Pollution 2022, 8(1): 45-56
DOI: 10.22059/POLL.2021.324514.1106

RESEARCH PAPER

Monitoring Nature's Kidneys with the aid of Macrobenthic Assessment: A casestudy in the city Wetlands of Guwahati, India

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Received: 31.05.2021, Revised: 29.09.2021, Accepted: 24.11.2021

ABSTRACT
Aquatic environments, including wetlands, are one of the most threatened ecosystems worldwide. Considering their ecological importance, wetlands are rightly appraised as 'natural kidneys'. In this current study, the city wetlands of Guwahati were viewed for the first time through the angle of lesser-explored bottom dwellers. Guwahati, a rapidly expanding metropolis,is the gateway to northeast India, part of an Indian biodiversity hot-spot region. This case study comprised the bridge between abiotic and biotic factors, thus directing the pave for characterization of wetlands through benthos analysis. The study, covering seasons, *viz.* winter, premonsoon and monsoon, revealed 15 definite taxa belonging to 10 orders. The dominance of Chironomidae and Culicidae in certain wetlands indicated high tolerance of Dipterans in a wide range of aquatic environments, including polluted water bodies. Similarly, the presence of Trichopterans, only in the wetland located distant from the mainland city, marked that with less anthropogenic impacts. The Shannon indices for benthos were in the range from 0.17 to 0.97. Density was found to have a significant positive correlation with dissolved oxygen (r = 0.567) and a negative correlation with free carbon dioxide (r = -0.377). In contrast to significant site-wise variation in density, there was no significant difference in benthic diversity across the sites and no significant seasonal variation of benthic density and diversity from the statistical point of view.
Keywords: limnology, water-quality, aquatic-ecosystem, bottom-dwellers

INTRODUCTION

Wetlands are regarded as natural kidneys because of their ability to recycle nutrients, regulate urban runoff, groundwater recharge, water purification etc. (Clarkson et al., 2013). Besides the direct services, wetlands are a source of historical information, cultural and scientific studies and artistic creativity (Pritchard & Papayannis, 2016). Wetlands can absorb carbon in them and are thus considered as a significant regulator for climate-changing parameters (Carlson et al., 2010). On the other hand, wetlands are one of the highly-threatened ecosystems worldwide due to anthropogenic activities and climate-change impacts (Desta et al., 2012). In the leap of urbanization, wetlands are vanishing rapidly along with the biodiversity supported by these (Xu et al., 2019). Lentic ecosystems are disadvantageous compared to a lotic ecosystem regulating and disbursing heavy metal pollutants (Rai et al., 2007). Therefore, regular check-up of 'nature'skidney' has become an imperative need of the hour to formulate good remedial prescription in the policymaking. Benthos are the bottom dwellers and their occurrences represent the aquatic environment they inhabit (Sarang &

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Sharma, 2009; Khiratkar et al., 2017). Given that the benthic fauna are a significant component of the lentic and lotic water food chain, aquatic invertebrates and vertebrates, including fish, consume those (Anbuchezhian et al., 2009; Asadujjaman et al., 2012). They are responsible for nutrient release as they recycle organic matter and debris by mineralizing and mixing oxygen flux into the sediment. Molluscan proteins are source of essential amino acids (Pawar & Al-Tawaha, 2017). Benthic community responds to water quality alteration and thus can be utilized as a bio-indicator of an aquatic ecosystem (Balogun et al., 2011). The change in benthic diversity may be an early alarm for eutrophication or invasion of exotic species, suggesting periodic monitoring of aquatic ecosystems (Pešić et al., 2018). The zoobenthos are incredibly diverse and represented by different animal phyla (Xu et al., 2019). They are termed Microbenthos, Meiobenthos and Macrobenthos, considering their size. They are also grouped based on their locations and types (Fatima et al., 2017). Macrobenthos generally carry a size of more than 500 µm (Bett, 2013). Moreover, their extended residency in a substratum, a wide range of diversity and easy procedure of their sampling have made benthos a good choice for researchers in monitoring freshwater to brine ecosystems, be it lentic or lotic (Sarker et al., 2016; Snelgrove,1998).

Assam is a part of the biodiversity hot-spot region and witnessing rapid ecodegradation in parallel to its rich biodiversity and endemism (Goswami et al., 2012). With the rapid urbanization of Guwahati, the capital of the Indian state of Assam, urban biodiversity is experiencing alarming habitat loss (Gogoi, 2013). Though macrobenthos have traditionally been a choice for assessing water quality at instances, there is rare report of prior studies on benthos from these city wetlands. In this current study, the macrobenthic diversity has been explored at diverse city wetlands to look into its possible linkage with water quality (Figure 1). Among the selected wetlands with varied forms, Dighali Pukhuri (DP) and Jor Pukhuri (JP) are manufactured tanks during the Ahom dynasty (Figure 2.a), (Figure 2.b). The Bharalu Channel (BC) runs throughout the city, carries the metropolis discharges and opens into the river Brahmaputra (Figure 2.c). Ghorajan beel (GH), the fourth wetland, is situated on the North bank, apart from the mainland metropolis and connected to river Brahmaputra (Figure 2.d). The study aimed to document macrobenthos' abundance at different seasons and diverse water bodies by bridging biotic and abiotic factors. Specifically, it had been designed to find the correlation between water quality parameters, benthic density and benthic diversity, the variation of benthic density and diversity among the four sites and the seasonal variation of benthic density and diversity. scientific exploration of the wetlands. This study would provide a platform for periodicand scientific monitoring of city wetlands in peril.

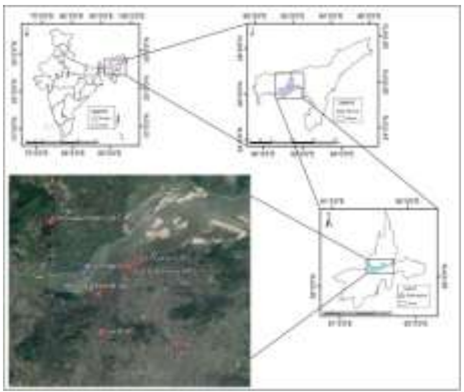

Figure 1: Study area map showing locations of selected city wetlands of Guwahati

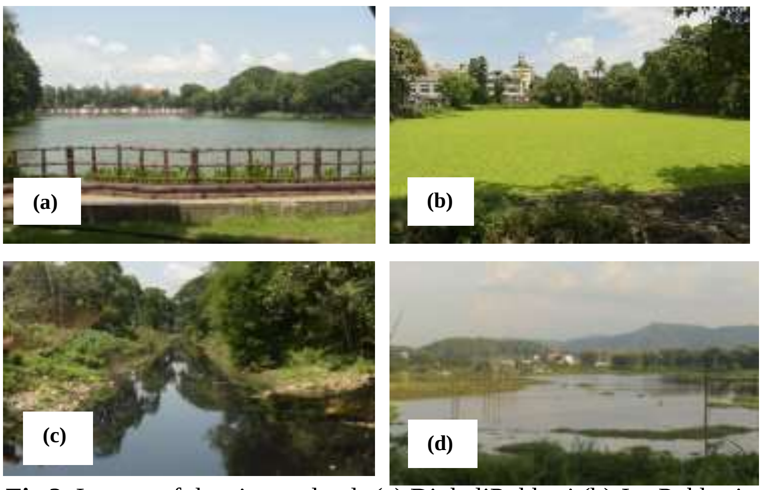

Fig 2. Images of the city wetlands (a) DighaliPukhuri (b) Jor Pukhuri (c) Bharalu Channel (d) Ghorajan beel

MATERIAL AND METHODS

For enumeration of benthic communities at four diverse wetlands under the greater Guwahati area, samplings were carried out for a year covering the seasons, *viz.* winter (November-February), premonsoon (March-June) and monsoon (July-October). Surface water samples were collected in triplicates for assessment of six abiotic parameters, *viz.* temperature, pH, dissolved oxygen (DO), free carbon dioxide (FCO_2), total alkalinity (TA) and total hardness (TH). *In-situ* analyses were carried out for temperature and pH. The APHA (1995) guidelines were utilized mostly for water quality estimation. The DO was estimated by Iodometric (Wrinkler's) method. Titration methods were adopted for estimation of TA with Phenolphthalein and Methyl Orange indicators, TH with Eriochrome Black-T indicator and FCO_2 with Phenolphthalein indicator.

Sediment samples were collected with a hand corer having a dimension of (15×15×15) cm^3 at a mean depth of 1.5m and secured in a tray. After removal of debris, using tap water slurry was prepared. About 5g sucrose was added for easy collection of benthos and 0.1-2.0% eosin was used to stain them. The entire sample was sieved by using 500-micron mesh. Benthos were picked up using forceps and brushes and preserved in 4% formaldehyde, sorted and labeled. The residual matter was transported to the laboratory for further collection of benthos after proper preservation. For qualitative and quantitative analysis, fauna were identified under stereozoom microscope. Foldscope (Cybulski et al., 2014), an origami-based paper microscope, had been used to look distinctly at different body parts such as 'proleg', 'head capsule' etc., to verify with the taxonomic keys. Due to the lack of previous studies on the benthos of Guwahati wetlands andthe absence of taxonomic information for them, fauna were identified only to family level within which they could be placed with certainty using universal identification keys (Bouchard, 2004; Kumar & Khanna, 1983; Biswas et al., 1995). The benthic density (numbers/m^2) was determinedusing the formula: Density (D) = $N/A \times 10^4$

Where N= number of individuals per sample and A= biting area of the sampler (15×15 cm^2)

Shannon index is based on measuring uncertainty. When there is a community with low diversity (dominated by one species), the uncertainty of prediction is low and a randomly sampled species is most likely going to be the dominant species. However, when there is a community with high diversity, uncertainty is high.

For diversity analysis, Shannon-Wiener indices were calculated as per the formula mentioned by Wilhm and Dorris (1966).

$$\text{Species diversity } \underset{i=1}{\overset{S}{H'}}, = -\sum Pi \ln Pi$$

Here, *Pi* is the proportion (*n/N*) of individuals of one particular species found (*n*) divided by the total number of individuals found (*N*), *ln* is the natural log, Σ is the sum of the calculations, and *S*is the number of species.

Statistical analysis was carried out using SPSS (Version 26.0). Initially, a normality test was performed to determine whether sample data has been drawn from a normally distributed population. In the frequentist statistics statistical hypothesis examination process, testing was done against a null hypothesis that assumes normal data distribution. In this study, the Shapiro- Wilk test was carried out to check whether the temperature, pH, density, Shannon index etc. of the four sites were statistically significant and different from a normal distribution. In addition, Zscore was calculated to determine how data were deviated from the mean, for 95% confidence, that should lie between -1.96 to +1.96. The Shapiro-Wilk test revealed various significant values which were less than 0.05 and thus, the null hypothesis stating the normal distribution of datawas rejected. In parallel, the data were found heavily skewed and kurtosis, advocating a non- parametric test for correlation and variation in density and diversity. Spearman correlation test was adopted between physico-chemical parameters and benthic diversity as the data were not normally distributed. Similarly, to find out a site-wise variation in benthic density and diversity among the four sites and seasonal variation in benthic density and diversity, the Kruskal-Wallis test was chosen, equivalent to one-way ANOVA in parametric analysis. The H statistic value calculated in the test denotes the variance in ranks between groups.

RESULTS AND DISCUSSION

The wetland DP is a cemented embankment pond and people's favourite recreational place for boating. There is no direct point source of pollution in DP as human activities have been restricted other than amusement. At the site of JP, twin ponds are situated closely, separated by a passage for commuters. One of the ponds, connected to a city temple, has an embankment. In this study, sampling was carried out from the pond other than the mounded one. Urban domestic sewage was the source of pollution in this lentic aquatic ecosystem. Domestic sewage, municipal solid waste, industrial discharge and surface runoff are the sources of pollution in BC. Although GH is not directly exposed to any source of pollution. Still, with the city expansion, the urban settlement around the aquatic ecosystems has been marked as a source of anthropogenic impacts in the near future. The water flow of BC and GH is regulated by sluice gates, primarily duringthe monsoon season. The mean depth of DP at the whole water level was reported as 6m. Similarly, the mean depth was 4m for JP and 3.5m for GH. The average depth and velocity of theflow of BC were reported 3m and 1.07 - 1.37 m/s, respectively. In terms of macrophytes, *Eichornia* and *Hydrilla* were found in DP, and the growth of *Ipomoea* and *Pistia* was abundantlyobserved in JP. Chlorophyta was found to be the dominant phytoplankton group in GH (Sharma, 2012). *Azolla*, *Eichornia*, *Hydrilla*, *Najas* were the dominant macrophytes in this wetland. The seasonal physico-chemical characteristics of the four wetlands are represented in Table 1. Throughout the study period, the water temperature was maximum in the monsoon season at GH (35.1℃) and the minimum was also found in the same wetland during the winter season (25.9 ℃). The pH was maximum in DP and BC during monsoon and winter respectively (7.8) and minimum in JP during premonsoon (6.9). The DO was maximum in DP during monsoon (10.4 mg/l) and minimum in BC during winter (0.16 mg/l). A pH below 5 and more than 10 are lethal, and DO more than 4mg/ml is generally considered optimum for the aquatic ecosystem's major vertebrate component, such as fish (Bhatnagar & Singh, 2010). The low DO in BC is probably attributed to the excessive decomposition of organic and inorganic debris that BC carried in the form of domestic waste and industrial discharge (Das et al., 2003). The free CO_2 was maximum at BC during monsoon (16.00 mg/l) and minimum free CO_2 (2.00 mg/l) was observed at the other three wetlands at different seasons. The increased level of free CO_2 observed during monsoon, when the temperature was higher comparatively, may be due to the high decomposition rate. Likewise, the low value of free CO_2 can be linked with the high photosynthesis of phytobenthos and macrophytes (Fatima et al., 2017). Excessive growth of aquatic vegetation was observed at JP in comparison to DP and GH. The total alkalinity was maximum at BC during winter and premonsoon (172.00 mg/l) and minimum at GH during winter (48.00 mg/l). All the wetlands qualified the minimum level of alkalinity to be categorized as productive, as reported by Mairs (1966). The total hardness was maximum at BC during winter (194.00 mg/l) and minimum at GH during winter (54.00 mg/l). Generally, total hardness lesser than total alkalinity is indicative of the association of bicarbonate and carbonate with sodium and potassium and more total hardness than total alkalinity indicates an association of calcium and magnesium with anions instead of bicarbonate and carbonate. Relationship of fish growth and alkalinity has been observed in unpolluted waters (Boyd et al., 2016).

Table 1. Season-wise variation of physico-chemical parameters of city wetlands of Guwahati

		Winter			**Premonsoon**			**Monsoon**		
		Range(mg/L)	**Mean**	**SD**	**Range(mg/L)**	**Mean**	**SD**	**Range (mg/L)**	**Mean**	**SD**
DP	Temp	27-27.3	27.2	0.17	29-29.3	29.17	0.15	33-33.2	33.1	0.1
	pH	7-7.2	7.1	0.1	6.9-7.1	7	0.1	7.7-7.8	7.77	0.6
	DO	6.88-7.2	7.04	0.16	7.84-8.32	8.05	0.24	9.92-10.4	10.19	0.24
	Free CO2	2.00-4.00	2.67	1.15	2.00-4.00	2.67	1.15	2.00-4.00	2.67	1.15
	Total Alkalinity	118.00-124.00	121.33	3.06	100.00-102.00	101.33	1.15	68.00-72.00	73.33	1.15
	Total Hardness	112.00-124.00	112.67	1.15	90.00-96.00	92.67	1.15	68.00-74.00	72.00	2.00
JP	Temp	27-27.3	27.17	0.15	28.9-29.3	29.1	0.2	34-34.3	34.17	0.15
	pH	7-7.1	7.07	0.06	6.9-7.2	7.07	0.15	7-7.1	7.07	0.06
	DO	6.72-6.88	6.83	0.09	6.88-7.36	7.15	0.24	8.64-9.28	9.01	0.33
	Free CO2	Upto 2.00	2.00	0.00	2.00-4.00	2.67	1.15	2.00-4.00	2.67	1.15
	Total Alkalinity	72.00-74.00	72.67	1.15	72.00-74.00	73.33	1.15	66.00-68.00	67.33	1.15
	Total Hardness	92.00-94.00	92.66667	1.15	110.00-114.00	112.00	2.00	86.00-94.00	90.67	1.15
BC	Temp	28-28.3	28.17	0.15	29.9-30.4	30.17	0.25	32.9-33.2	33.03	0.15
	pH	7.6-7.8	7.73	0.12	7-7.2	7.10	0.10	7.1-7.2	7.17	0.06
	DO	0.16-0.32	0.21	0.09	0.96-1.28	1.12	0.16	1.44-1.92	1.71	0.24
	Free CO2	6.0-14.00	10.67	4.16	8.0-14	11.33	3.06	8.0-16.00	12.00	4.00
	Total Alkalinity	152.00-172.00	160.00	10.58	162.00-172.00	167.33	5.03	142.00-168.00	153.33	13.32
	Total Hardness	172.00-194.00	184.67	11.37	162.00-176.00	170.67	7.57	142.00-158.00	148.67	8.33
GH	Temp	25.9-26.2	26.03	0.15	31.9-32.2	32.03	0.15	34.8-35.1	34.97	0.15
	pH	7.1-7.3	7.23	0.12	7.1-7.2	7.17	0.06	7.0-7.3	7.17	0.15
	DO	3.04-3.84	3.52	0.42	1.44-1.76	1.60	0.16	3.68-3.84	3.79	0.09
	Free CO2	6.00-12.00	9.33	3.06	2.00-4.00	2.67	1.15	8.00-12.00	10.00	2.00
	Total Alkalinity	48.00-54.00	50.67	3.06	60.00-64.00	61.33	2.31	60.00-62.00	61.33	1.15
	Total Hardness	54.00-68.00	61.33	7.02	62.00-66.00	64.00	2.00	74.00-78.00	76.00	2.00

At least 15 certain taxa of benthos were encountered in this whole study. The orders represented the benthic communities were Coleoptera, Oligochaeta, Diptera, Trichoptera, Gastropoda, Odonata, Hemiptera, Ephemeroptera, Amphipoda and Lepidoptera and five groups remained unidentified (Figure 3). Besides, three families from Coleoptera, two families from Amphipoda and one family from Trichoptera and Lepidoptera remained unidentified.

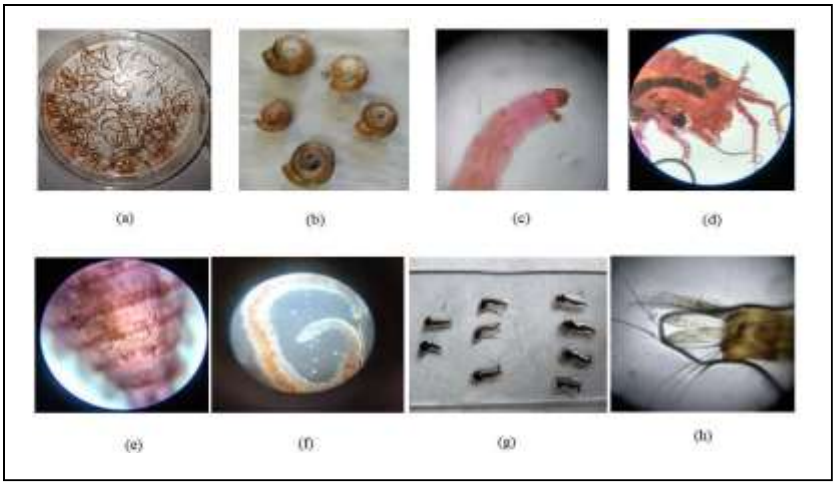

Figure 3: Benthic fauna and their identifying parts (a) Chironomidae larvae (b) Gastropoda (c) Proleg ofChironomidae (d) Amphipoda anterior part (e) Coleoptera body segments (f) Oligochaeta (g) Mosquitolarvae (h) Mosquito anal gills

The percentage contribution of benthos in DP showed the order Chironomidae (Diptera) 90.54% > unidentified family (Amphipoda) 2.05% > Physidae (Gastropoda) 0.77% > Culicidae (Diptera) 0.26% = unidentified family (Lepidoptera) 0.26% > Ceratopogonidae (Diptera) 1.28%. A 4.86% contribution by a group remained unidentified at the taxonomic levels described here. The percentage contribution of benthos in JP showed the order Chironomidae (Diptera) 46.15% > Thiaridae (Gastropoda) 15.38% > Ceratopogonidae (Diptera) 10.26% > Culicidae (Diptera) 7.69% > unidentified family (Coleoptera) 5.13% > Coenagrionidae (Odonata) 2.56% = Nepidae (Hemiptera) 2.56% = unidentified family (Amphipoda) 2.56%. There were two groups of unidentified benthos contributing 5.13% and 2.56%. The percentage contribution of benthos in BC showed the order Culicidae (Diptera) 48.48 % > Thiaridae (Gastropoda) 6.06% > Physidae (Gastropoda) 3.03% = Baetidae (Ephemeroptera) 3.03% = unidentified family (Coleoptera) 3.03%. A 33.33% and 3.03% contribution by two benthos groups remained unidentified at both the taxonomic levels. At the fourth station, GH, the percentage contribution of benthos showed the order Lymnaeidae (Gastropoda) 31.43% > Chironomidae (Diptera) 28.57% > Ceratopogonidae (Diptera) 20.00% > Lumbriculidae (Oligochaeta) 8.57% > Tipulidae (Diptera) 5.71% > unknown family (Coleoptera) 2.86% = unknown family (Trichoptera) 2.86%. Oligochaeta, Chironomidae and Mollusca are reported as the general dominant macro-benthos group found in tropical freshwater (Irin et al., 2017). Chironomidae (Diptera) was the most dominant group in DP and JP, whereas Culicidae (Diptera) was the dominant group in BC and Lymnaeidae (Gastropoda) in GH (Figure 4). An abundance of Dipterans and Chironomidae was reported in a recent study by Sun et al. (2019). Dipterans are known because of their presence at a wide range of aquatic environments, in contrast to Trichopterans, sensitive to pollution (Kabir et al., 2013). The abundance of Gastropods could be linked to the enormous presence of macrophytic vegetation (Garg et al., 2009).

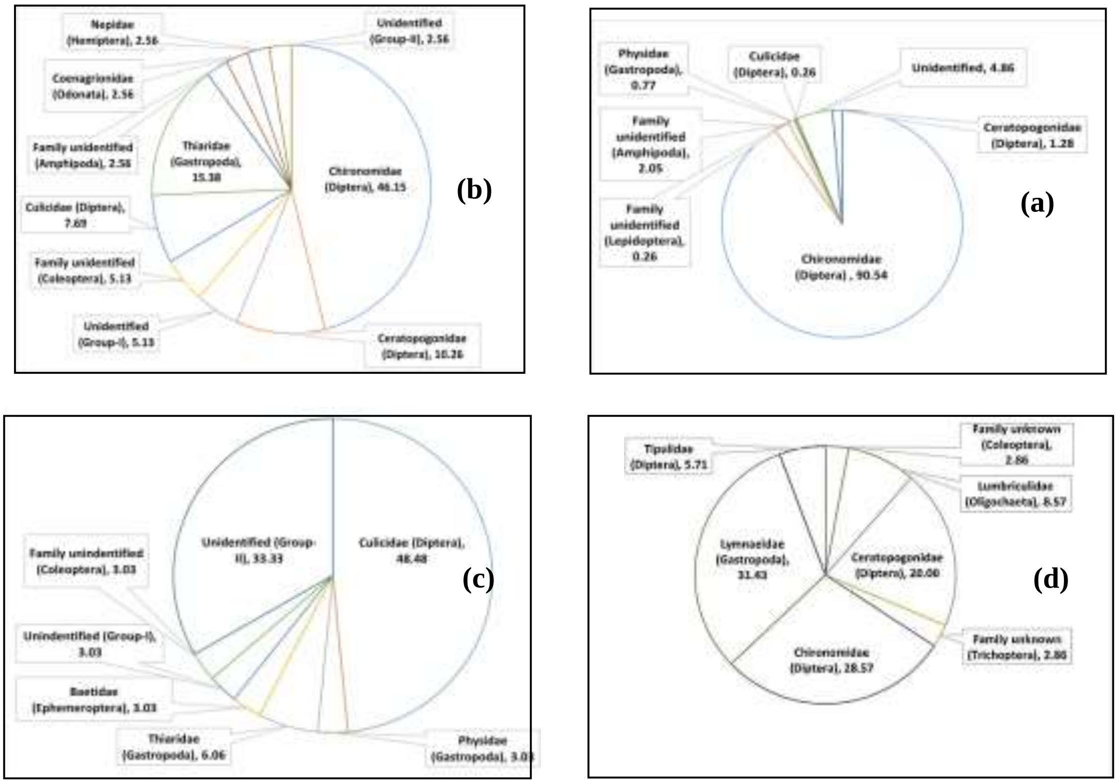

Figure 4: Percent composition of macrobenthos in the sampling sites (a) DP (b) JP (c) BC (d) GH

The density of benthos observed in DP was in the range of 1555- 2355 individuals/m^2. Likewise, for JP, BC and GH the ranges were 88-311, 44-355 and 44- 444 individuals/m^{2,} respectively (Figure 5). Density was found to have a significant positive correlation with DO ($r = 0.567$) and a negative correlation with free CO_2 ($r = -0.377$). Diversity (Shannon index) was also found to have a positive correlation with density ($r = 0.352$) (Table 2). The Shannon-Weaver indices for benthos were ranged from 0.40 - 0.61 for DP, 0.31 - 0.48 for JP, 0.22 - 0.71 for BC and 0.17 - 0.97 for GH (Figure 6). The diversity index advocated that JP and GH were comparatively more diverse. DP was the least diverse despite its high density because it was dominated by only one group making the uncertainty of prediction low.

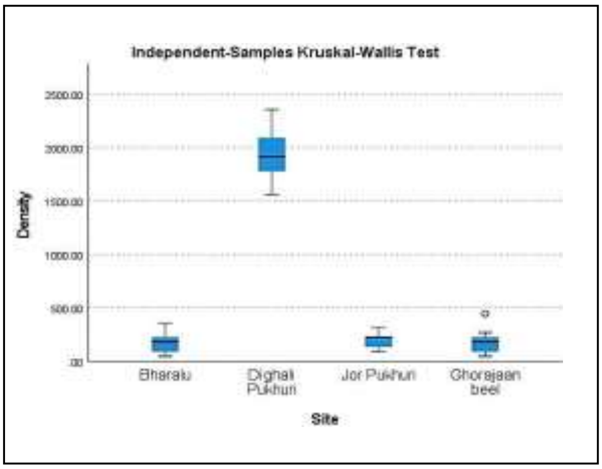

Figure 5: Site-wise variation inbenthic density

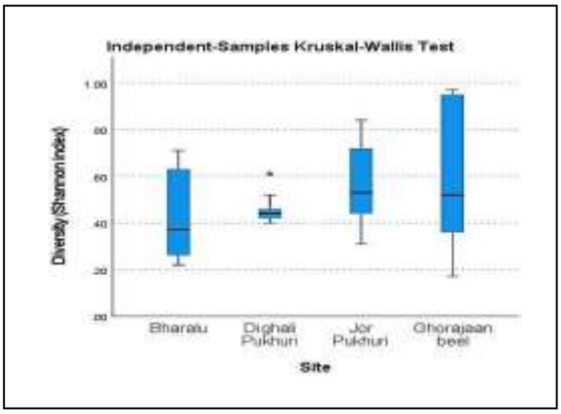

Fig 6: Site-wise variation inbenthic diversity

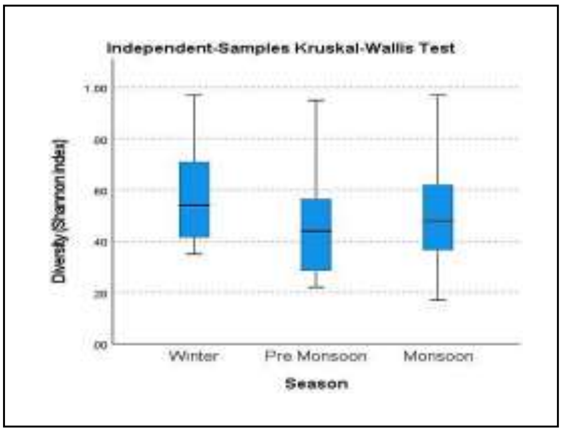

Fig 7: Season-wise variation inbenthic density

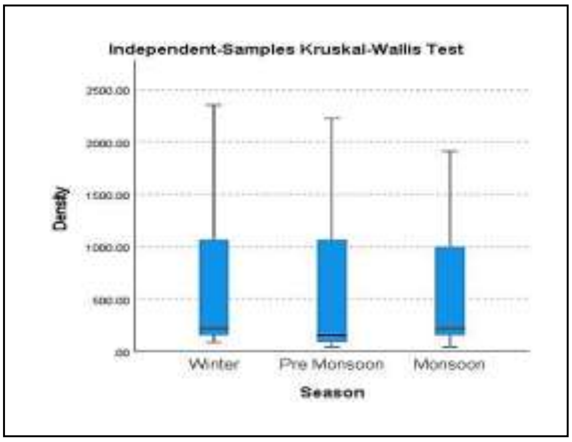

Fig 8: Season-wise variation inbenthic diversity

The obtained value H=20.415, p=0.000<0.05 from the Kruskal-Wallis test, indicated rejection ofnull hypothesis, corroborating that the benthos density was not the same across the study sites (Figure 5). Test results for pairwise difference checks revealed a significantly different nature of DP from the three other locations in terms of density. Similarly, the Kruskal-Wallis test for site- wise variation in diversity (Shannon diversity) pointed out that there was no significant difference in benthos diversity across the sites with H=2.325, p=0.508>0.05 retaining the null hypothesis (Figure 6). The test for seasonal variation of benthic density and diversity indicated that the distribution of benthos density was unchanged season-wise (H=0.626, p= 0.731>0.05) (Figure 7) and benthos diversity was the same in all the seasons (H=2.233, p=0.327>0.05), retaining the null hypothesis for both the cases (Figure 8).

Table 2: Correlation between physico-chemical parameters and density and density and diversity

			Temp	pH	DO	Free CO_2	Total Alkalinity	Total Hardness	Density	Shannon index
Spearman's rho	Temp	Correlation Coefficient	1.000							
		Sig. (2-tailed)	.							
		N	36							
	pH	Correlation Coefficient	.052	1.000						
		Sig. (2-tailed)	.764	.						
		N	36	36						
	DO	Correlation Coefficient	.156	-.217	1.000					
		Sig. (2-tailed)	.362	.203	.					
		N	36	36	36					
	Free CO2	Correlation Coefficient	.084	.286	-.638**	1.000				
		Sig. (2-tailed)	.628	.090	.000	.				
		N	36	36	36	36				
	Total Alkalinity	Correlation Coefficient	-.105	.039	-.262	.257	1.000			
		Sig. (2-tailed)	.542	.819	.122	.130	.			
		N	36	36	36	36	36			
	Total Hardness	Correlation Coefficient	-.144	-.078	-.353*	.296	.901**	1.000		
		Sig. (2-tailed)	.400	.653	.035	.079	.000	.		
		N	36	36	36	36	36	36		
	Density	Correlation Coefficient	-.050	.004	.552**	-.360*	.129	-.028	1.000	
		Sig. (2-tailed)	.773	.983	.000	.031	.455	.872	.	
		N	36	36	36	36	36	36	36	
	Shannon index	Correlation Coefficient	-.123	.145	.087	-.230	-.282	-.198	.344*	1.000
		Sig. (2-tailed)	.476	.398	.615	.177	.096	.247	.040	.
		N	36	36	36	36	36	36	36	36

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

The present investigation revealed the ability of macrobenthic fauna in the characterization of city wetlands of Guwahati. DP and JP are situated in close vicinity and dominated by Chironomidae (Diptera). In BC, the dominant group was mosquito larvae under the Culicidae family (Diptera). The sampling spot of BC was near the mouth of industrial and domestic waste discharge that it carried through the city. The findings corroborated the high tolerance of Dipterans in comprehensive environments, including polluted water bodies. Gastropod (Thiaridae) was the second dominant group after Chironomidae in JP. This wetland used to receive urban domestic sewage via an open drainage system and contained ample aquatic vegetation. The abundance of Gastropods could be correlated with the presence of macrophytes. In the fourth wetland, GH, the dominant group was Lymnaeidae (Gastropoda). GH is situated on the city's northern side and less exposed to metropolis load and anthropogenic impacts. Trichopterans, known for highly sensitive to pollution, were found only in GH. The statistical analysis marked DP differently from the other wetlands because of its high density with a single group of macrobenthic fauna. Besides particular water amusement, human activities are restricted in DP and the pond has been mounded under beautification projects. The studyestablished a significant positive correlation between density and DO and a negative correlation with free CO_2. Diversity (Shannon index) was also found to have a positive correlation with density. The existence of site-wise variation in density and absence of significant site-wise variation in diversity and season-wise variation in diversity and density remains inconclusive dueto the paucity of past benthic information from the sites. This study has developed a baseline for looking into probable correlation of water quality with other ecological components such as planktons, macrophytes and fishes to answer questions on ecological food web and their interdependent functioning in future research.

GRANT SUPPORT DETAILS

The present research has financially supported by the Department of Biotechnology (DBT), Government of India’s Foldcope scheme. bearing number BT/IN/Indo-US/Foldscope/39/2015 dated 20.03.2018.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the DBT, Government of India’s Foldscope scheme, for providing financial support to carry out the research. The authors also offer gratitude to Dr. Gurdeep Rastogi of Chilika Development Authority (CDA), Odisha (India), and his team in acquiring sampling skills at CDA and Sally Entrekin of Virginia Tech Entomology Department for helping with taxonomic materials on request.

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