

# BIOMONITORING THE STATUS OF AQUATIC BODIES USING ZOOPLANKTON AS SURROGATE SPECIES AMIDST URBAN LANDSCAPE

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## ABSTRACT

The concept of surrogates in biodiversity assessments has been widely accepted in the recent years. Surrogates are taxonomic groups that indicate the overall biodiversity at a particular site. Zooplankton is an important component of the aquatic ecosystem, playing a major role in energy transfer between the phytoplankton or producers and the consumers at higher trophic levels. In this study zooplanktons were considered as surrogates for biomonitoring status of two aquatic bodies amidst urban landscape at the southern fringes of Kolkata, West Bengal, India through different seasons. Zooplankton diversity and abundance was found to vary with seasons in both the ponds in correlation with limnological parameters. Pond 1 was found to be larger in size, having partial macrophyte cover in comparison with Pond 2 which is smaller and devoid of any macrophyte cover over the study period. The Pond 1 elucidated higher diversity of zooplankton having higher water pH and phosphate concentration and less nitrite concentration. Pond 2 elaborated less zooplankton diversity with lower pH, less phosphate and higher nitrite concentration. Diversity and abundance of zooplankton surrogates provided valuable information about the status of water bodies amidst urban landscape and can be utilised as a tool for biomonitoring.

**Keywords:** *surrogates, zooplankton, biomonitoring, limnological factors, urban landscape*

## INTRODUCTION

Surrogates are taxonomic groups that indicate the overall biodiversity at a surveyed site and play an important role in biological monitoring. The advantages of biological monitoring over separate physicochemical measurements to assess water quality are: (i) it reflects overall water quality, integrating the effects of different stress factors over time; and (ii) gives a direct measure of ecological

impact of environmental factors on aquatic organisms. In biomonitoring surveys of aquatic systems, zooplankton have played a key role for years, because they are ubiquitous and diverse, and plays major role in energy transfer between the producers and the consumers at higher trophic levels. Zooplankton are relatively inexpensive to sample, many laboratories have a good working knowledge of their taxonomy, and their abundance depends on a great variety of

abiotic and biotic factors [1, 2]. Zooplankton in this context serve the purpose of surrogation as it may reflect the overall health of aquatic bodies and be a sensitive indicator species [3 - 5].

Potentiality of zooplankton as a surrogate is immense as their growth and diversity are dependent on abiotic (e.g., temperature, salinity, stratification, pollutants) and biotic parameters (e.g., food limitation, predation, competition) of a waterbody [6, 7]. Nutrients from anthropogenic sources can deteriorate water quality and impact the balance of aquatic food webs [8]. Lying at the base of the trophic pyramid, plankton spontaneously respond to altered nutrient content in the water, that can have repercussions throughout the existing pelagic and benthic food webs, and thus they serve as a good bioindicator of water quality [9]. Alteration in limnological variables, macrophyte cover, morphology and water use by the people living in watershed greatly impact zooplankton surrogates and is related with its trophic status [9 - 12]. Higher trophic status leads to increased resource availability and hence increased abundance of zooplankton surrogates. Hence, qualitative and quantitative analysis of zooplankton community in temporal scale indicate degree of deterioration of the water body studied.

This work embodies the use of zooplankton as surrogate species in two aquatic bodies in the southern fringes of Kolkata, West Bengal, India. Both selected ponds were subject to differential anthropogenic stress and the study endeavours to correlate diversity and distribution pattern of zooplankton surrogates through different seasons along with associated limnological variables in selected ponds in the face of current urbanization pressure.

## MATERIALS AND METHODS

**Study site.** The sampling was conducted in two aquatic bodies located near Kavi Subhas Metro station in Garia, Kolkata. Pond 1 (Latitude: 22° 28' 33.78" N; Longitude: 88°23' 31.47" E

E) is situated near a construction site and it has been subjected to several anthropogenic sources of stress (domestic purposes, like bathing, washing, sewage discharge). The pond 1 is almost rectangular in shape (with dimensions of 440 m × 52 m) and it remains covered with water hyacinths (*Eichhornia crassipes*) occasionally in post monsoon months. Pond 2 (Latitude: 22° 28' 36.95" N; Longitude: 88° 23' 22.12" E) is located in the newly constructed urban park, beside Eastern Metropolitan Bypass, Kolkata. This pond is used for swimming, bathing, and pleasure boating (Figure 1). The Pond 2 is quadrangular in shape (with dimensions of 112 m × 111 m × 140 m × 28 m) and was free of any macrophyte cover throughout the study period.

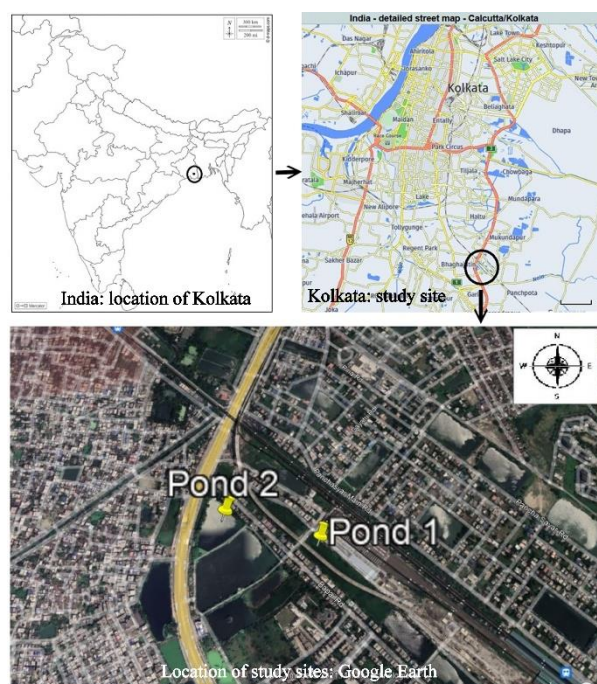


Figure 1. Location of study sites: pond 1 (Latitude: 22° 28' 33.78" N; Longitude: 88°23' 31.47" E) and pond 2 (Latitude: 22° 28' 36.95" N; Longitude: 88° 23' 22.12" E)

**Sampling and sample analyses.** Sampling was done over one year period (2019 - 2020) in three different seasons: monsoon (June to September 2019), post monsoon (October 2019 to January 2020) and pre monsoon (February to May 2020). All samples were collected early in the morning. Collection of water was done at early morning from the subsurface of waterbodies in a premeasured

thoroughly cleaned 250 - 500 ml inert plastic sample bottles. Water samples were carried to a laboratory and the limnological parameters were estimated following standard procedure [13]. Water temperature and pH were measured at the field using Systronics Digital pH meter 335. Sediment was collected from both the ponds at different seasons, dried, sieved with fine cloth to ensure passage of finest soil particles and then the physico-chemical parameters like soil organic carbon, soil phosphate, potassium were quantified according to standard protocol. The results were expressed as mean  $\pm$  SE format.

The subsurface water sampling was done randomly up to 50 litres of water filtered through the plankton net (mesh size 35  $\mu$ m). Zooplankton samples were then transferred to 100 ml appropriately labelled PVC plastic vials and carried to the laboratory. The samples were fixed with 4 % formalin solution, at the rate of 1 ml/litre of sample for preservation. Zooplankton taxa were identified to genus or species under a microscope at 100 - 450 $\times$  magnification and identified [14, 15]. Collected zooplankton samples were identified seasonally from selected ponds and quantified accordingly.

*Data analysis.* Diversity of zooplankton taxa was measured by the following indices: Shannon-Weaver index, Dominance Index, Evenness Index, Species Richness Index [16 - 19]. Limnological parameters, like water temperature, pH (using Systronics Digital pH meter 335), dissolved free carbon dioxide, dissolved oxygen (Winkler's method), alkalinity, hardness, dissolved inorganic phosphate, dissolved nitrite-nitrogen, dissolved silicate were estimated using collected water samples applying standard methodologies and results were expressed in the means and standard error (mean  $\pm$  SE) format [13]. Canonical Correspondence Analysis (CCA) was carried out using CANOCO (ver. 4.5) software to elucidate the possible relationships between abundance pattern of zooplankton and limnological variables studied [20, 21]. CCA permits the visualization of differential habitat preferences

of taxa via an ordination diagram [22]. CCA was carried out with forward selection of environmental variables and unrestricted Monte Carlo permutation test (499 permutation under reduced model,  $p < 0.05$ ). Interset correlations between the environmental variables and the ordination axes were used to select the adequate environmental parameters explaining variation in zooplankton abundance [20, 21].

## RESULTS AND DISCUSSION

The concept of surrogates in pond biodiversity assessments has been widely accepted in the recent years. Zooplankton in this context serve the purpose of surrogation as it may reflect the overall health of aquatic bodies by being an indicator species. In this study, monsoon was found to be the most zooplankton diverse season in both studied ponds. Six zooplankton taxa, *Brachionus* sp., *Asplanchna* sp., *Moina* sp., *Daphnia* sp., *Cyclops* sp. and *Filinia* sp. were found in pond 1 (P1) in the monsoon season. *Brachionus* sp., *Asplanchna* sp., *Moina* sp., *Cyclops* sp. were noted in the pond 2 (P2) in the monsoon season (Table 1). The zooplankton assemblage in P1 was characterised by presence of 5 taxa in the post monsoon season, while 4 taxa were noted in P2. The pre monsoon season appeared to be the least zooplankton diverse in both the ponds with only 4 species found (Table 1).

The present observation on the structural assemblage of zooplankton organization revealed a total dominance of rotifers in both sites. Rotifers represent 60 % and 69 % of the total planktonic abundance in P1 and P2, respectively (Figure 2). The rotifers are known to have a short life cycle, higher turnover rates and can adapt very fast with environmental variations and this makes them very good indicators of trophic level at freshwater conditions [9, 23]. The increased diversity of rotifers at the study sites could also be an indicator of unstable environment [24] with higher trophic level.

Table 1. Seasonal abundance pattern of zooplankton in study sites

Taxon		POND 1			POND 2		
		Mon	PoM	PrM	Mon	PoM	PrM
<i>Brachionus</i> sp.	Rotifera	+	+	+	+	+	-
<i>Asplanchna</i> sp.	Rotifera	+	+	+	+	+	+
<i>Moina</i> sp.	Cladocera	+	+	+	+	+	-
<i>Daphnia</i> sp.	Cladocera	+	+	-	-	-	+
<i>Cyclops</i> sp.	Copepoda	+	+	+	+	+	+
<i>Filinia</i> sp.	Rotifera	+	-	-	-	-	+

Abbreviations: Mon - Monsoon, PoM - Post monsoon, PrM - Pre monsoon

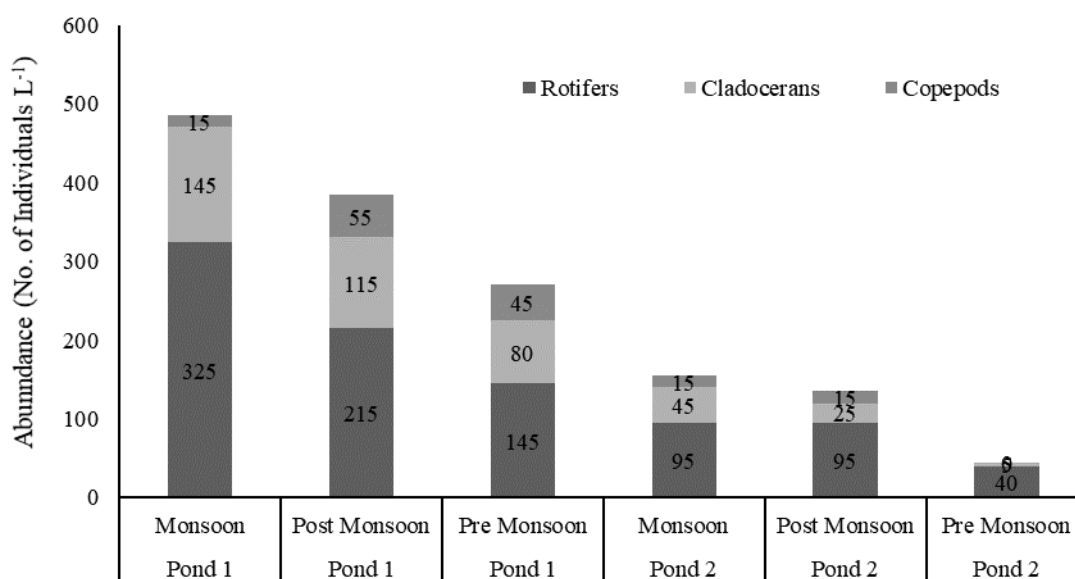


Figure 2. Seasonal variation in abundance of zooplankton at the study sites. Data labels depict abundance of zooplankton taxonomic groups

The cladocerans, which are known to be much more responsive against pollutants, represent 30 % and 22 % of the total planktonic abundance in P1 and P2 respectively. The cyclopoid copepods represent 10 % and 9 % of planktonic abundance in the study sites respectively (Figure 2). The cyclopoid copepods were known to be carnivorous and feed on smaller species of rotifers and cladocerans. The abundance of rotifers and cyclopoid copepods in both the ponds suggest higher productivity at the study sites [9]. One-way test of variance (ANOVA) detected changes in zooplankton abundance patterns and limnological parameters with significant differences among study sites as well as sampling seasons [25].

The diversity of zooplankton assemblage in the pond were measured through different

diversity indices (Figure 3). Shannon-Weaver diversity index, species richness, and evenness were higher in P1 in comparison with P2 in monsoon, post monsoon and pre monsoon seasons clearly suggesting that P1 had a greater diversity of species throughout the study period. Dominance Index was found to be higher in P1 (2.36, 2.75 and 3.38 respectively) in comparison to P2 (1.93, 1.68 and 1.12 respectively) in all three seasons. The distributional pattern of zooplanktonic species and the nature of dominance varied in different sites; *Brachionus* sp. was found to be the most abundant species in P1 whereas *Asplanchna* sp. was more abundant in P2 in the monsoon and post monsoon seasons. These variations in zooplanktonic species dominance and distribution over different seasons may be due to the influence of various limnological parameters [26, 27].



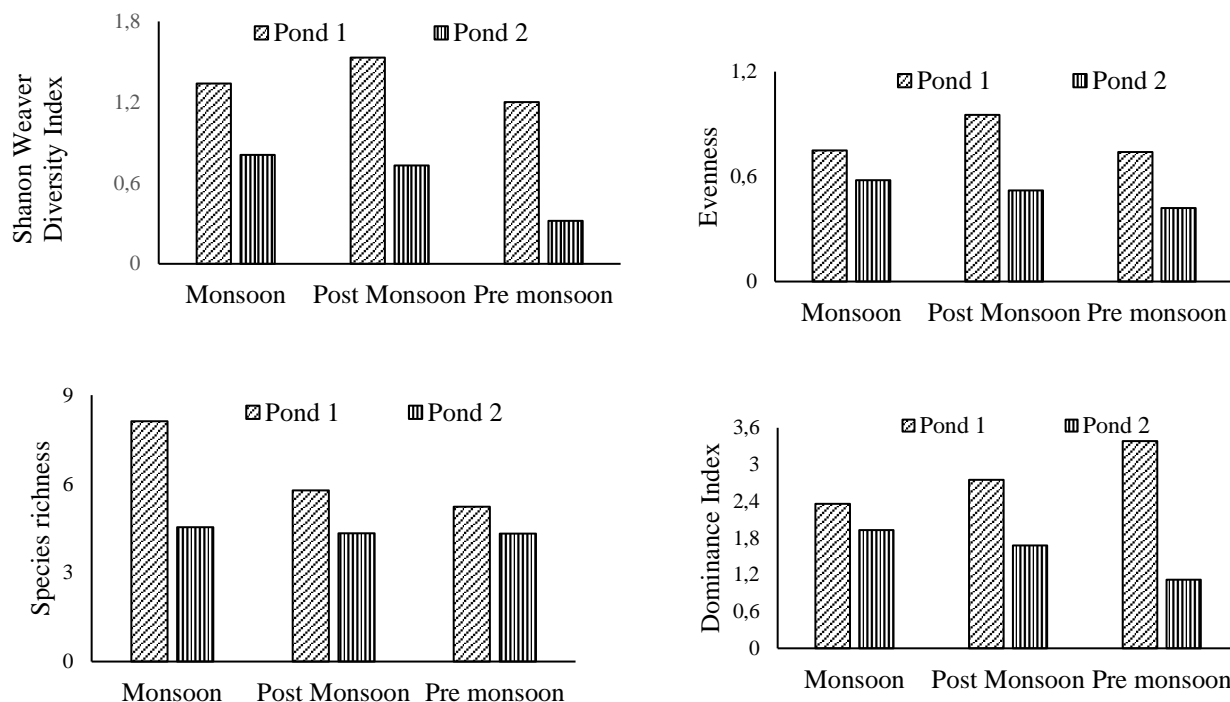


Figure 3. Diversity estimators calculated from zooplankton abundance data for both the ponds

Zooplankton species diversity was found to differ between two study sites situated in close vicinity as found in other cases [28, 29]. In order to find out relevant factors controlling species assemblage structure at the study site, limnological parameters were quantified [30 - 35]. The water pH was found to be slightly higher (Figure 4) in all the seasons in P1 ( $7.61 \pm 0.05$ ,  $7.6 \pm 0.034$  and  $7.2 \pm 0.015$  respectively) in comparison with P2 ( $7.1 \pm 0.032$ ,  $6.36 \pm 0.052$  and  $6.14 \pm 0.031$  respectively). The water temperature was found to be higher in P2 ( $27 \pm 0.62$ ,  $22 \pm 0.45$  and  $30.3 \pm 0.35$  respectively) in comparison with P1 ( $25 \pm 0.038$ ,  $21.3 \pm 0.32$  and  $28.5 \pm 0.25$  respectively) in all the seasons. The observed pH value and temperature recorded in this study was found to be conducive for planktonic growth [36, 37]. Among all the limnological parameters, dissolved oxygen is of crucial importance both as a regulator of metabolic processes of pond biota and as an indicator of water condition. The dissolved oxygen was also found to be slightly higher in P2 ( $8.30 \pm 0.54$ ,  $8.03 \pm 0.84$  and  $7.45 \pm 0.84$  respectively) in comparison with P1 ( $6.97 \pm 0.36$ ,  $7.52 \pm 0.54$  and  $5.45 \pm 0.43$  respectively) through all the year (Figure 4). The dissolved  $\text{CO}_2$  was found to be higher in P1 ( $17.87 \pm$

$1.24$  and  $10.4 \pm 0.89$  respectively) in comparison with P2 ( $14 \pm 0.85$  and  $8.65 \pm 0.945$  respectively) in the monsoon and pre monsoon season (Figure 4). However, the reverse trend was noted in post monsoon season with dissolved  $\text{CO}_2$  value in P2 ( $17.93 \pm 1.04$ ) in comparison with P1 ( $12.13 \pm 1.06$ ).

The alkalinity was found to be higher in P1 in all the seasons ( $73.80 \pm 2.85$ ,  $88.20 \pm 3.63$  and  $65.7 \pm 1.95$  respectively) in comparison with P2 ( $46 \pm 1.64$ ,  $53.06 \pm 1.63$  and  $42.4 \pm 1.36$ ). The hardness was found to be higher in P1 ( $283.58 \pm 2.764$  and  $174.89 \pm 2.835$ ) in comparison with P2 ( $223.20 \pm 2.314$  and  $167.98 \pm 1.634$ ) in the monsoon period. However, the hardness was found to be slightly higher in P2 ( $158.20 \pm 1.936$ ) in comparison with P1 ( $151.17 \pm 1.835$ ) in the post monsoon period. The dissolved  $\text{CO}_2$  and hardness value varied through the seasons explaining variation in plankter assemblage structure (Figure 5). The nutrients dissolved in water play an important role in zooplanktonic assemblage structure determining productivity of the aquatic body and ecologically phosphorus is often considered as the most critical single element in the maintenance of aquatic productivity [38].

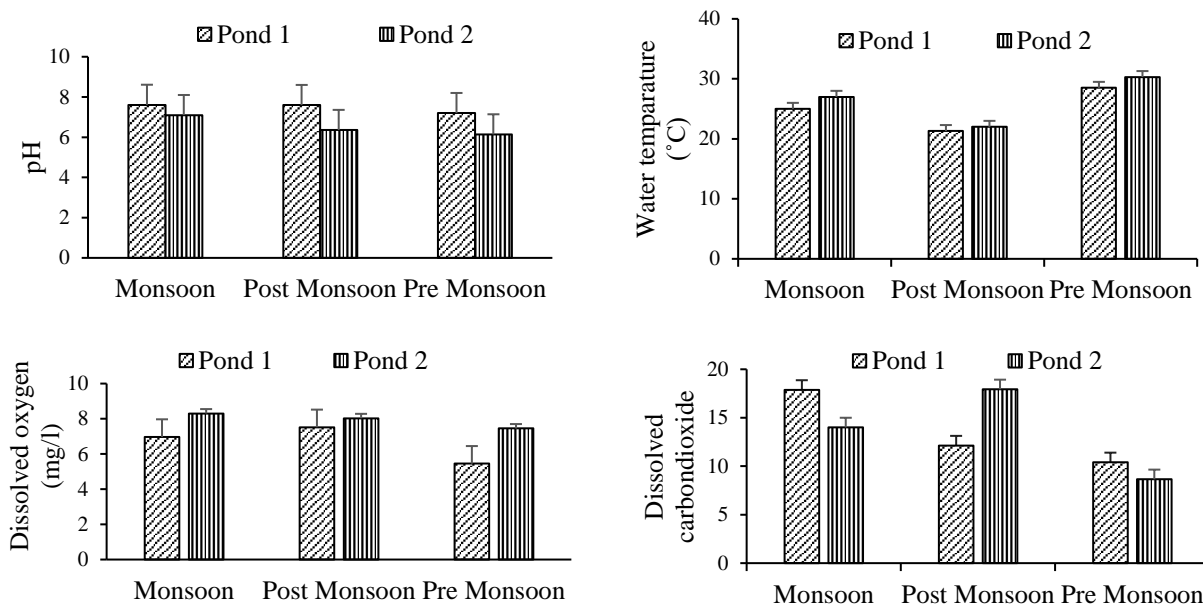


Figure 4. Estimation of limnological parameters in different seasons for both ponds

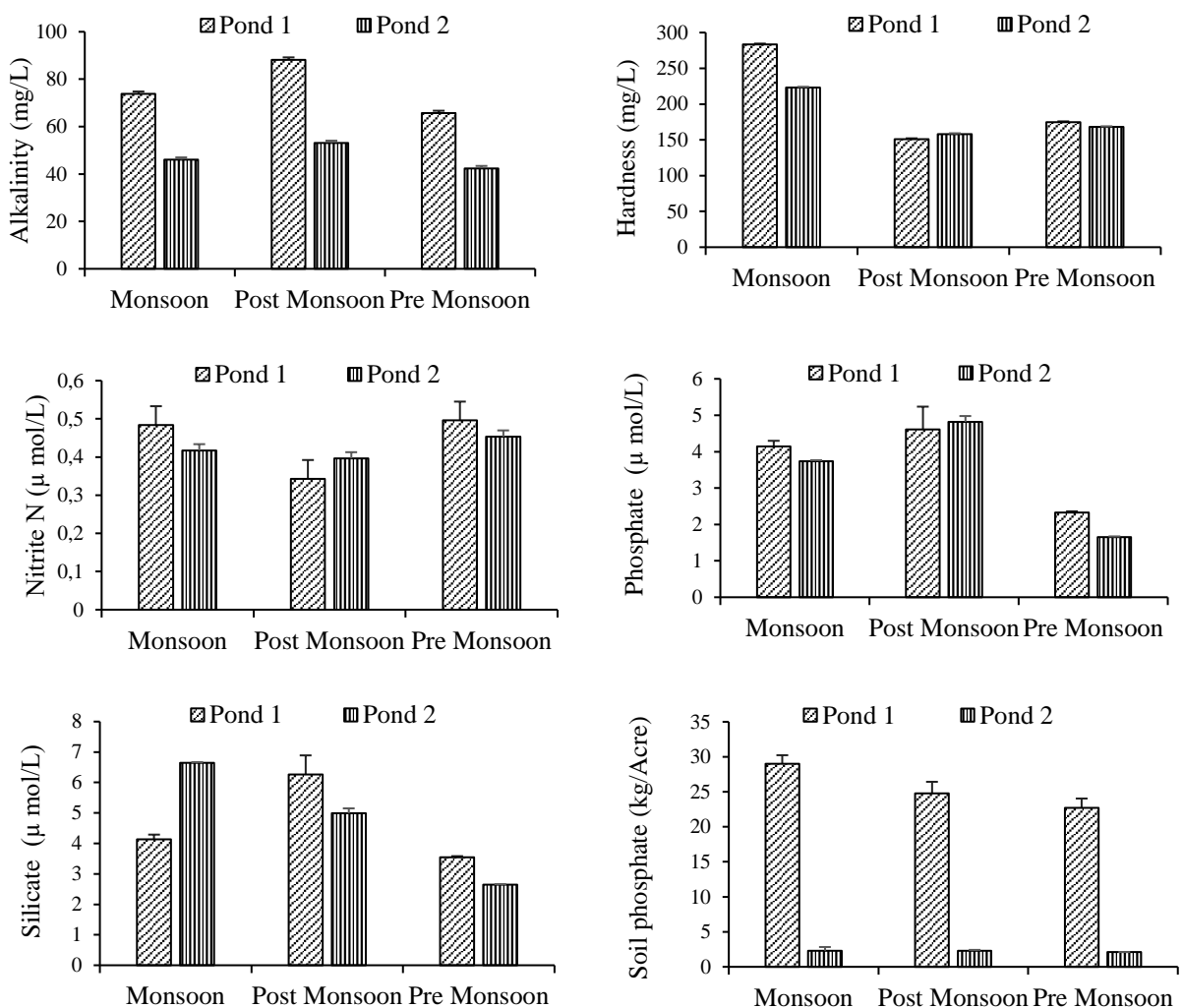


Figure 5. Estimation of limnological and edaphic parameters in different seasons for both ponds

The mean concentration of phosphate was found to be higher in P1 ( $4.146 \pm 0.152$ ,  $4.612 \pm 0.624$ ,  $2.324 \pm 0.036$ ) in comparison with P2 ( $3.74 \pm 0.024$ ,  $2.746 \pm 0.162$ ,  $1.654 \pm 0.017$ ) in all the seasons throughout the study period which is good for planktonic assemblage [39]. Likewise, the mean concentration of silicate was found to be higher in P1 ( $6.266 \pm 0.253$  and  $3.546 \pm 0.015$ ) in comparison with P2 ( $4.989 \pm 0.072$  and  $2.645 \pm 0.063$ ) in the post monsoon and pre monsoon period. The mean concentration of nitrite was found to be a little higher in P1 ( $0.484 \pm 0.004$ ) in comparison with P2 ( $0.417 \pm 0.003$ ) in the monsoon period. However, the mean concentration of nitrite was found to be higher in P2 ( $0.396 \pm 0.028$  and  $0.453 \pm 0.025$  respectively) in comparison with P1 ( $0.343 \pm 0.084$  and  $0.326 \pm 0.014$  respectively) in the post monsoon and pre monsoon period. The dissolved nitrite nitrogen, which is considered harmful for zooplankton, was found to be less in post monsoon season, leading to higher species diversity and evenness.

Regarding the ordination of CCA, the first two axes were considered, which expressed the highest variability in species data. They explained 86.8 % of the cumulative constrained variance in the species environment biplot (axis 1: 66.0 %, eigenvalue 0.247, axis 2: 20.8 %, eigenvalue 0.078) in P1 and 98.8 % of the cumulative constrained variance in the species environment biplot (axis 1: 74.9%, eigenvalue 0.277, axis 2: 23.9 %, eigenvalue 0.088) in P2 (Figure 6). Forward selection and Monte Carlo permutation (499 iteration) considered all environmental parameters as the important predictors of the species environment relationships at the study site. According to inter-set correlations, dissolved oxygen (DO), temperature (Temp), and hardness (Hard) were the most important environmental variables acting on the structure of the zooplankton assemblages in P1. There was a main sample ordination gradient in the fourth ordination quadrant related with temperature (inter-set correlations are -0.8348 and 0.0379 for the first and second axes, respectively) and hardness (inter-set correlations are -0.4934 and 0.1269 for the first and second axes,

respectively). Another major trend extended in the second ordination quadrant, which was related to dissolved oxygen with inter-set correlation of 0.4812 and -0.0891 for the first and second axes, respectively in P1. According to these gradients, four different species were distinguished in P1. *Asplanchna* sp. was abundant when temperature and hardness values were at the higher side. The second group consisted of *Daphnia* sp. and *Cyclops* sp. with higher dissolved oxygen and alkalinity whereas *Filinia* sp. and *Moina* sp. were abundant under high dissolved carbon dioxide concentration. On the other hand, *Brachionus* sp. seems to be dependent on soil phosphate concentration.

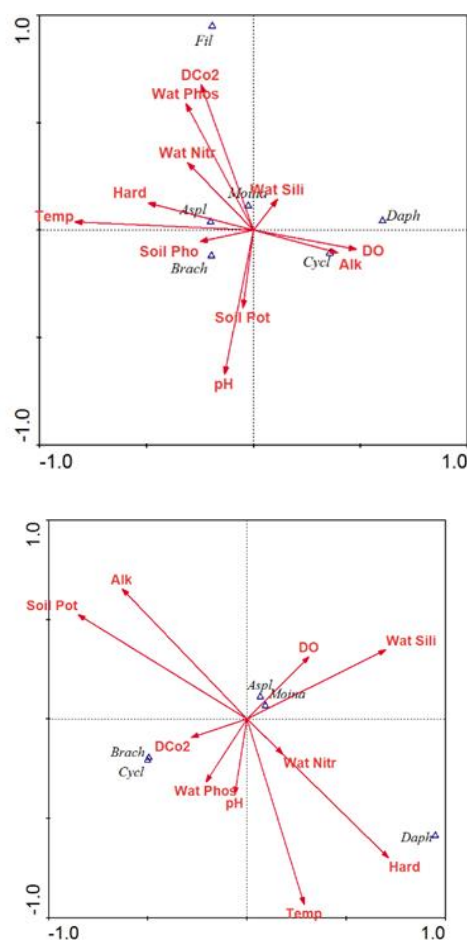


Figure 6. Canonical Correspondence Analysis (CCA) biplot for pond 1 (top) and pond 2 (bottom) respectively. Abbreviations: DO - dissolved oxygen, DCo2 - dissolved carbon dioxide, Wat Sili - water silicate, Hard - hardness, Alk - alkalinity, Temp - water temperature, Wat Phos - phosphate in water, Soil Pot - soil potassium, Wat Nitr - nitrate nitrogen in water, Soil Pho - soil phosphate

According to interspecies correlations, dissolved oxygen (DO), dissolved carbon dioxide (DCO<sub>2</sub>), water silicate (Wat Sili) and hardness (Hard) were the most important environmental variables acting on the structure of the zooplankton assemblages in P2. There was a main sample ordination gradient in the first ordination quadrant related with dissolved oxygen (interspecies correlations are 0.3137 for both the first and second axes) and water silicate (interspecies correlations are 0.6993 and 0.3473 for the first and second axes, respectively). Another major trend extended in the second ordination quadrant, which was related to hardness with interspecies correlations of 0.7138 and -0.6982 for the first and second axes, respectively. Another major trend extended in the third ordination quadrant, which was related to dissolved carbon dioxide (DCO<sub>2</sub>), with interspecies correlations of -0.2803 and -0.0930 for the first and second axes, respectively in P2. According to these gradients, four different species were distinguished in P2. *Asplanchna* sp. and *Moina* sp. was abundant when dissolved oxygen and water silicate values were at the higher side. The second group consisted of *Daphnia* sp. with higher hardness values, whereas *Brachionus* sp. and *Cyclops* sp. were abundant under high dissolved carbon dioxide concentration. On the other hand, *Brachionus* sp. seems to show slight affinity towards dissolved phosphate concentration.

## CONCLUSION

Both ponds, situated amidst urban area, are subjected to anthropogenic stresses by the local inhabitants. Pond 1 (P1), which was larger in size and had a partial macrophyte cover, showed greater biodiversity than pond 2 (P2). Correlating all the hydrological parameters with the zooplankton assemblage pattern during various seasons from the CCA biplot, higher dissolved oxygen, water temperature and hardness were found to play crucial role in structuring zooplankton assemblage structure in pond 1. The dissolved oxygen content above 7.0 mg/L (approx.) showed moderate aeration of the waterbody

with higher values for the post monsoon season, when zooplankton assemblage was found to be most diverse. A strong correlation between dissolved oxygen and species distribution that was found in the present study is corroborated with other studies in lentic and lotic system [40 - 43]. Pond 1, which is used for bathing, washing cloths and utensils, and sewage discharge gets more nutrients from the allochthonous sources and is therefore rich in planktonic diversity. Pond 2, which is used for swimming, bathing and pleasure boating, shows higher dissolved oxygen and nitrite, but less phosphate concentration. Phosphate in natural waters occurs in very small quantities, generally as calcium phosphate and is an important nutrient for maintaining fertility of the system [44]. Phosphorous is often considered as the most critical single element in the maintenance of aquatic productivity and nitrite is known to be harmful for planktonic assemblage [38, 45]. Pond 2, which had less phosphate and more nitrite accumulated, showed a lower zooplankton diversity, so according to the theory of surrogacy, it is less productive.

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