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Canonical Correspondence Analysis and Related Multivariate Methods in Limnology

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ABSTRACT

The sampling surveys covered three geographically distinct regions in the urban and suburban zones of Chennai city, The Chetpet pond (Urban Zone), Madhavaram pond (Sub urban) and Chenglepet lake (Suburban). The Canonical Correspondence Analyses (CCA) was performed for each of the three study sites separately. In general zooplankton distribution in lakes is influenced by a number of factors. Among the environmental factors, Temperature and Oxygen content stood first to restrict plankton occurrence. Such an influence in the water chemistry is bound to affect the flora and fauna. The general conclusions that can be inferred as will be seen below also suggest the negative impact of urbanization on the planktonic fauna through a comparison between the Chetpet and Madhavaram ponds on one side with the Chenglepet lake on the other.

Key words: Zooplankton, CCA, Physico-chemical parameters, Limnology

Zooplankton are passively drifting microscopic organism in an aquatic water body which occupy a central position between the autotrophs and heterotrophs in an aquatic ecosystem. They are good bio-indicator of environmental pollution as they response quickly to any changes in water quality. Zooplanktons are also known as staple food item of fishes especially the larvae of fishes. For success in an aquaculture activity, they are known to play very important role indicating the presence or absence of certain species of fishes or the determining the population densities of an aquatic farm. The present analysis reveals presence of good diversity of zooplankton in the lake. In this oxbow lake, three major groups of zooplankton were reported namely, Cladocera, Copepoda and Rotifera. Among the three groups, rotifers are found to dominate the lake ecosystem. Among the 37 taxa reported, 20 were from rotifera group followed by that of cladocera with 13 taxa and 4 taxa from copepoda. On quantitative analysis of zooplankton in the oxbow wetland, shows higher abundance percentage of 58% by rotifera which is reported to be much higher than that of cladocera and copepod. Higher presence of rotifer in the lake is an indication of its pollution leading to eutrophication in near future. Present investigation may help in conservation of the oxbow lake [1]. Zooplanktons encompass the microscopic animal species that freely float in aquatic ecosystem. They encompass a vast variety of taxonomic groups; of which the members belonging to Protozoa, Rotifera, Cladocera and Copepoda are most common and often dominate the entire consumer communities. They have many remarkable characteristics and are often armored with appendages such as spines, rostrum etc. which prevent their predation by next higher organisms. They are highly mobile and this ability of movement not only provide them an effective defense benefit but also enable them to actively search and feed upon the phytoplankton and establish the aquatic food chain. Hence, studying the members of zooplankton communities is important for their role in tropic dynamics and energy transfer in the aquatic ecosystem. They serve as a food for fishes in the freshwater ponds, lakes, tanks and other aquatic reservoirs and play a significant role in the fish production [2]. Zooplanktons are an important component of aquatic ecosystem and are microscopic, free-floating organisms**.** Maximum species were recorded in summer season and winter while minimum species were recorded in monsoon. The abundant organic detritus, waste water inflow in the basin, the open defecation by local residents causes the abundance of zooplankton species in the lake water thereby the quality of the Lake water gets deteriorated [3]. The plankton constitutes the basic food sources of any aquatic ecosystem, which supports fish and other aquatic animals. Zooplankton diversity is one of the most important ecological parameters in water quality assessment. Zooplankton is good indicator of the changes in water quality because they are strongly affected by environmental conditions and respond quickly to changes in water quality. They occupy an intermediate link between phytoplankton and fish. Hence

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qualitative and quantitative studies of zooplankton are of great importance [4]. Freshwater zooplankton of Uttarakhand are composed of the taxa of Protozoa, Rotifera, Copepoda, Cladocera and Ostrocoda. Ritifera contributes maximum (40.50%) with thirty-two species followed by Protozoa (22.78%) with eighteen species and Cladocera (22.78%) with eighteen species to the total zooplankton taxa of Uttarakhand. Copepoda contributes 8.86% with seven species, while minimum contribution (5.08%) with only four species is made by Ostracoda to the total zooplankton taxa of Uttarakhand [5].

The important groups of zooplankton in the study area were Cirriped larvae (88.5%), and Cladocera (10.6%). The highest value of average density of Cirriped larvae and Cladocera was recorded in spring. The highest value of average density of cladocera was recorded in spring [6]. Some of the changes in zooplankton population was found associated with monthly changes in physio chemical parameters [7[. Zoobenthos in freshwater lakes and to selecting effective bio-indicators for ecosystem health assessment of freshwater lakes [8]. Rotifer was most dominant group (31%) and copepod was least dominant group (19%). Zooplankton were recorded maximum in winter season and minimum in monsoon season at all sites [9]. A percentage comparison among the various zooplankton species reveals that the rotifers were the dominant group forming 50% of the zooplankton followed by cladocerans and copepods representing 13.7% each. This was followed by Ostracoda and Protozoa representing 9% each followed by Anostraca forming 4.6% of the total zooplankton. Thus, each group of zooplankters preferred to reach their peak in different months of the year [10]. A total of 38zooplankton taxa along with Nauplius larvae were observed belonging to 5 major taxonomic groups: Protozoa (6 species), Rotifera (21 species), Cladocera (6 species), Copepoda (3 species) and Ostracoda (2 species). Physico-Chemical parameters of Barnai pond revealed well marked fluctuations with maxima and minima values of each parameter during specific seasons and zooplankton analysis revealed seasonal variations with an increase during summer and a fall during winter and monsoon seasons [11]. Anzali International wetland is one of the most important places for various organisms such as fishes. Zooplanktons are the first consumers in the ecosystem, and they are perfect food for the larvae of fishes. Rotatoria were found dominating other groups of zooplankton. The water body is continuously receiving domestic discharge leading to large amount of nutrient inputs and high amount of phosphate and nitrate in the water body indicates that water is eutrophic in nature [12]. The status of health of any aquatic ecosystem is largely dependent on the diversity and density of zooplankton inhabiting the water body. Zooplanktons are considered to be the most vital primary consumer in any aquatic ecosystem. At the same time, they also influence to a greater extent in determining the total productivity of an aquatic body. Collection of the zooplankton samples and their quantitative analysis was done following the standard procedures. During the whole period of study 18 different species of zooplankton have been identified from the study area. Out of them 7 species belong to the Rotifera group, 6 species belong to Cladocera group, 3 species of Copepoda group and 2 species of Ostracoda group. Rotifera group comprise the dominant of all the groups constituting 39% of the total zooplankton species, followed by Cladocera (33%), Copepoda (18%) and Ostracoda (11%) [13]. One of the most intriguing environmental gradients connected with variation in diversity is ecosystem productivity. The role of diversity in ecosystems is pivotal, because species richness can be both a cause and a consequence of primary production. However, the mechanisms behind the varying productivity-diversity relationships (PDR) remain poorly understood. We found that species richness at all trophic levels was correlated with several environmental factors, and was also related to richness at the other trophic levels. This study showed that the PDRs in freshwaters show scale-dependency. We also documented that the PDR complies with the multivariate model showing that plant biomass is not mirroring merely the resource availability, but is also influenced by richness. This highlights the need for conserving diversity in order to maintain ecosystem processes in freshwaters [14]. The season-wise and month-wise zooplankton analysis showed an average abundance of species lower in monsoon due to inflow of water and less photosynthetic activity by primary producers and the maximum occurrence indicating that Kadwai reservoir contains more zooplankton during post monsoon season. On the contrary, the zooplankton showed a maximum occurrence in post monsoon indicating availability for stocking fish seed during that season [15]. Rotifers, copepods and protozoa had positive negative associations with some water variables. The zooplankton diversity indices (0.44 to 1.76) revealed a deteriorated environment [16].

The canonical correspondence analysis (CCA) showed a distinct smattering positive and negative correlation on the distribution of zooplankton indicating that the relative abundance of any species was dependent on specific environmental variables [17]. The canonical correspondence analysis (CCA) showed positive and negative correlation between the zooplankton and water quality of the river Ganga. The present study shows that the anthropogenic activities such as river bed mining disturbed the water quality through enhancing the turbidity and nutrients load in the aquatic system. However, these changes in water quality significantly affected the distribution and abundance of zooplankton [18]. The canonical correspondence analysis (CCA) confirms that the positive correlation of environmental parameters, such as water temperature, salinity, ammonia, nitrates, and silicates, on the distribution and assemblages of the plankton community [19]. Canonical Correspondence analysis (CCA) is a Multivariate method to elucidate the relationships between biological assemblages of species and their environment. The method is designed to extract synthetic environmental gradients from ecological data-sets. The gradients are the basis for succinctly describing and visualizing the differential habitat preferences (Niches) of taxa via an ordination diagram [20]. Monitoring programmes for phytoplankton and zooplankton ecology as a bioindicator for pollution were highly recommended in the study. In addition, this study suggests the usefulness of multivariate statistical methods in the analysis of water quality and recommendations for environmental recovery and restoration are proposed for preservation of El-Mex Bay and Naubaria sites in order to facilitate development of environmental and tourist activities [21]. Some species such as *Microcystis aeruginosa* were increased by organic and inorganic pollution in Tortum Lake [22].

Zooplankton population composed of five major groups namely Protozoa, Rotifera, Cladocera, Copepoda and Ostracoda. *Brachionus, Moina* and *Cyclops* were abundantly found in Bicherli pond which imparts a eutrophic status to the pond. Therefore, it is unfit for drinking and irrigation purposes. Richness of nitrates and phosphates were favourable for the growth of phytoplanktons. So, dumping of garbage and entry of sewage water should be restricted and for preventive measures, physical sediment removal, biological interaction and proper filtrations treatment should be applied for the conservation or restoration of the pond to increase its aesthetic values, making it suitable for aquacultural purposes [23].

MATERIALS AND METHODS

Brief description of the lakes with their historical significance

The fortnightly sampling surveys covered three geographically distinct regions in the urban and suburban zones of Chennai city. The Chetpet pond (Urban Zone), Madhavaram pond (Sub urban) and Chenglepet lake (Suburban). These water bodies have been receiving much attention because they are used for multiple purposes including irrigation, fishing, recreation and bathing etc.

Chepet pond

Chetpet pond is spread over 16 acres in Chetput, Chennai, India. It is located to the north of Chetpet railway station. It is the only existing pond at the centre of the city. The pond belongs to the Department of Fisheries of the Tamil Nadu government [24]. Of the total area of the lake, the waterbody is spread across 9.1 acres and the land area covers 6.9 acres. An anglers club was functioning till the 1940s in the lake, whose members visited the small island in the midst of the waterbody for fishing [25].

Although not used for drinking purposes, the lake was a source of ground water recharge for the surrounding areas. As the water quality is not saline, the lake has a few varieties of fishes such as rohu, Catla and mrigal. Breeding is monitored to assess the water quality.

Madhavaram pond

Madhavaram pond is a 150-acre lake in the Manali – Mathur-Madhavaram area of Chennai, India [26]. Due to indiscriminate dumping of garbage and sewage, the lake has shrunk to less than 100 acres. A recent study by Nature Trust, an NGO working on the flora and fauna recorded in the lake, showed that about 55 species of birds have been reported in the Wetland. However, about 500 birds were regularly sighted till the mid-1990s. The lake was cleaned by NSS volunteers of JHA Agarcen College in Madhavaram in December 2009 [27].

Chenglepet lake

Kolavai Lake is a lake adjoining the town of Chengalpattu in Tamil Nadu, India [28]. The lake is located about 60 Kilometers (37 mi) from Chennai, and is close to Paranur railway station and Chengalpattu Junction railway station. Chengalpattu Junction railway station [29]. During times of acute Water shortage in summer, this lake serves as an additional source of water for the city of Chennai [30-31]. The lake hosts migratory birds such as the Whiskered tern, Indian spot-billed ducks, Indian spot-billed ducks, moorhens, coots and small waders [29].

Methods of sampling and analysis

Totally nine sites were identified on the three habitats. Samples were collected twice a month in 1L polypropylene bottle for physical, chemical analysis and 100 mL filtered sample for biological analysis. The sampling period covers four seasons which prevail in this part of our country. Winter (January-March), Summer (April-June), South west monsoon (July-September) and North east monsoon (Oct-Dec).

Zooplankton samples for qualitative and quantitative analysis were collected by filtering 100 L of surface water through 64 um 64μm mesh plankton net and approximately 100 mL of concentrate was fixed with 5% formalin. Plankton samples were identified by using standard keys [32-44] and counted in Sedgewick – Rafter counting chamber. Both water samples and the plankton samples were taken at the surface at the limit of the euphotic zone. Qualitative and quantitative analysis of plankton samples were done using standard methods [45]. A wide mouth pipette was used for subsampling counting of plankton. Plankton samples were examined under inverted microscope "(Nikon) under 100x magnification.

Water samples

Key water quality parameters like pH, Temperature, Dissolved oxygen, Alkalinity, Hardness, Nitrate, Nitrite, Phosphate and Silicate were estimated by adopting the methods given in [46]. For the estimation of dissolved oxygen, water samples were collected separately in 250 mL Dissolved oxygen bottles (Winkler type) and fixed in the field itself.

Data analysis

The water quality data as well as plankton data were entered in the Excel (Microsoft 2000) worksheets separately. The data screening, verification and calculations were done using built-in function. The Canonical Correspondence Analyses (CCA) were done for each of the study area with full water of the study area with full water quality data but with restricted groups of zooplankton data. Only species that have been recorded in more than 10 samples were included in the analysis. Canonical Correspondence Analyses (CCA) was performed on the same subset of 29 taxa and ponds/lake to assess the influence of environmental factors on the distribution of zooplankton. CCA is a gradient analysis technique where the ordination axes are constrained to be linear combinations of environment factors.

Table 1 The list of abbreviations for canonical correspondence analyses for copepods and rotifers

Abbrevi-ation	Copepods	Abbrevi-ation	Rotifers	Abbrevi-ation	Rotifers	Abbrevi-ation	Rotifers
c1	*Cyclops sp.*	r1	*Asplanchna sp.*	r13	*Filinia sp.*	r25	*Platyias sp.*
c2	*Diaptomus sp.*	r2	*A. Priodonta*	r14	*F. longiseta*	r26	*P.quadricornis*
c3	*E. agilis*	r3	*Anuraeopsis sp.*	r15	*F. terminata*	r27	*Rotaria neptunia*
c4	*Eucyclops sp.*	r4	*B. diversicornis*	r16	*Horaella sp.*	r28	*Scaridium sp.*
c5	*Halicyclops sp.*	r5	*B. forficula*	r17	*Keratella tropica*	r29	*Trichocerca sp.*
c6	*Heliodiaptomus viduus*	r6	*B.Calyciflorus*	r18	*Lepadella sp.*		
c7	*Mesocyclops hyalinus*	r7	*B. quadridentatus*	r19	*Lecane sp.*		
c8	*M. leuckarti*	r8	*B. patulus*	r20	*Monostyla sp.*		
c9	*Macrocyclops sp.*	r9	*B. angularis*	r21	*Monostyla decipiens*		
c10	*Mesocyclops sp.*	r10	*B. falcatus*	r22	*M. bulla*		
c11	*Microcyclops sp.*	r11	*B. rubens*	r23	*Notholca sp.*		
c12	*Neodiaptomus sp.*	r12	*Conochilus sp.*	r24	*Philodona sp.*		

The canonical correspondence analyses (CCA)

The Canonical Correspondence Analyses (CCA) was performed for each of the three study sites separately. The total number of taxa included were restricted to those that have more than 10 occurrences during the entire study period. The list of copepods and the rotifers that meet the above criteria and their abbreviation used in the analysis are given in (Table 1) [47].

The (Fig 1) Shows the biplot, of species ordination for Chenglepet lake in the space defined by the first two canonical axes accounting for 53% of the total variance in the data. The environmental vectors are also sown as vectors in the same plot. The first axis shows a strong positive correlation with the pH and dissolved oxygen and a negative correlation with silicate and nitrite. The alkalinity and hardness show a strong positive correlation with axis 2 and phosphate and temperature show a negative correlation.

The Species ordination showed distinctive difference between copepods and rotifers in the arrangement. The copepods showed a closer relationship with the axis 1 than rotifers which shows a close relationship with axis 2. The *Cyclops* sp., *Macrocyclops* sp., and *Heliodiaptomus viduus* seems to be influenced by pH and dissolved oxygen conditions whereas *Eucyclops* sp., *Mesocyclops hyalinus* and *Neodiaptomus* sp., appear to be influenced by the silicate and nitrate. *Mesocyclops* sp., and *Halicyclops* sp., however, do not have any srong relationship with the above variables.

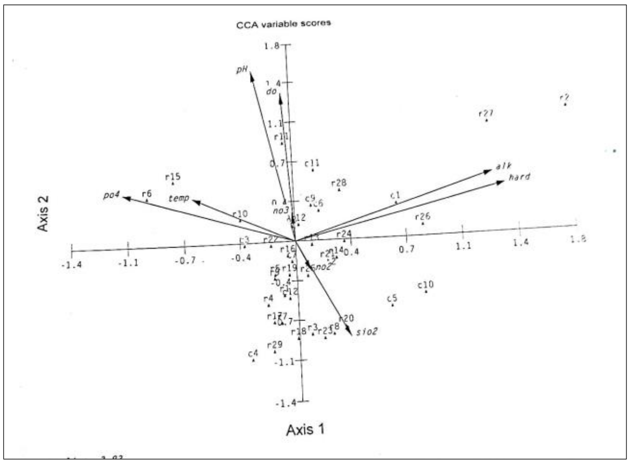

Fig 1 Canonical correspondence analysis variable sores of Chenglepet lake

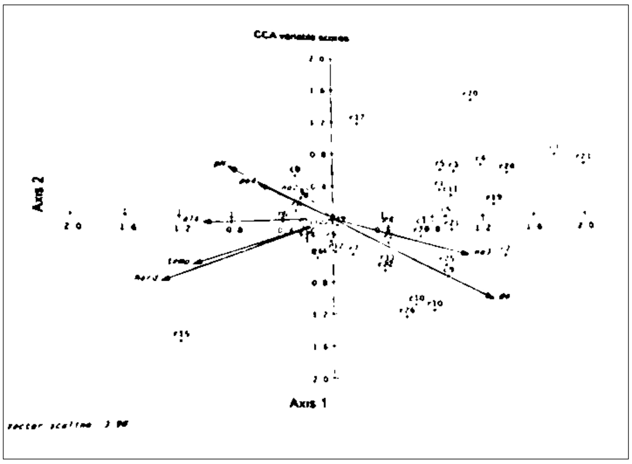

Fig 2 Canonical correspondence analysis variable sores of Madhavaram pond

The rotifers appear to be influenced positively by the carbonate system while negatively by temperature and phosphate. The excess of which may be limiting in the semi urban environment. The *Brachionus* genera (r4 to r11) however, is identified with the axis 1, influenced by pH and dissolved oxygen [19].

The ordination diagram for the Madhavaram pond (Fig 2) was different from the above the axis 1 accounted 25. 1% and axis 1 do not show any correlation with environmental vectors, while axis 2 15.6% respectively the axis 1 don not show any correlation with environmental vectors, while axis 2 showed a negative correlation with alkalinity, hardness and temperature. The pH, Phosphate and nitrite showed a negative correlation with dissolved oxygen and nitrate.

The species ordination also showed differences between rotifers and copepods. The copepods taxa showed a positive association with the axis 2 while the rotifer, distributions are less clear. The nitrate and dissolved oxygen appear to influence the rotifer distribution. The *Diaptomus* sp., and the *Cyclops* species are closely related to Dissolved oxygen and nitrate vectors.

The (Fig 3) show the ordination diagram of copepods and rotifers from the Chetpet pond. The axis 1 accounted for 18.2% while axis 2 accounted for 11.4%. The total variance explained by diagram is comparatively lesser than the other two sites. The copepods appear to be associated with the axis 1 rather than axis 2 in the other two sites. The rotifer species also showed closer associations with the axis 1 in this pond. The environmental vectors, the alkalinity, silicate, temperature and hardness appear related to the axis 2 which apparently do not seem to show influences either with copepod or rotifer species distribution. The axis 1 on the other hand indicated a negative association with the axis 1. The total variance accounted by the ordination diagram itself is low (30%). Therefore, it is possible that there may be more influencing factors may be present in this study site other than these environmental vectors measured.

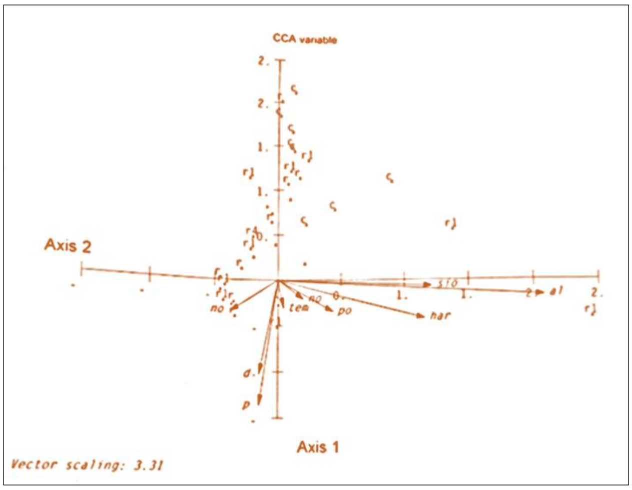

Fig 3 Canonical correspondence analysis variable sores of chetpet pond

Such an influence in the water chemistry is bound to affect the flora and fauna. The general conclusions that can be inferred as will be seen below also suggest the negative impact of urbanisation on the planktonic fauna through a comparison between the Chetpet and Madhavaram ponds on one side with the Chenglepet lake on the other.

Therefore, to probe further to understand the wild fluctuations in zooplanktonic abundance and the possible interrelations with the quality of water, a set of Multivatiate analysis were done for each of the study sites. The ordination diagram of the canonical correspondence analysis (CCA) is given in the (Fig 1-2).

The ordination diagrams also brought out the differences in the species distribution, arrangement and correlation with the environmental vectors. In this analysis also, the total variation explained by the ordination diagram was less in the case of Chetpet pond only. This may probably suggest that there may be unknown factors present and influencing zooplanktonic fauna in Chetpet pond, yet have not been measured and included in the study.

The ordination diagrams also brought out the differences in environmental preferences between rotifers and copepods in Madhavaram pond and Chenglepet lake. The pH and Dissolved oxygen appear to be the influencing factors on the distribution and abundance of copepods, while the alkalinity and hardness of the water, control the occurrence of the rotifers. Also, the rotifers indicated a negative influence of temperature and phosphate in these two study sites. The lack of clear separation of zooplankton taxa had been attributed to the biological factors also by other authors. For example, in a study on distribution and ecology of copepods in alps, Switzerland [48] suggest that the predation by fish had been an important factor in limiting the calanoid copepods. The differences between the presence or absence of fish in limiting the abundance of zooplankton taxa had been found to be significant in a study on small lakes from Wisconsin, USA by [49]. It is likely; therefore, in this study also, the biological factors apart from the deleterious impact of the Urbanization process on the study sites may have had a role in limiting the occurrence and abundance of zooplankton taxa.

In general zooplankton distribution in lakes is influenced by a number of factors. Among the environmental factors, Temperature and Oxygen content stood first to restrict plankton occurrence. Many temperate lakes lack a cold-water refuge for zooplankton in the summer [50] because the hypolimnion becomes anoxic as summer progress. Most of the observations are in good agreement with the similar results obtained elsewhere. The three habitats have a diverse zooplankton community represented by a variety of phytoplankton and zooplanktons. The total zooplankton abundance followed a typical. The results suggest that zooplankton provide a blunt tool for distinguishing wetlands from small lakes. Clearly none of the three habitats exhibited consistency through time for all limnological variables. In conclusion, local abiotic factors are important in structuring the zooplankton communities of lakes and ponds.

Crustacea: *Bosmina sp*., *Daphnia sp*., stagnant polluted waters indicators of pollution *Cyclops sp*., step wells in tropical climate carrier host of guinea worm.

Rotifers: *Anurea sp*., Rotaria, Philodina polluted and algae laden waters feed on algae (Table 2).

Table 2 Illustrative list of microscopic organisms present in water

S. No.	Classification of microscopic organism	Group and name of the organism	Habitat	Effect of the organisms and significance
Zooplankton	Crustacea	*Bosmina, Daphnia*	Stagnant polluted waters	Indicators of pollution
		Cyclops	Step wells in tropical climate	Carrier host of guinea worm
	Rotifers	Anurea, Rotaria, Philodina	Polluted and algae laden waters	Feed on algae

As pollution indicator species of Bicherli Pond were also 37 noted. The presence of these indicator species, hence, confirm the highly organically polluted nature of the pond water. Zooplankton species are indicators of trophic status. The analysis of pollution indicator species is based on the presence of particular species, which is indicative particular environmental condition. Rotifers such as *Brachionus caudatus, Brachionus caliciflorous, Keratella tropica* and *Asplancha sp*., have been identified as eutrophic indicator species in India and elsewhere in world by [51-53] considered Cladocerans as bioindicators for eutrophication. [54] characterized *Moina sp*., and the Copepoda *Cyclops sp*., as indicators of eutrophication. High densities of *Brachionus sp.*, *Asplanchna sp*., and *Fillinia sp.*, in Bicherli pond indicate the eutrophic nature of the pond water. Similar observation also reported by [55]. The presence of pollution indicator zooplankton species shows that the pond was under pollution and shows a trend of increasing eutrophication. Water of Bicherli pond unfit for drinking and irrigation purposes. Richness of nitrates and phosphates were favorable for the growth of phytoplanktons [56]. So, dumping of garbage and entry of sewage water should be restricted and for preventive measures, physical sediment removal, biological interaction and proper filtration treatment should be applied for the conservation or restoration of the pond to increase its aesthetic values, making it suitable for aquacultural purposes.

CONCLUSION

Rotifers are important group of zooplankton which can be considered as a valuable component of freshwater ecosystem. Their community structure can be used as bio-indicator of water quality assessment whereas their long-term changes need to be monitored. Presumably, the abundance of rotifers is strongly dependent on the trophic state of the water bodies.

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