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Rotifer Diversity in Coal Mine Generated Pit Lakes of Raniganj Coal Field Area, West Bengal, India

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ABSTRACT

The research looks at the rotifer diversity in five different coal mine generated pit lakes from Raniganj Coal Field Area (RCF), West Bengal, India. The collection methodology was involved monthly sampling (n = 120) to analyze the rotifer diversity using surface hauling with standard plankton net of mesh size 55 µm and water column at different depths (20 cm to 50 cm) for the periods of two years (February 2018 – January 2020). Analyzes of some limnological parameters and macrophytes were also performed following standard protocol. Statistical analysis based on the physicochemical parameters showed that Harabhanga and Dhandardihi 1 Pit Lakes were more similar while Dhandardihi 1 Pit Lake and Dhandardihi 2 Pit Lake were more alike in terms of rotifer community structure. Seventeen taxa of rotifers under the five families were found with varying densities and diversity indices. The highest diversity was observed in the Searsole Pit Lake, and the dominant species was *Keratella tropica* Apstein. The five pit lakes can be separated from each other based on the variations in rotifer diversity and water quality parameters, advocating the implementation of limnological management. Our results indicated different abiotic and biotic variables influencing the rotifer assemblages and diversity of the pit lakes studied.

Keywords: Rotifer, pit lake, limnological variables, density, macrophyte

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Introduction

Members of the phylum Rotifera, a significant part of the aquatic metazoans, can be observed in almost any form of aquatic habitats having worldwide distribution and comprise two main groups: Bdelloidea and Monogonta while the taxon Seisonacea covers marine species (Segers 2007, 2008; Fontaneto and De Smet 2015). The morphological peculiarities and high richness of rotifers are key aspects in many investigations on freshwater ecosystems (Sharma and Michael 1980). The descriptive features of the rotifers were applied by many scientists for ecological analysis (Bai et al. 2006) and also for the water quality assessment. Besides, the impact of various abiotic parameters affecting the biological environment is also mirrored in population patterns and shifts in the rotifers' species architecture and functions (Chovanec et al. 2002; Dong 2004). The rotifer diversity is considerably large globally with at least 544 taxa reported from the Oriental region (Segers 2001, 2002, 2008). Approximately, 500 rotifer species have been reported from Indian water bodies and about 1700 of them have been reported from various regions of the world (Kiran et al. 2007). A substantial number of studies on the faunal diversity of Rotifera from aquatic biomes of the protected parts of the region of Northeastern India is seen in Indian literature. While taxonomic analyses of Indian rotifers were started more than a century ago, there has been still insufficient data on rotifer biodiversity in Indian aquatic bodies (Sharma and Sharma 2011). About 303 rotifer species has been reported from the Northeast India (Sharma and Sharma 2019). Rotifers play a significant part in the cycle of freshwater fish breeding, as it is an important live food for larval and early juvenile fish stages (Velasco-Santamaría and Corredor-Santamaría 2011; Shil et al. 2013).

Therefore, the nutrient dynamics and freshwater production are significantly governed by the rotifers (Snell 1998; Lin et al. 2005). Evaluation of the rotifers' diversity is key to demonstrate their importance in sustaining freshwater habitats (Gannon and Stemberger 1978; Dumont 1983). A negligible quantity of work was performed about the population ecology, habitat distribution of the zooplankton community in multiple wetlands, lakes and ponds in India (Sinha et al. 1994; Kumar 2001; Khan 2003; Bhalla et al. 2012; Deepthi and Yamakanamardi 2014). Furthermore, less number of data supporting the comprehensive review of rotifers with reference to the various physicochemical parameters in the man-made pit lake ecosystem is known. Though pit lakes are prevalent in several regions of India, these aquatic ecosystems have not been adequately studied. Water chemistry can vary significantly in different types of mining activities. Relatively high conductivity is observed in these huge water bodies (Ciszewski et al. 2013; Wołowski et al. 2013; Sienkiewicz and Gasiorowski 2015; Geller et al. 2013). These lakes formed by mining operations or extraction appear to be huge in-depth with sloppy sides (Blanchette and Lund 2016). Remarkably few biotic investigations were executed in these water bodies (Ferrari et al. 2015) since these researches are complicated to perform and needs specialized methodologies (Woelfl and Whitton 2000). Researchers have, therefore, started to find out different ecological components in these ecosystems, biotic succession and population dynamics (Geller et al. 2013; Wołowski et al. 2013; Sienkiewicz and Gasiorowski 2015; Vucic et al. 2019). The available information is inadequate to identify the trophic position of planktonic species in the mine lakes food system, which is different from that of more traditional lakes. Previous research has found that a few organisms dominate these pit lakes, like *Brachionus* sp., *Cephalodella* sp., *Rotaria* sp., *Elosa* sp., etc. (Deneke 2000). The work can demonstrate the application of rotifers as water quality indicators (Saksena 1987), thereby facilitating the monitoring of the resources of the aquatic ecosystem in the region (Kar 2014). The present work is a groundbreaking attempt which offers the first comprehensive evaluation of the rotifer ecology from the pit lake ecosystem of RCF. In addition to identifying and listing the inventory of different rotifer organisms from five distinct pit lakes, the findings of this analysis would also illustrate the variability of rotifers from the RCF. This study also highlights the association between aquatic macrophytes and rotifer assemblages in the pit lake ecosystem for the first time. To explore the ecosystem framework and its interaction with the limnological characteristics of these regions, some experimental works are required to establish a theoretical foundation for ecological regeneration, fair use and conservation of pit lake resources and an awareness of a healthy aquatic ecosystem. The finding represents valuable information to the diversity of Indian Rotifera. This result also allows us to enhance our understanding of rotifers' ecology in the pit lake system and unearth important connections between rotifer species and their ecosystem. The aims of this study were to (i) examine the spatial variability of rotifer populations in relation to various biotic and abiotic factors in the pit lake ecosystem created by the coal mine, West Bengal, (ii) provide and contribute to fill the research gap concerning the composition of rotifer communities in pit lakes, and (iii) assess the ecological integrity of the studied lakes utilizing pertinent data gathered during this research. This data will support the development of a comprehensive management strategy for developing in these water bodies a foundation for sustainable water protection and the development of fish stocks.

Materials and Methods

Study Sites

The sampling was made for two years running between February 2018 and January 2020, using five pit lake habitats: Harabhanga (H) Pit Lake, Dhandardihi 1 (D1) Pit Lake, Dhandardihi 2 (D2) Pit Lake, Searsole (S) Pit Lake and Dalurbandh (Dal) Pit Lake under RCF located in West Bengal, India. The detailed study area is represented in Table 1 and Figure 1.

Table 1. Physiography properties of the study sites

Study sites	Block	Mean Depth	Location
HARABHANGA (H)	RANIGANJ	~ 22.86 m	23°36'39.65"N, 87°3'49.18"E
SEARSOLE (S)	RANIGANJ	~ 31.50 m	23°37'35.38"N, 87°6'9.88"E
DHANDARDIHI 1(D1)	ANDAL	~ 30.48 m	23° 37' 26.58 " N, 87° 9 '22. 85" E
DHANDARDIHI 2(D2)	ANDAL	~ 25.91 m	23° 37'41.14"N, 87° 9'26.20"E
DALURBANDH(Dal)	PANDABESWAR	~ 22.86 m	23°42'48.68"N, 87°15'35.70"E

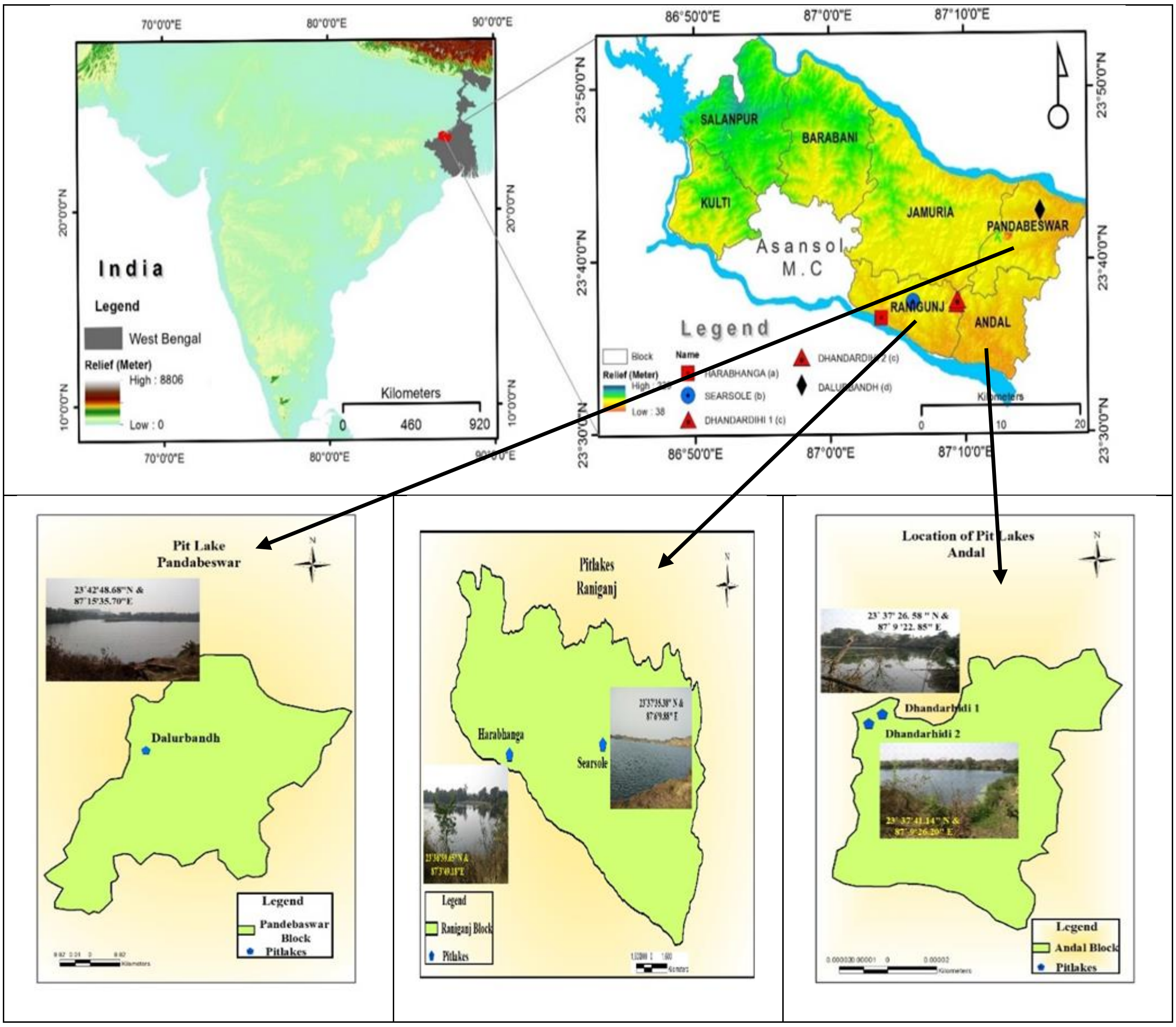


Figure 1. Map showing the study sites

Rotifer and Water Sampling

Random stratified sampling was implemented in compliance with the plankton selection standards (n= 24 per site) (Edmondson 1959; Battish 1992). The methodology involved monthly sampling of the mentioned sites to analyze the zooplankton using surface hauling with standard conical plankton net of mesh size 55 µm, 50 cm diameter and water column at surface water between 20 and 50 cm. The plankton net was drawn for a specific sample at a distance of between 5 and 10 m from the bank of the pit lakes. The equal force was exerted in the process of pulling the net across the water column to ensure a steady flow of water through the net. For a specific sample, a repetition of at least four hauls was conducted from four different locations of the investigated water bodies. This was packed in plastic containers after the plankton was harvested from the sites (100 mL), filtered and were fixed and preserved in a neutral formalin solution (4%) (May and O'Hare 2005).

The density and composition were calculated using the Sedgwick-Rafter counting chamber and compound microscope (Olympus Magus, Ch20i) (×10 and ×40) for identification up to species level. The rotifer species count was consequently represented in a unit liter of sample. The photos of the respective individuals were taken during the evaluation of the specimens in the collections to evaluate and validate the identity (Edmondson 1959; Battish 1992; Sharma 1998). Shannon and Weaver diversity index (Shannon and Weaver 1963), evenness index (Pielou 1966), and species richness index (Margalef 1958) were determined. Such indices help to interpret a specific ecosystem's population framework. The similarity index (Jaccard 1901) was also determined. Analyzes of some limnological variables from the same pit lakes were also made. Water temperature, pH, salinity, total dissolved solids (TDS) and conductivity were taken *in situ* using a multi-parameter probe (Oakton PCSTestr 35). Dissolved oxygen (DO) was measured following Winkler method (APHA 2005) and for the other variables such as total alkalinity, hardness, turbidity, biochemical oxygen demand (BOD), nitrate, and phosphate, the samples were evaluated in the laboratory following the standard protocol (APHA 2005).

Macrophyte Analysis

Aquatic macrophyte abundance was reported at each site on a five-point scale (1: scarce or non-vegetable, 2: many individuals, 3: tiny clusters of vegetation, 4: consistent vegetation, and 5: 100% covers) by visual assessment and walking along the embankment of the studied pit lakes during sampling (Stefanidis and Papastergiadou 2010).

Statistical Analysis

Hierarchical cluster analysis (HCA) focused on physicochemical parameters and rotifer density was provided in dendrogram to demonstrate the association among study areas using PAST statistical software (Hammer et al. 2001). The non-parametric Spearman correlation was done to portray the association between the physicochemical and biological variables using SPSS statistical software. The Mann–Whitney test and Kolmogorov–Smirnov test were performed to establish the differences in the rotifer community. Canonical correspondence analysis (CCA) was also performed between the limnological variables and different indices as the response variables. PAST statistical software was used to conduct all the statistical analyses.

Results

The rotifer community of the five pit lakes consisted of 17 rotifer taxa (Table 2). Dhandardihi 1 and Searsole Pit Lakes represented the highest number of species (11), Harabhanga Pit Lake by 10 species, while Dhandardihi 2 and Dalurbandh Pit Lakes by 8 and 9 respectively. *Brachionus angularis*, *B. calyciflorus* Pallas, 1766, *B. falcatus* Zacharias, 1898, *B. forficula* Wierzejski, 1891, *B. diversicornis* Daday, 1883, *Keratella tropica* Apstein, 1907 and *Filinia longiseta* Ehrenberg, 1834 were observed in all the study sites. The species richness was the lowest in Dhandardihi 1 Pit Lake, with 8 species while it was the highest in Dhandardihi 2 Pit Lake and Searsole Pit Lake with 11 species (Table 2) each. The number of rotifer organisms in the samples ranged from 57 to 153 individuals / L (mean 98.40 ± 1.65 SE; n = 120 samples). The relative abundance was varied in different study sites (Figure 2). The rotifer per samples in the Harabhnaga Pit Lake was: ranges 120–153; mean 140.10 ± 1.16 SE; n = 24; in Dhandardihi 1 Pit Lake: ranges 60–80; mean 68.30 ± 0.73 SE; n = 24; in Dhandardihi 2 Pit Lake: ranges 38–65; mean 58.72 ± 0.75 SE; n = 24; in Searsole Pit Lake: ranges 95–125; mean 113.06± 0.99 SE; n = 24 and in Dalurbandh Pit Lake: ranges 80-120; mean 109.48 ± 1.44 SE; n = 24. In Harabhanga, Dhandardihi 1 and Dalurbandh Pit Lakes, *K. tropica* was the dominant species (Figure 2), whereas in Searsole Pit Lake, *Brachionus diversicornis* was the dominant one. In Dhandardihi 2 Pit Lake, *B. forficula* was the dominant one. The Harabhanga Pit Lake showed the highest rotifer abundance accounting for 31.27% of total rotifer and the lowest in Dhandardihi 2 Pit Lake (11.58%) (Figure 2).

Different ecological indices values including the Shannon and Weaver diversity index (H′), Pielou's evenness (E) and Margalef's richness index (D) are represented in Table 3. The results showed that the Shannon and Weaver index and Pielou's evenness were the highest in Searsole Pit Lake. These indices are useful for understanding the community structure of a given ecosystem. The Jaccard's similarity index (SJ) for the study sites was compared and highlighted in Table 3 to contrast the similarities between the rotifer compositions among the study sites. Both the Harabhanga and Searsole Pit Lakes showed the highest similarity in terms of rotifer composition (0.429) while least similarities between Dhandardihi 1 and Searsole Pit Lakes (0.318), and between Dhandardihi 1 and Dalurbandh Pit Lakes (0.318) also.

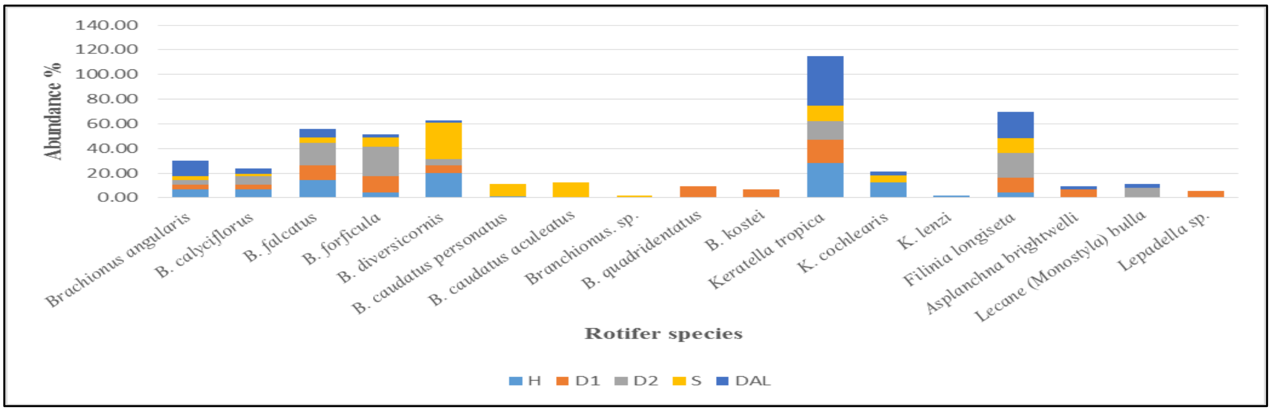


Figure 2. The abundance (%) of different rotifer species at study sites

Table 2. Inventory of rotifer taxa collected in the five pit lake habitats (n=24 per habitat)

Sl. no.	Scientific name	Acronym	(H) 10	(D1) 11	(D2) 8	(S) 11	(Dal) 9
	Family: Brachionidae						
1	*Brachionus angularis* Gosse, 1851	Ba	+	+	+	+	+
2	*B. calyciflorus* Pallas, 1766	Bc	+	+	+	+	+
3	*B. falcatus* Zacharias, 1898	Bf	+	+	+	+	+
4	*B. forficula* Wierzejski, 1891	Bfo	+	+	+	+	+
5	*B. diversicornis* Daday, 1883	Bd	+	+	+	+	+
6	*B. caudatus personatus* Ahlstrom, 1940	Bcp	+	-	-	+	-
7	*B. caudatus aculeatus* Hauer, 1937	Bca	-	-	-	+	-
8	*Branchionus* sp.	Bs	-	-	-	+	-
9	*B. quadridentatus* Hermann, 1783	Bq	-	+	-	-	-
10	*B. kostei* Shiel, 1983	Bk	-	+	-	-	-
11	*Keratella tropica* Apstein, 1907	Kt	+	+	+	+	+
12	*K. cochlearis* Gosse, 1851	Kc	+	-	-	+	+
13	*K. lenzi* Hauer, 1953	Kl	+	-	-	-	-
	Family: Filiniidae						
14	*Filinia longiseta* Ehrenberg, 1834	Fl	+	+	+	+	+
	Family: Asplanchnidae						
15	*Asplanchna brightwelli* Gosse, 1850	Ab	-	+	-	-	+
	Family: Lecanidae						
16	*Lecane* (*Monostyla*) *bulla* Gosse, 1851	Lb	-	-	+	-	-
	Family: Lepadellidae						
17	*Lepadella sp.*	Le	-	+	-	-	-

'+' represents present of species and '-' represents the absence of species

Table 3. Different indices reflecting the community structure of the study sites

Ecological indices	**Study sites**				
	H	**D1**	**D2**	**S**	**DAL**
Shannon and Weaver diversity index (H')	1.978	2.285	1.921	2.559	1.783
Pielou's evenness index (E)	1.978	2.194	2.128	2.457	1.783
Margalef's richness index (D)	1.769	2.323	1.724	2.089	1.946

Study sites	**Jaccard's similarity index**				
	H	**D1**	**D2**	**S**	**DAL**
H					
D1	0.333				
D2	0.389	0.368			
S	**0.429**	**0.318**	0.368		
DAL	0.400	**0.318**	0.389	0.381	

Physicochemical -Parameters of Water

Mean values (with standard deviation) of physicochemical parameters obtained from the different pit lakes were given in Table 4. The water temperature was recorded to be highest in Dhandardihi 2 Pit Lake (32.20°C) and lowest in Harabhanga Pit Lake (28.13°C). The pH was recorded highest in Dhandardihi 2 Pit Lake (8.10±0.24), whereas lowest in Searsole Pit Lake (6.14 ± 0.06). TDS and conductivity were recorded highest from Searsole Pit Lake (581.33 ± 11.85 and 949±5.29 respectively). Total alkalinity was lowest at Harabhanga Pit Lake (22.22 ± 6.41), while the mean total alkalinity was highest in Dhandardihi 1 Pit Lake (28.00 ±4.67). Mean total hardness was recorded highest from Searsole Pit Lake (405 ±5). DO content showed highest in Dalurbandh and lowest in Dhandardihi 1 Pit Lakes. BOD was highest in Searsole and lowest in Dhandardihi 1 Pit Lake. The highest phosphate content was observed at Harabhanga Pit Lake and lowest in Dhandardihi 2 Pit Lake. The lowest and highest nitrate values were recorded from Searsole and Dalurbandh Pit Lakes, respectively. The lowest and highest turbidity values were recorded from Dalurbandh and Dhandardihi 2 Pit Lakes, respectively.

Table 4. The physicochemical parameters recorded at five different pit lakes of the study area

Sites	Temp (°C)	pH	TDS mg/l	CON µS/cm	TH mg/l	TA mg/l	SAL mg/l	DO mg/l	BOD mg/l	PHOS mg/l	NIT mg/l	TUR NTU
H	28.13 ± 4.40	6.97 ± 0.12	184 ± 6.24	186.66 ± 4.14	219.77 ± 3.80	22.22 ± 6.41	211.33 ± 4.73	5.08 ± 0.52	2.28 ± 0.11	3.40 ± 2.91	1.33 ± 1.27	5.27 ± 1.80
D1	31.77 ± 3.49	7.85 ± 0.37	249.88 ± 13.42	258.33 ± 5.78	83.11 ± 5.67	28.00 ± 4.67	330 ± 15.13	4.25 ± 0.32	2.19 ± 0.13	1.60 ± 1.43	1.79 ± 1.00	5.98 ± 0.95
D2	32.20 ± 3.36	8.10 ± 0.24	250.78 ± 5.67	244.17 ± 21.36	189.56 ± 13.54	23.55 ± 0.38	192.33 ± 20.55	4.60 ± 0.36	2.37 ± 0.14	0.835 ± 0.50	1.34 ± 0.21	21.33 ± 3.27
S	28.9 ± 3.56	6.14 ± 0.06	581.33 ± 11.85	949 ± 5.29	405 ± 5	27.5 ± 2	412 ± 2	5.87 ± 0.70	3.26 ± 0.04	2.47 ± 0.26	0.91 ± 0.39	4.56 ± 0.86
DAL	29.73 ± 2.80	7.29 ± 0.60	308.44 ± 2.91	448.41 ± 8.07	204.22 ± 2.77	27.11 ± 1.68	247 ± 3	6.46 ± 0.81	2.94 ± 0.46	2.60 ± 1.64	2.02 ± 1.03	2.09 ± 0.09

TDS – Total dissolved solids, CON – Conductivity, TH – Total hardness, TA – Total alkalinity, SAL – Salinity, DO – Dissolved oxygen, BOD – Biochemical oxygen demand, PHOS – Phosphate, NIT – Nitrate, TUR – Turbidity.

Aquatic Macrophytes

During the investigation 20 aquatic macrophytes comprising 19 families were recorded (Table 5). Maximum macrophytes vegetation was found in the Harabhanga Pit Lake. Among the macrophytes, *Hydrilla verticillata* was the most abundant submerged macrophytes, *Azolla pinnata* was the most abundant free floating macrophyte while *Ipomoea aquatica* was the most abundant floating macrophytes, and *Marsilea minuta* was the most abundant rooted floating type macrophytes.

Statistical Analysis

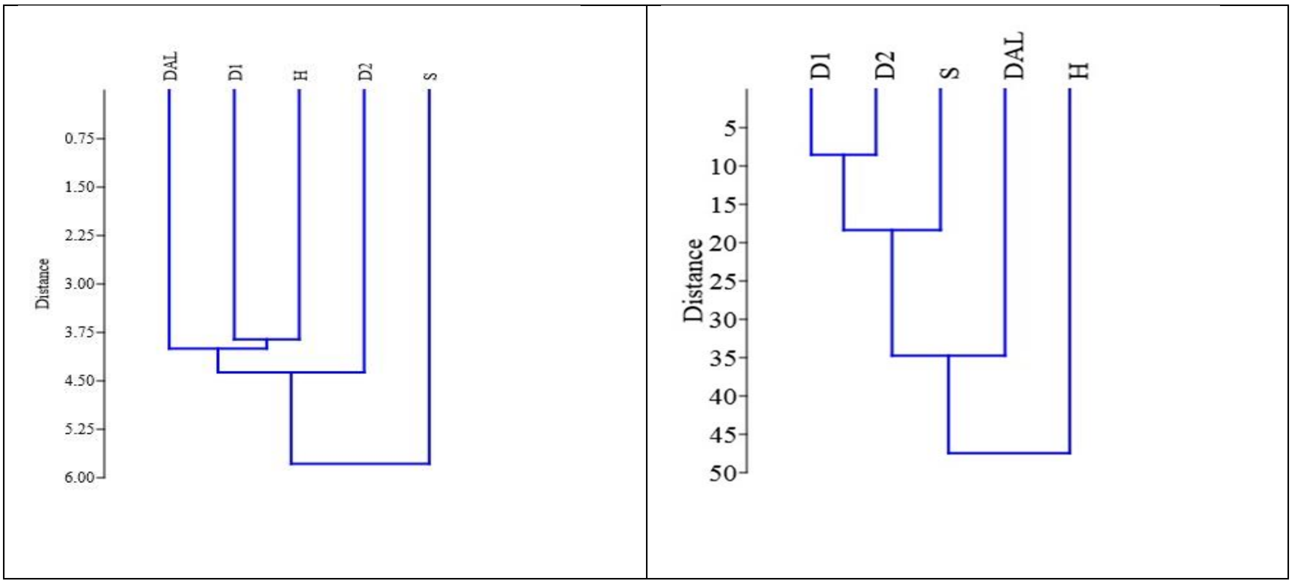
Hierarchical cluster analysis based on the different physicochemical factors placed Dhandardihi 1 and Harabhanga Pit Lakes in a cluster (D1+H) while the remaining lakes viz., Dhandardihi 2, Searsole and Dalurbandh did not form any separate cluster. Here Dalurbandh Lake being more similar to (D1+H) united to forma (D1+H+DAL) cluster. Next Dhandardihi 2 Lake joined individually to make a (D1+H+DAL+D2) cluster and finally the least similar Searsole Lake united with (D1+H+DAL+D2) cluster to complete the linkage (Figure 3a). However, when the analysis was determined regarding the zooplankton abundance, it showed different results (Figure 3b) that Dhandardihi 1 and Dhandardihi 2 Pit Lakes together formed a cluster (D1+D2), whereas other lakes did not make any separate cluster among them. Here Searsole Lake being more alike with (D1+D2) compared to other individual lakes, it joined to form a (D1+D2+S) cluster. With this cluster next closer Lake Dalurbandh joined and finally least similar Harabhanga Lake in rotifer community united to complete the linkage. *Brachionus calyciflorus* showed a significant negative correlation with the conductivity, salinity and alkalinity ($r = -0.900$; $p < 0.05$).

Table 5. The table presents the list of aquatic macrophytes observed at the study sites along with their ecotype

Sl. No.	Scientific name	Family	Habitat	D1	D2	S	D	H
1	*Chara* sp.	Characeae	S	-	-	-	-	+
2	*Eichhornia crassipes*	Pontederiaceae	FF	+	-	-	+	+
3	*Enydra fluctuans*	Asteraceae	F	-	+	+	-	-
4	*Hydrilla verticillata*	Hydrocharitaceae	S	+	-	+	+	+
5	*Ipomoea aquatica*	Convolvulaceae	F	+	-	+	-	+
6	*Nymphaea* sp.	Nymphaeaceae	F	-	-	+	-	+
7	*Nymphaea nouchali*	Nymphaeaceae	F	-	-	-	+	+
8	*Phragmites karka*	Poaceae	E	-	-	-	-	+
9	*Potamogeton* sp.	Potamogetonaceae	S	-	-	+	+	+
10	*Trapa natans*	Lythraceae	F	-	-	-	-	+
11	*Vallisneria spriralis*	Hydrocharitaceae	S	-	-	+	+	+
12	*Ceratophyllum demersum*	Ceratophyllaceae	S	-	-	+	+	+
13	*Lemna minor*	Lemnaceae	FF	+	-	+	+	+
14	*Spirodela polyrrhiza*	Araceae	FF	+	-	+	+	+
15	*Azolla pinnata*	Salviniaceae	FF	+	+	+	+	+
16	*Ludwigia perennis*	Onagraceae	FC	+	-	-	+	+
17	*Ottelia alismoides*	Hydrocharitaceae	S	-	-	+	-	+
18	*Hygrophila auriculata*	Acanthaceae	E	-	-	+	-	+
19	*Ipomoea carnea*	Convolvulaceae	S	+	-	+	-	+
20	*Marsilea minuta*	Marsileaceae	RF	+	+	+	+	+

Table 5. D1 – Dhandardihi 1, D2 – Dhandardihi 2, S – Searsole, D – Dalurbandh, H – Harabhanga. E – emergent, S – submerged, FF – free floating, F – floating, FC – floating and creeper. "+" represents present and "–" represents absent.

Brachionus forficula showed significant positive correlation with the turbidity (r= 0.900; p<0.05) while negative correlation with the phosphate (r= -0.900; p<0.05); *Brachionus falcatus* showed significant negative correlation with the conductivity and salinity (r= -0.900; p<0.05). *Brachionus diversicornis* significantly negatively correlated with the nitrate (r= -0.900; p<0.05); *Brachionus caudatus personatus* showed significant positive association with the hardness (r= 0.894; p<0.05) and significant negative correlation with both the pH and nitrate (r= -0.894; p<0.05). *Lecane* (*Monostyla*) *bulla* showed significant negative correlation with the temperature (r= -0.894; p<0.05) (Table 6). Mann–Whitney (p=0.0001) and Kolmogorov–Smirnov test (p= 0.001) demonstrated that there was a significant difference regarding the abundance of different rotifers taxa between the studied pit lakes.



(a) **(b)**

Figure 3. Hierarchical cluster analysis of the study sites based on the (a) physicochemical criteria and (b) rotifer community structure. H – Harabhanga Pit Lake, D1 – Dhandardihi 1 Pit Lake, D2 – Dhandardihi 1 Pit Lake, S – Searsole Pit Lake, DAL – Dalurbandh Pit Lake

Table 6. Spearman correlation matrix showing significant correlations between the physicochemical parameters and the biotic community

Rotifer	Temp	pH	TDS	CON	HARD	ALK	SAL	DO	BOD	PHOS	NITR	TUR
Ba	.205	-.103	-.308	-.154	-.051	-.205	-.103	.410	-.205	.718	.616	-.564
Bc	-.200	.300	-.700	-.900*	-.100	-.900*	-.900*	-.100	-.400	.300	.100	.300
Bf	-.200	.700	-.700	-.900*	-.500	-.600	-.900*	-.600	-.600	-.300	.100	.800
Bfo	-.100	.600	-.100	-.200	-.500	.200	-.200	-.800	-.300	-.900*	-.200	.900*
Bd	.500	-.700	0.000	.100	.600	.100	.500	-.100	.100	.200	-.900*	0.000
Bcp	.224	-.894*	.224	.224	.894*	-.112	.447	.335	.447	.447	-.894*	-.335
Bca	0.000	-.707	.707	.707	.707	.354	.707	.354	.707	0.000	-.707	-.354
Bs	0.000	-.707	.707	.707	.707	.354	.707	.354	.707	0.000	-.707	-.354
Bq	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354
Bk	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354
Kt	.100	.100	-.400	-.300	-.200	-.300	-.300	.300	-.300	.600	.700	-.400
Kc	.205	-.872	-.051	-.051	.872	-.462	.154	.564	.308	.872	-.564	-.564
Kl	.354	-.354	-.707	-.707	.354	-.707	-.354	0.000	-.354	.707	-.354	0.000
Fl	-.600	.600	.400	.300	-.500	.200	-.200	.200	.200	-.400	.800	-.100
Ab	.447	.335	-.112	.224	-.671	.671	.335	-.224	-.447	-.112	.783	-.112
Lb	-.894*	.671	.224	-.112	-.335	-.335	-.671	.112	.224	-.447	.447	.224
Le	.707	.354	-.354	0.000	-.707	.707	.354	-.707	-.707	-.354	.354	.354

*Correlation is significant at the 0.05 level (2-tailed). For rotifer, acronym see Table 2.

Canonical Correspondence analysis (CCA) diagram showed a correlation between the different diversity indices and species richness with the different water quality parameters. The two canonical axes explain more than 98% of variations of the dataset with the Eigenvalues being 0.0026 and 0.0004, respectively. From the CCA diagram, it can be observed that Shannon and Weaver index (H') was positively correlated with salinity, hardness, TDS, conductivity and BOD. Evenness (E) showed an affinity with the turbidity whereas species richness (D) was linked with nitrate (Figure 4). Also, CCA reveals the influence of different aquatic macrophytes on the rotifer community structure (Figure 5). The two canonical axes explain about 72% of variations of the dataset. From the CCA diagram, it is observed that *B. caudatus personatus*, *B. caudatus aculeatus*, *B. diversicornis*, *K. cochlearis* and *K. lenzi* are influenced by *Enydra fluctuans*, *Chara* sp., *Trapa natans*, *Ludwigia perennis* and *Ottelia alismoides*. *Keratella tropica*, *Brachionus angularis* and *Lecane (Monostyla) bulla* are influenced by *Nymphaea nouchali* and *Ceratophyllum demersum*. *Filinia longiseta* and *B. calyciflorus* are influenced by *Azolla pinnata*. *B. falcatus* are influenced by *M. minuta*, *Phragmites karka* and *H. verticillata*. *Asplanchna brightwelli* is influenced by *Eichhornia crassipes*, *Spirodela polyrrhiza* and *Ludwigia perennis*. *Brachionus forficula*, *B. quadridentatus*, *Lepadella* sp., and *B. kostei* are influenced by *Ipomoea carnea* and *Ipomoea aquatica.*

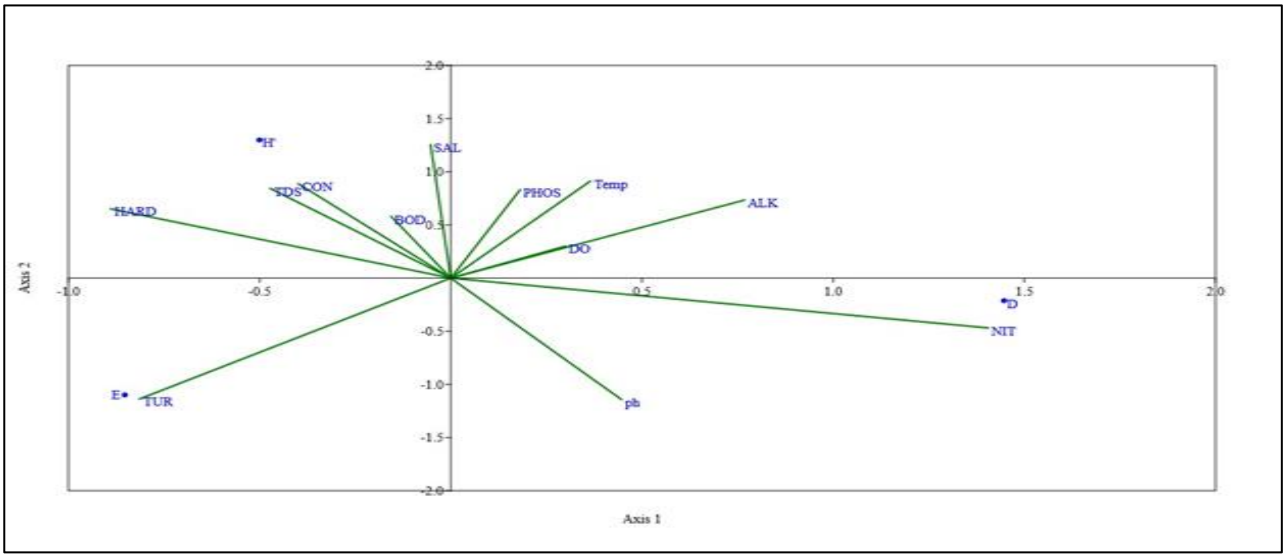


Figure 4. CCA biplot of the Shannon and Weaver diversity index (H'), evenness index (E) and species richness (D) as dependent variables against the physicochemical parameters shows a strong correlation. Temp – Temperature, TDS – Total dissolved solids, CON – Conductivity, TH – Total hardness, Alk – Total alkalinity, SAL – Salinity, DO – Dissolved oxygen, BOD – Biochemical oxygen demand, PHOS – Phosphate, NIT – Nitrate, TUR – Turbidity.

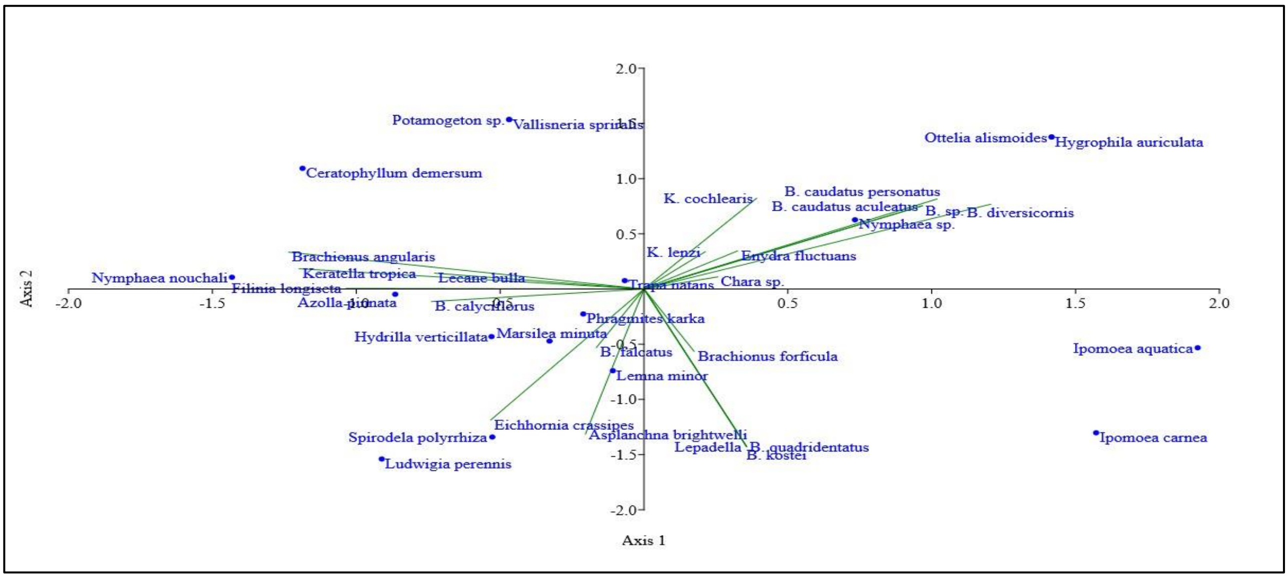


Figure 5. CCA biplot showing the relationship between aquatic macrophytes and rotifer communities

Discussion

Six different genera of rotifers have been identified during the study, where the genus *Brachionus* is most speciose while *Keratella* is least speciose. The lower alkalinity level in this study showed the strong buffering potential of the surface water of the pit lakes. At Harabhanga Pit Lake the lower DO value was liable for its higher alkalinity. Specific causes of hardness appears to be the presence of calcium and magnesium ions in water. The usage of soap, detergents and other cleaning products for washing practices in the Searsole Pit Lake contributed to a greater hardness level than other locations. In some sampled Pit Lakes (Harabhanga, Searsole and Dalurbandh) elevated phosphate concentration was found, suggesting enrichment of the nutrients. This in turn leads to greater plankton abundance which is reflected in our study. This culminated in relatively large nitrate levels, as the Harabhanga, Searsole and Dalurbandh Pit Lakes specifically receive domestic sewage through drainage systems from the nearby household. The rotifers' correlation tests against the environmental variables indicate a strong positive connection to the TDS, conductivity, hardness, salinity, DO, BOD, phosphate, nitrate, and turbidity as per this study. Several surveys across the globe have recorded the density of the rotifers and other zooplanktons with different relevant physicochemical parameters of different water bodies, including eutrophic freshwater lakes (Anton-Pardo et al. 2016; Sharma et al. 2016; Gupta and Devi

2014; Sharma and Pachuau 2013) as well as in the gravel-pit lakes (Vucic et al. 2019). However, it is possible that this is the pioneer work about the rotifer composition of the coal mine generated pit lake ecosystem in RCF. The important finding of this research is the mean density of rotifers is 98.40 ind/l, which is quite low. This may appear to be the condition of the studied pit lakes. Such a trend was also apparent in many reports on the prevalence of zooplankton and limnological variables encountered throughout the globe (Joniak and Kuczyńska-Kippen 2016; Burdis and Hirsch 2017). The younger pit lakes and the harsh environmental conditions resulted in less species diversity compared to elderly normal lakes. During the succession of this ecosystem when the community will reach its climax, diversity may increase (Lipsey 1980; Ejsmont-Karabin 1995; Hindák and Hindáková 2003). Pawlikiewicz and Jurasz (2017) found that Shannon diversity index value was increased along with the size of a water body. Mimouni et al. (2018) have described water body size as one of the significant local variables that influences variability like the zooplankton population. However, it was not noticed during this investigation. In Searsole and Dhandardihi 1 Pit Lakes, Shannon diversity index value was found to be higher compared to the other pit lakes tested, as these two pit lakes are relatively older and ecological succession got adequate time than the other studied pit lakes. The correlation found between the rotifer density and physicochemical variables like temperature, pH, conductivity, hardness, salinity, phosphate, nitrate and turbidity reflects the changes of rotifers' population composition mostly. In the Dhandardihi 2 Pit Lake, high pH illustrated the resalinization of the lakes linked to the freshwater inputs. The role of pH in structuring the rotifer community has appeared in the correlation and CCA analysis. Several experiments have demonstrated the vulnerability of the freshwater rotifers to elevated pH values (Bērzinš and Pejler 1987). Besides the geographical influences, the variations in the composition of the rotifers are probably related with the water quality variables such as nitrate and phosphate content, turbidity and temperature regime, as it is obvious from the observation of tropical lakes across the globe (Burdis and Hirsch 2017). This study also showed that *L.* (*Monostyla*) *bulla* is positively correlated with the temperature. The correlation study represents the sensitivity of a single zooplankton to the environmental factors (water quality parameters) seemed to be different a little from those observed in Manipur, Maharashtra, and Tamil Nadu because of the habitat conditions and the limnological variables of the pit lakes (Gupta and Devi 2014; Sharma et al. 2016; Rajagopal et al. 2010; Shinde et al. 2012). Previous findings indicated that the frequency and distribution of individual rotifer were generally affected by the trophic environment (Duggan et al. 2001; Wen et al. 2011). Throughout this analysis, *Brachionus* spp. dominated rotifer assemblages. *Brachionus* spp. are stated to be the most common rotifer in eutrophic lakes as they are strong pollution tolerant (Tasevska et al. 2012). Ismail and Adnan (2016) further reported that *Brachionus* spp. are one of the potent trophic indicators, as they are less influenced by algal bloom. The comparatively large rotifer abundance observed in both Harabhanga and Searsole Pit Lakes may have been correlated with macrophyte cover. During field sampling, large submerged macrophyte cover was recorded in these pit lakes. In a few experiments in other temporary and permanent waterways and stormwater storage systems, a clear beneficial association between zooplankton richness and macrophyte abundance has been demonstrated (Mimouni et al. 2018; Sun et al. 2019). Feasible macrophyte impacts that might drive richness of zooplankton species involve ecological gradients along with food availability and low visibility of predators (Mimouni et al. 2018). Many reports support this observation (Basu et al. 2000; Kuczyńska-Kippen and Nagengast 2003). In comparison, reports suggest that planktonic organisms such as *Keratella* spp. and *Brachionus* spp. can sometimes be bound to macrophytes (Green 2003). Submerged macrophyte vegetation is a very significant biotic factor for sustaining healthy lake ecosystem. Although several studies have examined the relationships in different lake environments between macrophytes and zooplankton, no research has yet been performed in these pit lakes formed by open cast mining operation. Taking the findings of this report as a foundation, further assessment of these pit lakes may be conducted to determine the overall ecological health and integrity. The present findings of correlation between the zooplankton assemblages and their responses to various biotic and abiotic factors will certainly pave the way for estimating the susceptibility of the native species of these major water bodies to specific kinds of stress. In turn, to conserve and allow judicious use of such tremendous water bodies, improved monitoring of these lake ecosystems would be required to take necessary measures towards their management and conservation. These findings showed the rotifer assemblages and their diversity at the different coal mine generated pit lakes.

The findings of this analysis show that the distribution and abundance of various rotifer organisms were determined by specific water quality parameters and aquatic vegetation. Despite the heavily impacted environment of the studied pit lakes, several littoral species contributed to the total zooplankton diversity and densities, emphasizing the role of aquatic vegetation in providing habitat for many zooplankton species. This study marks an important contribution on the diversity of freshwater zooplankton of India in general and that of the pit lakes of West Bengal as well as the tropics and subtropics in particular. Using the outcomes of this study as a framework, subsequent monitoring of the pit lakes concerned can be pursued to evaluate the ecological quality and integrity of the aquatic community. Despite this report's limitations, this analysis still presents a holistic basis for further studies related to rotifer distribution in the RCF regions' pit lakes. This can provide a vital framework for local and national resource protection and fisheries management. The output of this reseach can be utilized for the effective pit lake management strategy that includes involoving ecologists in the construction of pit lakes, prioritizing ecosystem development and proactive treatment in mine closure preparation and eventually providing residents with post-mining alternatives for their livelihood.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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