions, including the widely used Saprobic Index (Reynoldson and Metcalfe-Smith, 1992) and the Biological Monitoring Working Party (BMWP) index (Hilsenhoff, 1987). As the benthic macroinvertebrate families having an extremist adaptability by their prevalence of tolerant and intolerant families with respect to pollution the biological monitoring based an integrated biological metrics i.e., Saprobic Index (based on BMWP score (adopted by CPCB)) is considered to assess the health of the lower stretch of the BHE. The calculated saprobic scores for Group-I and Group-II for the 2020, 2021 & 2022 are 5 and 4.53, 5.19 and 4.78 & 4.83 and 5.00 respectively. This indicates that the water quality status of Group-I (upstream/rural & semi-urban area) was improved from 2020 to 2021 and stands at Good and it slightly deteriorated and fell into moderately polluted saprobic index during 2022. Whereas Group-II (lower stretch/urban region) showed continuous improvement from 2020 to 2022. A notable observation in our study was the high presence of the Chironomidae family in areas where the Saprobic score was declined. This finding is consistent with similar observations made in Danish streams and Dikhow River (Friberg et al., 2010 by Dutta et al., 2016). Furthermore, it is important to mention that Chironomidae presence has also been documented in the small streams of Melbourne metropolitan area by Walsh et al. (2001). As a whole, based on the presence or absence of macroinvertebrates the saprobic score for the entire study period revealed that Group-I (5.09) was good, and Group-II (4.95) was moderately polluted.

# 3.5. Evaluation of synergistic impact of environmental variables on macroinvertebrate composition and biodiversity

The diversity and composition of macroinvertebrates are subject to substantial influence from a diverse array of abiotic and habitat factors, encompassing water quality, substrate quality, and ecological interactions like competition and predation (Chibsa et al., 2022; Bendary et al., 2023). Understanding the impact of these environmental variables is paramount for untangling the complex relationships between macroinvertebrates and their ecosystem, providing insights into the dynamics of biodiversity in aquatic environments.

To evaluate the synergy between the environmental factors and the macroinvertebrate community, Redundancy Analysis (RDA) was



Fig. 3. Cluster and nMDS (powered with SIMPROF analysis) on Bray–Curtis similarities based square root-transformed macroinvertebrate assemblages data showing discrete assemblages at various similarities with a) on overall data (43%), b) Postmonsoon-2022 (34%), c) Postmonsoon-2021 (40%) & d) Postmonsoon-2020 (40%), based on this a classification showing spatial segregation of sampling locations named after Gr-I & II.



Fig. 4. Comparative distribution of macroinvertebrates in Semi-Urban(Gr-I) and urban (Gr-II) across the Bhagirathi Hooghly River-Estuarine System.

#### Table 4

Alpha diversity metrics, Detrended correspondence analysis (DCA) and Redundancy analysis (RDA) showing the extent of environmental regulation of Macroivertebrate families in Bhaghirathi Hooghly river-estuarine system.

	Group 1	Group 2			
Total Number of families encountered (S)	44	37			
Total No. of individuals encountered (N)	2300	3430			
Margalef richness (d)	5.555	4.422			
Pielou's Evenness (J')	0.7444	0.5543			
Shannon-Wiener Index (H')	2.817	2.002			
Saprobic Score	5.09	4.95			
DCA	1	2	3	4	Total inertia
Eigenvalues	0.553	0.137	0.017	0.008	1.403
Lengths of gradient	2.669	1.616	1.464	1.483	
Cumulative percentage variance of species data	39.4	49.2	50.4	51	
Sum of all					1 403
eigenvalues					1.405
RDA					
Eigenvalues	0.45	0.251	0.131	0.065	1.000
Cumulative	45.0	70.0	83.1	89.6	
percentage					
variance of species					
data					
Cumulative	45.0	70.0	83.1	89.6	
percentage					
variance of species-					
environment					
relation					
Sum of all					1.000
eigenvalues					
Environmental Variab	les	0 101	0 1 1 4 1	0.0000	
BOD	0.873	0.191	0.1141	-0.0039	
COD	-0.8452	-0.1014 -0.1675	-0.0391 -0.0714	0.0339	
TDS	-0.6304	0 1506	0 2339	-0.0069	
TAlk	-0.7426	0.2625	0.2597	-0.0466	
THard	-0.7971	0.0707	0.0087	0.0801	
PO43-	-0.7066	0.0554	-0.056	0.2527	
NO3-	-0.6387	-0.2063	-0.0702	-0.2111	
NH4+	-0.4419	-0.1112	0.1125	0.3206	
d	0.4851	-0.1917	-0.3529	-0.4018	
J.	0.3828	-0.6469	-0.3461	-0.1932	

employed. The scores extracted for axes 1 and 2 explained 45% and 70% of the variations in macroinvertebrate families, respectively (Table 3). The RDA plot (Fig. 5) clearly indicates that the Group-II locations appear to be affected by pollution, as evidenced by their positive correlation with pollution-tolerant species, including Chironomidae, Culicidae Parathelphusidae, and Thiaridae, which have been confirmed as resilient organisms in environments subjected to both domestic and industrial pollution (Ordoñez-Sierra et al., 2020; Castro et al., 2018; Chua et al., 2015; Ojunga et al., 2010; West et al., 2021). The river stretch of Group-II receives a mixed drains carrying both domestic and industrial runoff (as per the CPCB unpublished data) inference the increase in the no. of pollution-tolerant families in this stretch. Also, this is in corroboration with the reports associated with pollution tolerant families and their tolerance towards industrial pollutants reported elsewhere (Quanz et al., 2021; Nedeau et al., 2003 Ojunga et al., 2010). In particular, Qunaz et al., reported that Chironomidae sustained in the boat harbour stabilization lagoon, at Canada receives discharge from pulp industry effluents. Ordoñez-Sierra et al. (2020) and Castro et al. (2018) found in their studies that the preponderance of small-bodied organisms such as Chironomidae and Culicidae in the disturbed sites is attributed to their relatively short life cycles, enabling them to recover quickly after

disturbance activities. Sand mining/surface sediment runoff removes the benthic macroinvertebrates living in the sediment (sand), mostly the organisms like Thiaridae that have higher fecundity in that environments can tolerate such disturbances and survive (Appleton et al., 2009; Karatayev et al., 2009; López-López et al., 2009: M. E. Raphahlelo et al., 2022). Also, Ephimeroptera, Plecoptera and Tricoptera (EPT) taxa, were not reported during the entire study period in these location which are generally considered most sensitive to urban perturbations (Haidekker and Hering, 2008). The resilience of these families likely to explain their abundance in the Group-II locations, where environmental variables are associated with both industrial and domestic pollution. In contrast, Group-I locations exhibit a positive correlation with Dissolved Oxygen (DO), families diversity (d) and eveness (j) and support populations of moderately to less pollution-tolerant/sensitive organisms, such as Coenagrionidae, Dytiscidae and others (Roth et al., 2020; Dolný et al., 2021). Also, indicates that diversity of macroinvertebrate family distribution is rich in the Group-I locations. This suggests that these areas are subject to relatively lower pollution levels compared to Group-II locations

Overall, the results of the RDA analysis provide valuable insights into the distribution patterns of macroinvertebrates in relation to environmental variables, highlighting the impact of pollution on species composition and diversity. This information can serve as a basis for designing effective conservation and management strategies to safeguard the delicate balance of aquatic ecosystems and promote the sustainability of the biodiversity.

### 4. Conclusion

The integrated approach of multivariate analysis in this study provided valuable insights into the spatio-temporal variability of macroinvertebrate community structure and ecological health status in the BHE system. The water quality parameters exhibited significant variation, with urban locations showing higher pollution indicators compared to rural locations. The lowest DO concentration (4 mg  $L^{-1}$ ), highest BOD (9 mg  $L^{-1}$ ) and COD (35 mg  $L^{-1}$ ) were reported among the Gr-II (urban) locations during 2021. Benthic macroinvertebrates, particularly Chironomidae and Culicidae, were found to be resilient in polluted environments. The alpha diversity metrics indicated higher diversity and evenness in rural locations, indicating healthier ecological conditions. However, urban locations displayed moderately polluted with saprobic index scores of 4.78 & 4.83 and 5.00 during 2020, 2021 and 2022 respectively. Beta similarity based on Brav- Curtis similarity revealed significant spatio-temporal variation in macroinvertebrate community distribution. The redundancy analysis (RDA) provided a clear correlation between pollution indicators and pollution-tolerant macroinvertebrate species in urban locations (Group-II), indicating the impact of anthropogenic pressures (both industrial and domestic pollution) on the community structure. In contrast, rural locations (Group-I) exhibited positive correlations with Dissolved Oxygen (DO) and supported less pollution-tolerant organisms. The findings of this study highlight the importance of comprehensive ecological monitoring to assess the health status of river-estuarine ecosystems. Understanding the dynamics between pollution indicators and macroinvertebrate community composition is crucial for effective conservation and sustainable management of aquatic environments. The results can guide policymakers and environmental agencies in formulating targeted strategies for pollution control and biodiversity conservation in the BHE system. Implementing measures to reduce pollution and protect sensitive habitats will be crucial to maintain the health and integrity of these valuable ecosystems.

## Credit author statement:

S. C. Mohapatra: Investigation, Resources, Formal analysis, Writing – original draft, V. V. Kanuri: Project administration, Conceptualization,



Fig. 5. Redundancy analysis (RDA) shows how various abiotic and biotic factors persuade Macroinvertebrate community assemblage patterns in the Bhagirathi Hooghly River-Estuarine System.

Investigation, Supervision, Formal analysis, Writing – review & editing, V. K. Kumar: Investigation, Resources, Formal analysis, Writing – original draft, S. Saha: Resources, S. Y. Ali: Resources, A. Verma: Resources, M. K.Biswas: Project administration, Investigation, Co-ordinating, Review, A. K. Vidyarthi: Funding acquisition, Project administration & Review.

### Declaration of competing interest

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

# Data availability

Data will be made available on request.

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