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Assessment of Benthic Macro-Invertebrates to Assess Water Quality of Treated Sewage Fed Pond at Pahari, Patna

Neetu, Anupma Kumari, Dilip Kumar Kedia, Ravindra Kumar Sinha

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ABSTRACT

Studies on the diversity, composition and abundance of benthic macro-invertebrates and water quality of a sewage fed fish pond located at Pahari close to south Patna was undertaken for two years. This pond receives treated sewage from an aerated lagoon Sewage Treatment Plant of 25 MLD capacities. Samples were collected from September 2007 to August

Dr Neetu*

Former Research Scholar, Environmental Biology Laboratory, Department of Zoology, Patna University, Patna 800005, Bihar, India

e-mail : neetu0513@gmail. com

Dr Anupma Kumari Associate Professor, Environmental Biology Laboratory, Department of Zoology,Patna University, Patna 800005, Bihar, India

e-mail : anupmakumari 10@yahoo.com

Dr Dilip Kumar Kedia Former Research Association, Environmental Biology Laboratory, Department of Zoology, Patna University, Patna 800005, Bihar, India e-mail : dilipkkedia@gmail.com

Prof (Dr) Ravindra Kumar Sinha University Professor (on lien), Environmental Biology Laboratory, Department of Zoology, Patna University, Vice Chancellor, Nalanda Open University, Patna 800005, Bihar, India e-mail : rksinha.pu@gmail.com *Corresponding author 2009 on monthly basis. Twelve species of benthic macro-invertebrates were recorded, out of which one belonged to Bryozoa, four to Oligochaeta, two Insecta and five Gastropoda. The range, mean and standard deviation of macro-benthos have been calculated. Species composition (%) was found to be highest in Gastropods (41.67) followed by Annelida (33.33), Insecta (16.67) and then Bryozoa (8.33). So far the number of individuals are concerned, insecta dominated the benthic community in the pond constituting 58.15% (2007-08) and 43.89% (2008-09) followed by Oligochaeta, Gastropoda and Bryozoa. The Shannon's species diversity index of benthic macro-invertebrates in the pond ranged between 0.9-2.1 with a mean value of 1.58 ± 0.325 showed that the pond is moderately polluted. Statistical relationship between different physico-chemical parameters and macro-invertebrates was also computed.

Keywords Benthic macro-invertebrates, Patna, Physico-chemical parameters, Sewage fed pond, Species diversity index.

INTRODUCTION

Benthos are bottom dwelling organisms ranging from microscopic to macroscopic fungi, protozoans, bryozoans, crustaceans, mollusks, insects, annelids

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and many lower vertebrates. They are defined as organisms that live on or inside the deposit at the bottom of a water body (Idowu and Ugwumba 2005) and play an important role in the ecosystem by feeding on living or decaying organic matter settled on the bottom and serving as food for invertebrate, fish and other higher vertebrates (Prabhakar and Roy 2008, Moulton et al. 2010, Copatti et al. 2013 Basu et al. 2013). In polluted lentic ecosystems and intolerant macro-benthos may be eliminated and tolerant remain abundant due to less competition and /or tolerance to adverse conditions (Myslinski and Ginsburg 1977). Thus benthic macro-invertebrate communities play a two fold role : First, they act as a connecting link in the food web and secondly, they purify the polluted water (Brraich and Kaur 2017).

Various methods have been applied to measure the impacts of human activities on the integrity of water resources such as chemical, physical and biological measures. Benthic macro-invertebrates have largely been used due to their very slow movements or static nature (Mason 2002). Biological communities integrate the effects of different stressors such as reduced oxygen, excessive nutrients; toxic chemicals and habitat degradation that water resource agencies are struggling to address (Tamiru et al. 2017). There are many studies that used macro-invertebrates assemblages for assessing the ecological quality of aquatic ecosystems, as they are affected by the physical, chemical and biological conditions of the stream (Menetrey et al. 2011). Also, some of the macro-invertebrates are extremely sensitive to organic pollutants, are widely distributed, easy and economical to sample and show clear responses encountering any adverse environmental conditions (Setiawan 2009, Kalyoncu and Gulboy 2009, Moreno et al. 2009). They may show the cumulative impacts of multiple stressors, like habitat loss, which are not always detected by the traditional water quality assessments using physico-chemical measurements.

Bio-indices have been recognized as suitable criteria for understanding the quality of aquatic environment. These are numerical expressions that combine quantitative values of species diversity with qualitative information on ecological sensitivity of each taxon (Czeniawska 2005). A large number of biotic indices have been developed to assess water quality (Metcalfe 1996). The most widely used indices of diversity are based on information theory, the most frequent measure being the Shannon diversity index, which assumes that individuals are randomly sampled from an indefinitely large population. The more species present in a community and the more equal abundance, the greater the uncertainty and hence, the greater the diversity.

The present study was carried out in a sewage fed fish pond at Pahari Patna. This pond receives treated effluents from Pahari Sewage Treatment Plant. The pond is used both for fish culture and irrigation purposes. The aim of the present study was to examine composition and diversity of benthic macro-invertebrates in this pond to assess the water quality of the pond.

MATERIALS AND METHODS

Study area

Pahari Sewage Treatment Plant, is aerated lagoon type with 25 MLD (million liter per day) capacity for the treatment of city sewage from Southern Zone of Patna (Fig.1). The sewage after passing through screen chamber and grit chamber flows to distribution chamber of aerated lagoon system. The sewage is then mechanically aerated in two parallel facultative aerated lagoons. The effluents from these lagoons are taken to the fish pond within 12 h. Total area of the fish pond is 0.83 hectare. The fish yield of 4.16 tons /year was estimated, @ 5 tons/ha.

Analysis of physico-chemical parameters

Water samples were collected once a month during September 2007 to August 2009 following Standard Methods (APHA 1998). Water samples were collected manually from a depth of about 15—20 cm from the pond using plastic bottles. Samples for analysis were kept in 1 l capacity bottles and transported to the laboratory in ice-box for further analysis. Separate samples were collected for the parameters that required specific preservation. Air temperature, water temperature, pH, conductivity, total dissolved solids



Fig. 1. Map showing sewage fed fish pond at Pahari Patna.

(TDS) and dissolved oxygen (DO) were determined *in situ* with the help of water analysis kit. Total alkalinity, chloride, total hardness, calcium, phosphate, nitrate, COD and BOD were analyzed in the laboratory following the Standard Methods (APHA 1998, Trivedy and Goel 1986). All the data were pooled and statistical mean and standard deviation were calculated.

Sampling of benthic macro-invertebrates

Monthly grab samples for benthic macro-invertebrates were collected using Eckman dredge (15.2 ×15.2 cm in size) from September 2007 to August 2009. The dredged materials were sieved through a metallic sieve (mesh size 425 micron) and preserved in 70% alcohol (Trivedy and Goel 1986). The organisms were identified up to species level and their abundance was calculated as number of organism per square meter, following Jhingran et al. (1969). Species identification was done with the help of Subba Rao (1989), Subba Rao et al. (1995), Ward and Whipple (1992), Nesemann and Sharma (2005a, b) Nesemann et al. (2007). Physico-chemical parameters were correlated with benthic fauna and statistical analyses were done using SPSS (Statistical Package for Social Science) software and Excel spreadsheet.

Community analysis

The basic statistical calculations such as range, statistical mean and standard deviation (SD) were made. Biological indices such as Shannon-Weiner index of diiversity (Shannon and Weiner 1949), evenness index (Pielou 1966), species composition and relative abundance were used for further analysis.

Shannon-Weiner diversity index (H') :

S
Diversity index (H') = -
$$\Sigma$$
 (P_i in P_i) or, - $\sum_{i=1}^{N} \left(\frac{ni}{N}\right) \log\left(\frac{ni}{N}\right)$

Where, H' = Shannon Weiner index of diversity, NI = Number of individuals in each species and, N = Total number of individuals.

Margalef's Index (d) (Richness) is given as :

Richness =
$$\frac{S-1}{\ln(N)}$$



Fig. 2. Number of species, species composition (%) and relative abundance (%) of benthic macro-invertebrate group in the sewage fed pond during September 2007 to August 2009.

Where, S = Total number of species, ln = Natural log and N = Total number of individuals.

Evenness =
$$\frac{H}{\ln(S)}$$

Where, H = Shannon–Weiner index and S = Total number of species in the sample.

Species composition (%) is given as :

Where, n = Total number of benthic macro-inver-

tebrate species in each taxonomic group, N = Total number of benthic macro-invertebrate species in all taxonomic group.

Relative abundance (%) is given as :

$$% RA = n (100)/N$$

Where, n = Total number of individuals in each benthic taxonomic group, N = Total number of individuals in the entire benthic taxonomic group.

RESULTS AND DISCUSSION

Benthic macro-invertebrates diversity

In the present study, a total of 12 species of benthic macro-invertebrates were recorded, out of which 1 belonged to Bryozoa, 4 to Oligochaeta, 2 to Insecta and 5 to Gastropoda (Fig. 2).Species composition (%) was found to be highest in Gastropods (41.67) with relative abundance of 14.94%, followed by Annelida (33.33) with relative abundance of 30.26% and lowest in Bryozoa (8.33) and Insecta (16.67) with relative abundance of 3.43% and 51.38% respectively (Fig.2).

The total abundance of benthic macro-inverte-

 Table 1. Annual quantitative analysis of macro-invertebrates (individuals/m²) of the sewage fed fish pond during September 2007–August 2009.

Benthic macro-invertebrates	2007-2008	2008-2009	Average density	Range
Bryozoa				
Lophopodidae	24.5 ± 30.80	38.5 ± 62.57432	31.5 ±48.76	21-210
Oligochaeta				
Dero spp.	40.25 ± 42.43524	56 ± 47.66359	48.125 ± 44.86	21-189
Limnodrilus hoffmeistri	103.25 ± 118.611	145.25 ± 116.5653	124.25 ± 116.99	21-399
Aulodrilus spp.	42 ± 39.03146	42 ± 45.65881	42 ± 41.54	21-168
Branchiura sowerbyi	70 ± 68.9743	57.75 ± 68.26835	63.875 ± 67.40	42–231
Insecta				
Chironomus spp.	523.25 ± 285.4846	364 ± 156.9794	443.625 ± 239.54	147-1155
Culicoids larva	38.5 ± 28.07943	19.25 ± 20.9203	28.875 ± 26.14	21-84
Gastropoda				
Bellamva bengalensis	56 ± 40.37776	56 ± 36.1889	56 ± 37.50	21-126
Pila globosa	10.5 ± 14.1582	5.25 ± 9.497607	7.875 ± 12.09	21-42
Indoplanorbis exustus	43.75 ± 20.9203	42 ± 28.31639	42.875 ± 24.36	21-84
Quickia bensoni	1.75 ± 6.062178	10.5 ± 14.1582	6.125 ± 11.55	21-42
Lymnaea acuminata	12.25 ± 14.03972	36.75 ± 39.15964	24.5 ± 31.37	21-126



Fig. 3. Monthly variation in total abundance (individuals /m²) of benthic macro-invertebrates and number of species during 2007-2009.

brates during the present study is represented in Table 1. The density of Bryozoa which was represented by Lophopodidae spp. ranged from 21 to 210 individual/m² (Annual mean 31.5, SD \pm 48.76). Out of four species of Oligochaeta identified in this pond, the density of Aulodrilus spp. was lowest and ranged from 21 to 168 individual/m² (Annual mean 42, $SD \pm 41.54$) while of *Limnodrilus hoffmeisteri* was highest which ranged from 21 to 399 individual/m² (Annual mean 124.25, $SD \pm 116.99$). In group Insect the density of Culicoids larvae was lowest and ranged from 21 to 84 individual /m² (Annual mean 28.875, $SD \pm 26.14$) while of *Chironomus* spp. was highest which ranged from 147 to 1155 individual/m² (Annual mean 443.625, $SD \pm 239.54$). Out of 5 species of Gastropoda the density of Ouickia bensoni was lowest and ranged from 21 to 42 individual/m²(Annual mean 6.125, SD \pm 11.55) while the *Bellamya* bengalensis was highest which ranged from 21 to 126 individual/m² (Annual mean 56, SD \pm 37.50). According to Pamplin et al. (2006), Higuti and Takeda (2002), the Diptera order, including the Chaoboridae and Chironomidae families and Oligochaeta class, represent the most noticeable and relevant organisms of macro-invertebrate benthic assemblies in small water bodies such as pond rich in organic pollutants.

Figure 3 shows monthly variation in total count (individuals/m²) of different groups of benthic macro-invertebrates and number of species present during September 2007 to August 2009.The number of benthic invertebrates was higher during 2007-08 compared to 2008-09 during the present study which could be due to the nutrient content as they have positive



Fig. 4. Seasonal variations of Shannon-Wiener diversity, indices of richness and Pielou's evenness recorded during September 2007 to August 2009 in sewage fed fish pond Pahari Patna.

correlation with the occurrence of these organisms. Other researchers have made similar observations and concluded that some macro invertebrates are highly sensitive to water quality and slight change of nutrients can cause fluctuations in the growth of these organisms (Ahmadi et al. 2011). However the statistical analysis showed that there was no significant difference in maximum abundance of benthos during 2007 to 2009 (t $_{stat}$ =0.955, p = 0.356). The abundance of benthic macro-invertebrates in sewage fed fish pond ranges from 546 to 1827 individuals/ m^2 during 2007-08 and 483 to 1323 individuals/ m^2 during 2008-09. Minimum number of species (5) was recorded in January 2007 and maximum (10) in October 2007 and August 2008 during first year of study and minimum species (4) was recorded in August 2009 and maximum (11) in April 2009 during the next year of study period. The distribution and abundance of benthic macro-invertebrates in this may be attributed to the availability of the food, shelter and physico-chemical properties of this pond. As the abundance, population density and diversity of benthic fauna mainly depend on physical and chemical properties of its habitats it responds quickly to any change in water quality (Sharma and Rawat 2009).

Biological indices

The occurrence of macro-invertebrate diversity can be used as a good indicator for biological and water quality assessment of pond. Biological indictes like Shannon-Weiner diversity index, richness index, and evenness index were used for the analysis. As the species diversity index and species richness index depend upon the number of species as well as number of individuals each species they contribute equally to these index values (Ludwig and Reynolds 1988). Hence decrease or increasse in any one of these two variables will influence the overall values of these indices. The seasonal patterns of diversity, richness and evenness are shown in Fig. 4.

Shannon-Weiner diversity index measures the number of species and the number of individuals in each species. A healthy benthic macro-invertebrate community should have a higher Shannon-Weiner diversity index. According to the Welch (1992) pattern, H'>3 represents unpollted regions H'<1 represents polluted status and 1 < H' < 3 represents moderate pollution status. In this study, the value of Shannon index ranged between 0.9 - 2.1 with a mean value of 1.58 ± 0.325 which indicates the pond was moderately polluted. Seasonal Shannon-Weiner diversity index varied from 1.5 in summer to 1.7 in monsoon season (Fig.4).

Species richness indicates the presence of various species and is calculated by the number of species in an area. An increasing number of taxa can be due to habitat diversity, suitability of water or its improved quality. The value of richness varied from 0.96 in winter to 1.14 in Monsoon (Fig. 4). The high value of indices showing high taxa richness and high relative abundance of benthic macro-invertebrates was due to availability of food and physico-chemical factors at the study site.

Evenness index measures the evenness or equitability of the community by scaling one of the heterogeneity measures relative to its maximal value that each species in the sample is represented by the same number of individuals (Brraich and Kaur 2017). Evenness index ranges from 0 (low equitability) to 1 (high equitability). Pielou's evenness index varied from 0.68 in summer to 0.8 in winter during the present study (Fig. 4). Higher evenness index values of 0.656-0.865 supporting high equitability of benthic macro-invertebrates have also been reported by Basu et al. (2013) at East Calcutta Wetlands. However evenness index value studied by Brraich and Kaur (2017) 0.9762 – 0.9819 indicates that benthic macro-invertebrates species found in the studied habitat

 Table 2. Mean value of physico-chemical parameters of water in the sewage fed fish pond.

Parameters	Range	Mean value
Water temperature (°C)	15.7 - 32.5	25.7 ± 5.2
pH	8.2 - 9.4	8.6 ± 0.3
Transparency (cm)	6.0 - 46.0	16.6 ± 10.47
DO (mg/l)	5.4 - 16.2	12.0 ± 2.87
Toatal alkalinity (mg/l)	5.6 - 34.4	19.0 ± 8.8
Total hardness (mg/l)	112 - 313	211.0 ± 65.0
Calcium (mg/l)	31.4 - 83.7	54.5 ± 16.4
Phosphate (mg/l)	0.163 - 0.930	0.469 ± 0.247
Nitrate -nitrogen (mg/l)	0.32-7.04	2.80 ± 1.89
BOD (mg/l)	16.7 - 34.4	23.6 ± 4.0
COD (mg/l)	53.1 - 132.6	81.4 ± 23.4
TDS (mg/l)	225 - 510	378.7 ± 82.3

was almost evenly distributed because the calculated value is closer to 1. The evenness index values of 0.68 to 0.8 supporting high equitability of benthic invertebrates.

Physico-chemical parameters

The mean and standard error values of physico-chemical parameters are given in Table 2. Water temperature ranged between 15.7°C-32.5°C with mean value of 25.7 ± 5.2 . Molluscs generally appear to be favored by the elevated temperature up to 30°C (James and Evison 1979). The pH value was always higher and ranged between 8.2 to 9.4. Pond water remained alkaline throughout the study. The great majority of species and the largest numbers of individuals of Gastropoda occur under alkaline condition (Pennak 1978). Increased value of pH in this pond during the study could be related to the increased level of photosynthesis carried out by phytoplankton and macrophytes; where CO₂ is consumed and hence pH is raised. Alkaline nature of the pond (ranged 120 mg/L-434 mg/L) may have supported the highest species composition of Gastropoda which was recorded in the study. Transparency ranged between 6 cm to 46 cm with the mean value of 16.63 ± 10.47 cm. Similar results have been recorded by Basu et al. (2013). Nitrate-nitrogen ranged between 0.32-7.04 mg/L with the mean value of 2.80 ± 1.89 . Phoshphate ranged between 0.163-0.930 mg /L with the mean value of 0.469 ± 0.247 . The crucial level of phosphate that causes eutrophication is somewhere near 0.005 mg/L, 0.01 mg/L of phosphorus is acceptable while

	Water temp (°C)	Trans- parency (cm)	рН	DO	BOD	COD	СТ	Elect conduct (µS/cm)	NO ₃ -N
Lophopodidae	103	142	198	.346	083	.115	.291	.366	223
Dero sp. Limnodrilus	-260	.289	.484*	.075	-276	.257	.069	.066	.046
hoffmeisteri	.472*	270	003	.049	418*	.485*	.542**	.296	449*
Aulodrilus sp.	088	.178	.520**	.422*	372	195	068	.054	039
Branchirua									
sowerbyi	131	.317	218	.065	059	132	046	.143	.290
Chironomus sp.	.137	.051	.006	052	.289	.250	.193	.290	068
Culicoids larva Bellamya	.506*	.235	.093	123	038	.017	378	573**	275
bengalensis	188	.086	.022	.141	.109	248	.026	.221	.308
Pila globosa Indoplanorbis	064	.320	009	.162	252	042	203	182	.062
exustus	157	.255	119	059	.170	157	338	020	.427*
Quickia bensoni Lymnaca	.359	199	137	133	.189	056	.108	.140	.250
acuminata	323	.071	.031	.101	137	574**	397	.104	.123

 Table 3. Pearson's correlation coefficient between the physico-chemical variables and benthic fauna of fish pond. All parameters are in mg/L, except water temperature, transparency, pH and electrical conductivity.

Table 3. Continued.

	PO ₄ -P	SO_4	Total alkalinity	Carbonate alkalinity	Bicarbonat alkalinity	e Hard- ness	Ca++	Mg++	TDS
Lophopodidae	.229	.031	.328	.455*	.267	.303	.322	.218	.259
Dero sp. Limnodrilus	.360	.048	.067	492*	.123	.059	.167	154	.106
hoffmeisteri	.436*	.357	.307	054	.306	.187	.234	.230	.425*
Aulodrilus sp. Branchiura	.207	171	.072	183	.090	001	.098	225	.097
sowerbyi	072	131	.199	365	.234	.128	.132	.044	.085
Chironomus sp.	.423*	.178	.301	613**	.365	.218	.161	.304	.363
Culicoids larva Bellamya	164	434*	371	344	329	493*	252	598**	504*
bengalensis	.287	098	.301	188	.315	.454*	.417*	.271	.153
Pila globosa Indoplanorbis	091	312	189	.236	209	089	.018	260	236
exustus	256	025	.045	.040	.037	.052	108	.132	104
Quickia bensoni Lymnaea	.228	.063	.316	067	.318	.374	.340	.250	.147
acuminata	.133	224	.092	149	.096	.263	.361	021	070

0.02 mg/L is excessive. BOD and COD showed different values depending on the amount of organic matter received.

Correlation between physico-chemical parameters and benthic macro-invertebrates

Table 3 presents the Pearson's correlation analysis between the physico-chemical variables and benthic

macro-invertebrates. A significant positive correlation of the abundance of the benthic macro-invertebrates with total alkalinity (r=0.434, p>0.05), phosphate (r = 0.547, p>0.01) and TDS (r=0.435, p>0.05) was observed. The availability of maximum number of Gastropods could be correlated with the cumulative effect of alkaline nature of water and high calcium content (Martinez et al. 2014). Similar studies have also been carried out by other workers (Basu et al. 2013, Mishra et al. 2015). Total abundance of Oligochaeta was positively correlated with phosphate (r = 0.444, p>0.05). According to James and Evison (1979) Oligochaeta are favored by organic enrichment and are often the dominant invertebrates in severely polluted conditions. Gastropoda showed positive correlation with total hardness (r = 0.434, p>0.05), Ca^{++} (r = 0.416, p>0.05) as calcium carbonate is the essential for shell formation (Pennak 1978).

The species whose abundance was significantly correlated with abiotic factors were Dero sps. (pH), Limnodrilus hoffmeisteri, (water temperature, phosphate, chloride, BOD, COD, nitrate and TDS); Aulodrilus sps. (pH, DO); Chironomidae sps. (phosphate); Culicoids sps. (water temperature, sulfate, total hardness, mg++, EC and TDS), Bellamya bengalensis (total hardness and Ca++), Indoplanorbis exustus (nitrate) and Lymnaea acuminate (COD). As the pond is sewage fed pond which receives heavy discharge of treated sewage from sewage treatment plant, this provides favorable condition for the development of Oligochaetes such as Limnodrilus hoffmeisteri and Branchiura sowerbyi. In this pond, presence of Limnodrilus hoffmeisteri and Branchiura sowerbyi indicates heavy pollution as they are frequently very abundant in heavily polluted waters (Mason 2002). Chironomus larva are known to thrive in polluted environment probably due to possession of hemoglobin a pigment that transport and store dissolve oxygen (Mason 2002). According to Myslinski and Ginsburg (1977) Oligochaetes and leeches are important indicators of pollution.

In this study, Mollusca, Oligochaeta and Insecta were positively associated with increasing nutrient concentration. Sensitive organisms will be replaced by organisms such as Chironomus and Oligochaeta which can tolerate lower dissolved oxygen concentrations. Some of the Oligochaetes like Tubificides show negative Bohr effect, that is why they survive even in water body with dissolved oxygen as low as 05 mg/L. Nutrient enrichment lead to eutrophication which ultimately leads to very low dissolved oxygen. Such conditions lead to decreased macro-invertebrates richness and elimination of sensitive taxa mostly represented by insect orders Ephemeroptera, Plecoptera and Trichoptera (Martinez et al. 2014). The absence of most of the taxa in this pond supports this explanation. In the polluted site the nutrient entrichment and organic loading, only organisms with special physiological and morphological adaptation could be find such as Hydrobidae, Phyceaedae and Viviparidae snails (Tamiru et al. 2017).

Presence or absence of particular benthic species in a particular environment and habitat condition make them significant indicators for bio-assessment (Sharma et al. 2010). The occurrence and dominance of pollution tolerant sps. such as *Chironomidae* larva, *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi* in this pond depicts deterioration of water quality. The correlation that exist between physico-chemical quality of the pond and the population of macro-benthic invertebrates indicates that the physico-chemical quality influence the abundance of macro benthic fauna (Mishra et al. 2015).

CONCLUSION

This study provides information on the diversity and community structure of benthic macro-invertebrates in the sewage fed fish pond. The higher abundance of tolerant species and lower of sensitive species in this pond indicated polluted water in the pond. Species diversity index of benthic macro-invertebrates and presence of certain pollution indicator species showed that the pond is moderately polluted. Presence of *Chironomus* and Oligochaetes as bulk of benthic fauna indicates species richness was affected as the species which could not tolerate low oxygen tension were absent.

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