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PHYTOPLANKTON NUTRIENT DYNAMICS OF TWO LENTIC HABITATS IN EASTERN INDIA

PARTHA TALUKDAR*, AMIT SWARNAKAR AND RUMA PAL

Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, W.B., India

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ABSTRACT

In the present investigation we studied seasonal fluctuations in composition and community structure of micro-phytoplankton in relation to the environmental drivers from two lentic habitats. The phytoplankton nutrient dynamics from two different ecological niches are represented as one industrial wastewater pond (Pond-I) and the otherdomestic pond, also used for fish production (Pond-II). The phytoplankton productivity was determined in terms of chlorophyll content for both the ecosystems and their diversity was determined by species diversity, richness and evenness index. The results indicated that major nutrients influenced the seasonal phytoplankton population together with temperature and pH of the water bodies. More productivity (mg/L) in relation to chlorophyll (Chl.) was recorded in Pond-I (31.71) compared to Pond-II (10.12), especially in winter period. Wide seasonal variation in nutrient concentrations (mg/L) were recorded for both sites, showing much higher levels of total nitrogen (nitrate 2.9-30.72, ammonium nitrogen 0.2-8.6), phosphate (0.37-1.89), and silicate (9.1-46.8) in Pond I, compared to that of pond II (nitrate 0.6-10.1, ammonium nitrogen 0.01-0.27, phosphate 0.005-0.11 and silicate 1.3-7.5). Dominant micro-planktonic flora of wastewater pond was represented by members of Chlorophyceae, mainly the genera *Actinastrum* and *Kirchneriella*, whereas in Pond-II the Cyanobacterialgenus, *Aphanocapsa* was the dominant one. The species richness was higher in wastewater fed pond showing blooming of *Kirchneriella lunaris* in winter. Multivariate statistical analysis especially Principal Component Analysis (PCA) showed the possible relationships between productivity and other abiotic and nutrient variables like nitrate and silicate, and moderately with phosphate and ammonium nitrogen availability. In conclusion, it was found that the seasonal cycle of production and succession was majorly controlled by temperature, transparency, BOD, DO, nitrate and phosphate for pond-I while only temperature and pH played important role in pond-II.

KEY WORDS : Phytoplankton diversity, Nutrient dynamics, Environmental variables, Wastewater fed pond, Fishpond, Seasonal variations

INTRODUCTION

Phytoplankton especially the microplanktonic cyanobacteria and algae are important primary producers of aquatic ecosystems and give valuable information regarding water quality assessment. The populations consist of a very large number of species in spatially and temporally dynamic assemblages. The interactions of nutrients, environmental factors and biological traits like, growth rate, functional type, physical and chemical requisites etc. (Dove and Chapra, 2015), determine the local occurrence of varying subsets of the regional pool of species (Das *et al.,* 2018; Descy *et al.,* 2012). The contribution of microplanktonic cyanobacteria and algae to total standing stock and primary production have a significant impact on the food webs supporting the productive fisheries (Cermeno *et al.,* 2006). Over the last two decades the fish farming in India has been developed tremendously, subsequently phytoplankton monitoring program is necessary for betterment of fish productivity (Vale *et al.,* 2008).

On the other hand, different anthropogenic factors including industrial effluents change thephysico-chemical composition which in turn affect phytoplankton composition (Das *et al.*, 2018). It is also known that high nutrient pulses from industrial waste are rapidly transformed into high amounts of biomass (Cloern and Dufford, 2005) and trigger phytoplankton succession (Margalef, 1963; 1978) followed by the development of 'blooms' of different species (Anderson, 1997; Wilkerson *et al.*, 2006). The elevated levels of nutrients mainly nitrogen and phosphorus obtained due to some activities such as water supply, sewage disposal, fisheries, wastewater management, recreation, etc., enriched phytoplankton populations, leading to extensive blooms (Moss, 1983).

Seasonal variations of light intensity, temperature and nutrient availabilities greatly affect phytoplankton composition of a pond ecosystem (Heydari *et al.*, 2018). Phytoplankton community of different ecological niches differ significantly as they are controlled by physicochemical parameters of habitat water. Thus different population pattern of microplanktonic cyanobacteria and green algae have been reported by researchers viz. fresh water ecosystem (Bose *et al.*, 2016), East Kolkata Wetland – a Ramasar site (Singha Roy *et al.*, 2018), coastal west Bengal, India (Chaudhury and Pal, 2010; 2011) and Sunderbans mangrove ecosystem (Satpatiand Pal, 2019).

Mathematical measurement of species diversity and species richness in terms of diversity indices explain these parameters in more comprehensive and better understandable way. Quantification of diversity using these factors is a valuable tool to understand community structure. In this context, monitoring of water quality and nutrient status in waterbodies, phytoplankton are suitable indicators (Bellinger and Sigee, 2010; Choudhury and Pal, 2012).

In the present investigation we examined monthly samples of two years long study of one industrial wastewater pond and one domestic fishpond for studying seasonality of the phytoplankton assemblage and its dynamics in relation to the fluctuations of major environmental conditions.

MATERIALS AND METHODS

Study area

Two sites were selected from West Bengal, India for investigation, oneindustrial wastewater fed pond of Dankuni (22.671679N latitude and 88.300209E longitude-marble cutting-cum-chemical fertilizer storage industry), designated as Pond-Iand a pond used for domestic purpose and fish farming (Pond-II) situated at Serampore (22.8893N latitude and 88.38385 Elongitude).

Physico-chemical parameters analysis

Samplings were done at bi-weekly intervals for two years from July 2017 to August 2019. All data are summarized into 4 seasons viz. summer (March-May), monsoon (June-August), autumn (September-November) and winter (December-February). Sample water was collected in a 1L PVC bottle keeping immersed beneath the surface water from four different transects of the water body. The sample bottles were stored in a cool place and brought back to the laboratory for determination of nutrient concentrations. The water temperature (temp.) and transparency (transp.) were recorded in situ using a Celsius thermometer and a Secchi disc respectively. In the laboratory, pH was measured using an electronic pH meter. Different chemical parameters including nitrate (NO_3^-), dissolved inorganic phosphate (DIP), dissolved inorganic silicate (DIS) and ammoniumnitrogen (NH_4^+) were measured spectrophotometrically following the standard protocols of APHA (2017; 2018). Dissolved oxygen (DO) was measured using Winkler iodometrictitration method (Winkler, 1988). BOD was estimated as per the standard protocols of APHA (2017) and (2018).

Phytoplankton sample collection

From each site 1L of the water sample was collected at regular intervals from surface between 7:00 – 8:00 a.m. Brought to the laboratory centrifuged and concentrated to 5 mL. Slides were prepared and identified following Standard literatureof Desikachary (1959), Presscott (1964), Komarek and Fott (1983) and Algae Base (http://www.algaebase.org).

Total count of planktonic cyanobacteria and algae were counted by Sedgewick rafter cell counter following the equation:

Number/mL = C *1000 mm/(L*D*W*S)

Where, C = Number of organisms counted, L = Length of each strip (Sedgewick rafter cell length) mm, D = Depth of each strip (Sedgewick rafter cell length) mm, W=Width of each strip (Sedgewick rafter cell length) mm and S = Number of the strip counting.

Statistical analysis

The correlation matrix was prepared between chlorophyll content and otherphysico-chemical variables (pH, temp., transp.,DO, BOD, GPP, NPP, CRR, NO_3^-, NO_2^-, NH_4^+, PO_4, SiO_2). Moreover, Principal component analysis (PCA) was performed to envisage the possible relationships between biotic and abiotic variables and to group these variables based on similarity by using PAST (PAleontological STatistics) software (version 3.26) developed by researcher (Hammer *et al.*, 2001).

RESULTS

Diversity study of phytoplankton

The diversity study of phytoplankton was investigated throughout the study period at 15 days intervals, the population pattern of two sites during their peak growth (winter) have been represented here. The winter population revealed that the species belonging to Chlorophyceae (48%) had major contribution followed by other groups viz. Cyanophyceae (29%), Bacillariophyceae (11%), Euglenophyceae (9%) and Chrysophyceae (3%) in Pond-I (Fig 1a) while in Pond II, members of Cyanophyceae (48%) were found to be dominant followed by other groupslike Chlorophyceae (40%), Bacillariophyceae (7%) and Euglenophyceae (5%) (Fig. 1).

The Shannon Weiner's diversity indices were obtained for both Pond-I (H2 = 3.104) and Pond-II (H2 = 3.698) showing more value in Pond II,while opposite trends recorded for Simpson index values of Pond-I (SI = 0.0548) and Pond-II (SI = 0.0349) having larger value (2a and b). In case of Evenness index, higher value was obtained in Pond-I (2.028) compared to Pond-II (1.981) (Fig 2c). In pond I dominant genera recorded as *Kirchneriellalunaris* (Kirchner) Möbius, *Merismopedia minima*G. Beck, *Chlorococcumhumicola* (Nägeli) Rabenhorst, etc. during the study period whereas phytoplankton like *Synechococcuselongatus* Nägeli, *Aphanocapsa incerta* (Lemmermann) G. Cronberg and Komárek and *Anabaenopsisluzonensis*W. R. Taylor were dominating in Pond II. The major genera in Pond-I and Pond-II is depicted in Fig. 3.

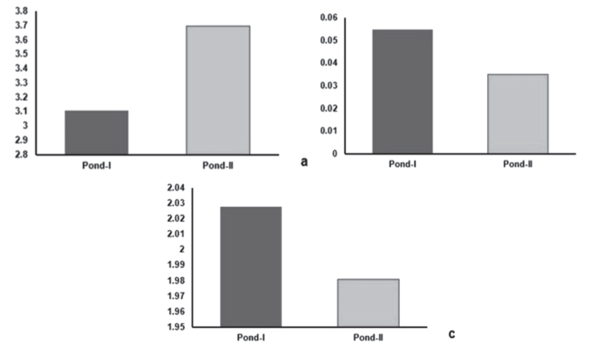

Fig. 2. Comparative study of different diversity indices at Pond-I and Pond-II during winter season (a = Shannon's diversity index, b = Simpson index and c = Evenness index)

The mean ± standard deviation (M ± SD) values of eachparameter of biotic and physicochemical parameters were recorded as two datasets at fifteen days intervals ineach month and seasonal datasets were prepared.

Seasonal variations of biotic parameters

Phytoplankton productivity was estimated in terms of total chlorophyll (Chl.) content and obtained highly significantly ($P<0.001$) lower value in Pond-II varying from 7.57 to 12.98 compared to Pond I having much higher value 25.39 to 39.24 mg/L, for all four seasons(Fig. 4a). Also highly significant ($P<0.001$) lower values for GPP and NPP were obtained from Pond-II (222.1 to 388.8 and 111.0 to 277.7 $mgC/m^3/hr$ respectively) compared to that of

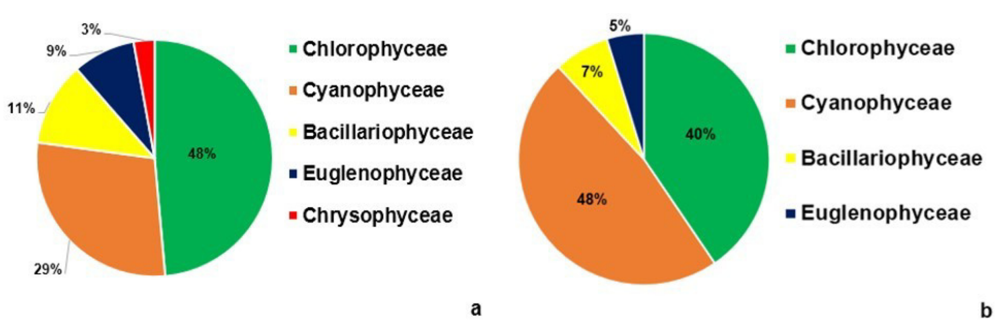

Fig. 1. Comparative study of different algal groups during winter season (a = Pond-I and b = Pond-II)

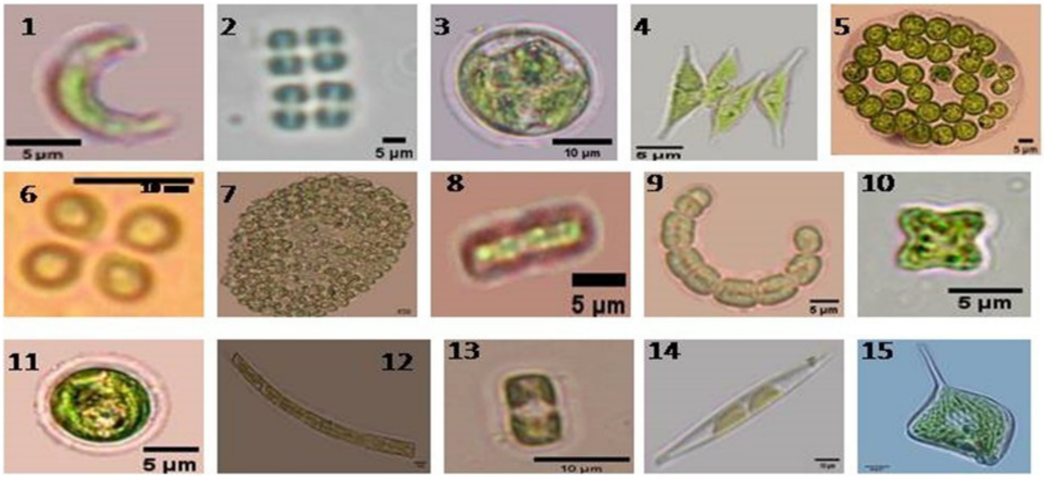

Fig. 3. Plate showing some of the major genera in Pond-I and Pond-II (1 = *Kirchneriella lunaris*, 2 = *Merismopedia minima*, 3 = *Chlorococcum humicola*, 4 = *Acutodesmus dimorphus*, 5 = *Eudorina elegans*, 6 = *Crucigenia quadrata*, 7 = *Aphanocapsa incerta*, 8 = *Synechococcus elogatus*, 9 = *Anabaenopsisluzonensis*, 10 = *Tetraedronmuticum*, 11 = *Cyclotella meneghiniana*, 12 = *Leptocylindrusdanicus*, 13 = *Thalassiosieraweissflogii*, 14 = *Nitzschia* sp. and 15 = *Phacuscaudatus*)

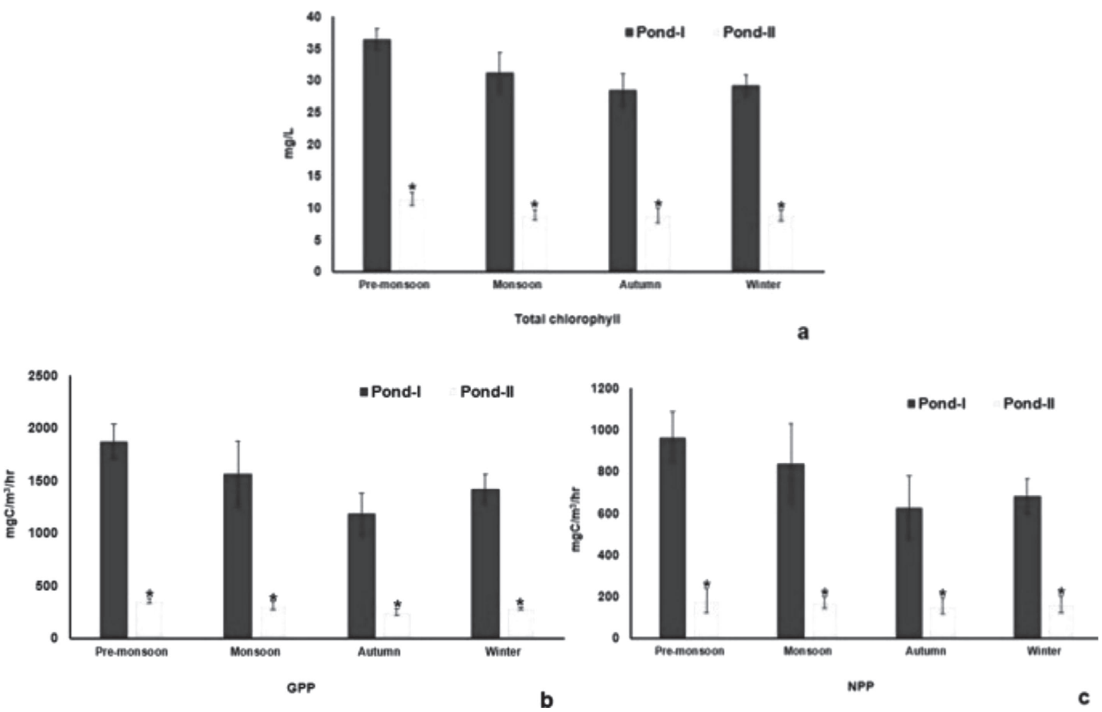

Fig. 4. Seasonal variations of biotic parameters in the study sites (a = total chlorophyll; b = GPP; c = NPP. *P<0.001)

Pond-I (916.6 to 2055.4 and 472.2 to 1111.08 mgC/ m^3/hr respectively), (Fig. 4b and c).

Seasonal variations of physico-chemical factors

Variations among four seasons for DO, BOD, pH, Temperature (Temp.) and Transparency (Transp.) of studied Pondsare represented in Fig. 5 (a-e). Among them highly significant (P<0.001 and P<0.01) higher value of DO (Fig. 5a) was recorded in Pond-I (4.8 to 18.7) compared to Pond-II (3.1 to 12.7), but BOD values were significantly higher (P<0.001 and P<0.01) in fishpond (Fig. 5b). Temperature variation was almost similar for both the ponds (Fig. 5c). Pond II was slightly alkaline in nature (Fig. 5d) andthe water transparency (Fig. 5e) were higher for all seasons in Pond-II (0.78 to 1.02) compared to Pond-I (0.62 to 0.96) (P<0.001 and P<0.01).

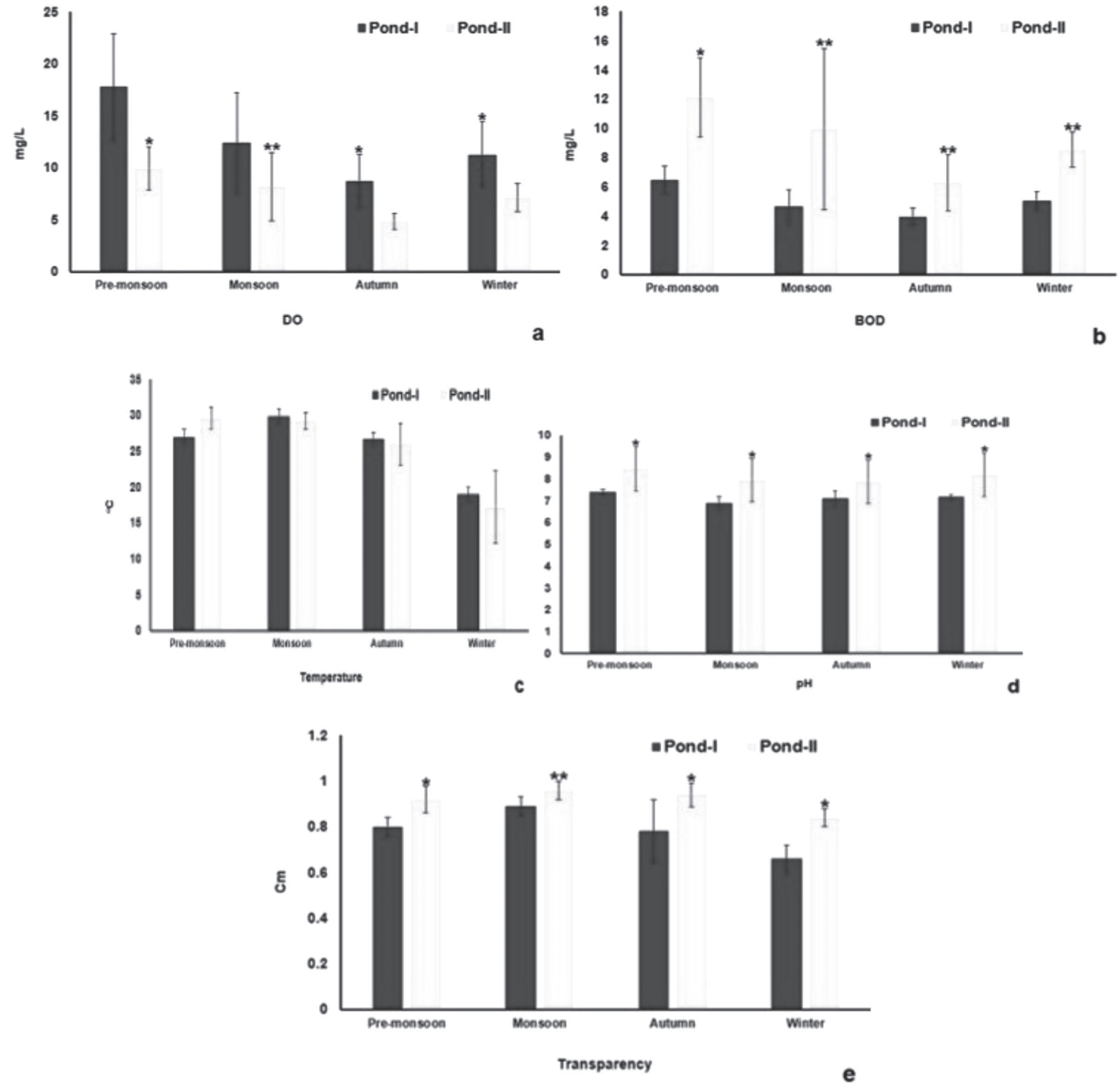

Fig. 5. Seasonal variations of physico-chemical parameters in the study sites (a = DO; b = BOD; c = Temp., d = pH, e = Tansp.*P<0.001, **P<0.01)

Seasonal variations of nutrient parameters

Industrial waste fed pond i.e. Pond I was nutritionally rich showing higher levels of nitrate(2.9 to 30.72mg/L), ammonium nitrogen (0.2 to 8.6 mg/L) andphosphate (0.34 to 1.89 mg/L) compared to those of pond II showing the range of values of 0.6 to 10.1mg/L,0.01 to 0.35mg/L and0.005 to 0.110mg/L respectively(Fig 6a-c). Due to mixing of regular marble cutting waste the silicate level is significantly higher in Pond 1 showing maximum value in summer (23.0 to 46.8 mg/L) (6d).

Correlation study between chlorophyll and abiotic factors

The correlation matrix (Table 1) as per R^2 values revealed for Pond-I that Chl. had significant positive correlation with temp. ($R^2 = 0.21$; $P<0.001$), BOD ($R^2 = 0.83$; $P<0.001$), DO ($R^2 = 0.84$; $P<0.001$), nitrate ($R^2 = 0.65$, $P<0.001$) and phosphate level ($R^2 = 0.81$, $P<0.001$) while in Pond-II, it was observed that Chl. had significant positive correlation with temp. ($R^2 = 0.32$, $P<0.001$), and pH ($R^2 = 0.46$, $P<0.05$).

The Principal Component Analysis (PCA)showed the possible relationships between productivity

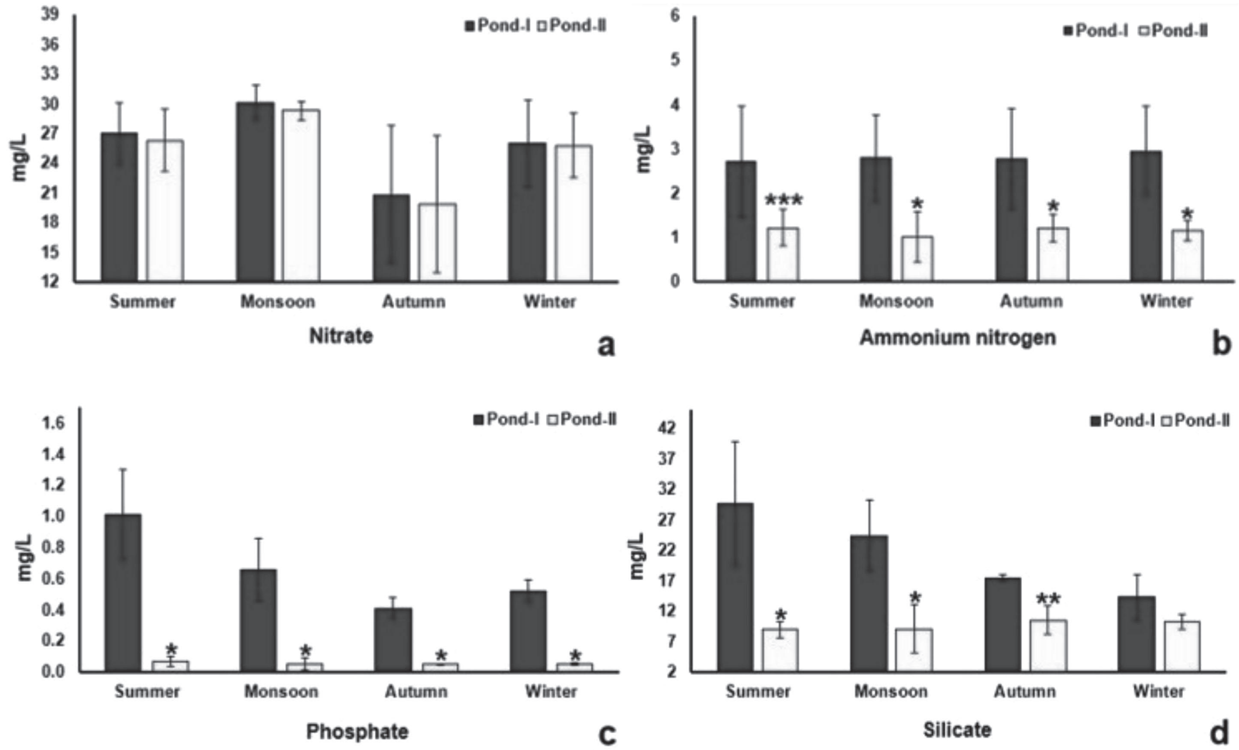

Fig. 6. Seasonal variations of nutrient parameters in the study sites (a = nitrate; b =ammonium nitrogen; c = phosphate; d = silicate. *P<0.001; **P<0.01; ***P<0.05)

Table 1. Correlation matrix for Chlorophyll content versus abiotic environmental variables in studied sites

Name	Total Chl	Temp.	pH	Transp.	Ammonium nitrogen	BOD	DO	Nitrate	Phosphate	Silicate
					Pond-II					
Total Chl	1									
Temp.	0.320509[a]	1								
pH	0.468865[a]	-0.1701	1							
Transp.	0.215929	0.708389	-0.40724	1						
Ammonium nitrogen	0.075196	0.298181	-0.098	0.116198	1					
BOD	0.04994	0.239767	-0.06907	0.308942	0.291069	1				
DO	0.066434	0.117799	0.124151	0.244614	0.088952	0.880758	1			
Nitrate	0.07653[b]	0.434546	0.49119	0.230307	0.106712	0.510775	0.553416	1		
Phosphate	0.066720	0.172981	0.282204	0.130003	0.054863	0.673445	0.828154	0.568975	1	
Silicate	0.016042	0.399869	-0.1076	0.410438	0.121025	0.707162	0.77066	0.545154	0.803931	1
					Pond-I					
Total Chl	1									
Temp.	0.40957[a]	1								
pH	-0.0846	-0.27575	1							
Transp.	-0.25104	0.606778	0.222778	1						
Ammonium nitrogen	0.071593	0.263786	-0.14782	0.130674	1					
BOD	0.83261[a]	0.362369	-0.13276	0.035271	0.345343	1				
DO	0.84079[a]	0.094628	-0.02221	-0.10794	0.206241	0.899231[a]	1			
Nitrate	0.65864[a]	0.348016	-0.27402	-0.12225	0.333121	0.550308	0.432133[a]	1		
Phosphate	0.81884[a]	0.201182	-0.02931	-0.01973	0.192505	0.819919[a]	0.794838	0.432666	1	
Silicate	0.077075	0.040698	0.099541	-0.00349	0.232337	0.78239[a]	0.865738[a]	0.401235[a]	0.905165[a]	1

[a]P<0.001,[b]P<0.05; n = 26

(total chlorophyll) and abiotic variables and group these variables based on similarity during winter (most productive) for Pond-I (Fig. 7) and Pond-II (Fig. 8) separately. PCA amongbiotic and environmental variables extracted four significantfactors (eigenvalues greater than 1). PC1 and PC2 jointly contributed to 88.48% of the variations within the data at Pond-I while 80.06% of the variations within the data at Pond-II.For Pond-I (Fig. 7), positive loading wasfound in which higher values of temperature, BOD, DO, nitrate and phosphate considering the controlling factors; while for Pond-II (Fig. 8) positive loading was found higher for temperature and pHwith respect to Chl., whichsignifies these two factors are the controlling parameters in phytoplankton dynamics.

Furthermore, the PCA showed the possible relationships between productivity (total chlorophyll) and abiotic variables and group these variables based on similarity during monsoon i.e. least productive for Pond-I (Fig. 9) and Pond-II (Fig. 10) separately. PCA among biotic and environmental variables extracted four significant factors (eigenvalues greater than 1). PC1 and PC2 jointly contributed to 92.38% of the variations within the data at Pond-I while 95.78% of the variations within the data at Pond-II. For Pond-I (Fig. 9), also found positive loading in which higher value of temperature, BOD, DO, nitrate and phosphate and are the controlling factors; while for Pond-II (Fig. 10) with respect to Chl., it was found positive loading in which higher values of temperature and pH with respect to Chl. were obtained, which signifies these two factors are the controlling parameters in phytoplankton dynamics.

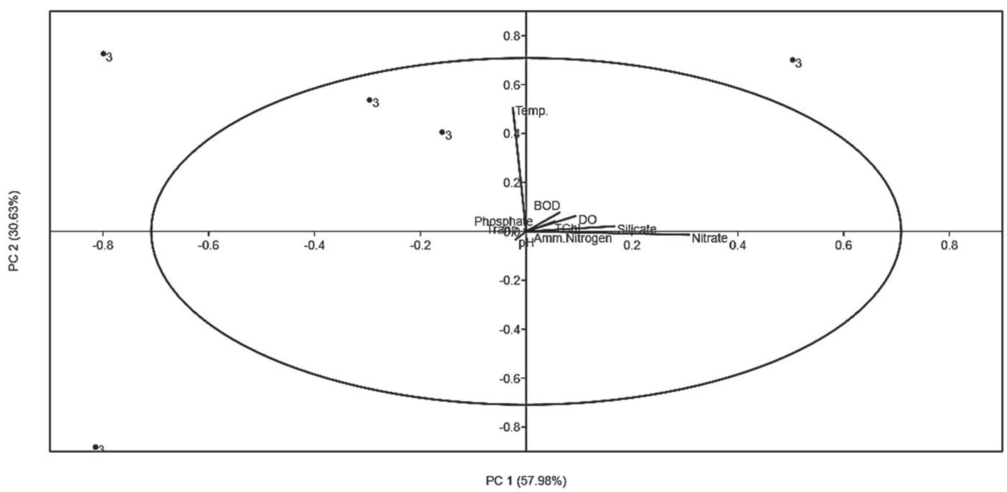

Fig. 7. Principal component analysis (PCA) for PC1 vs. PC2 of Pond-I for winter

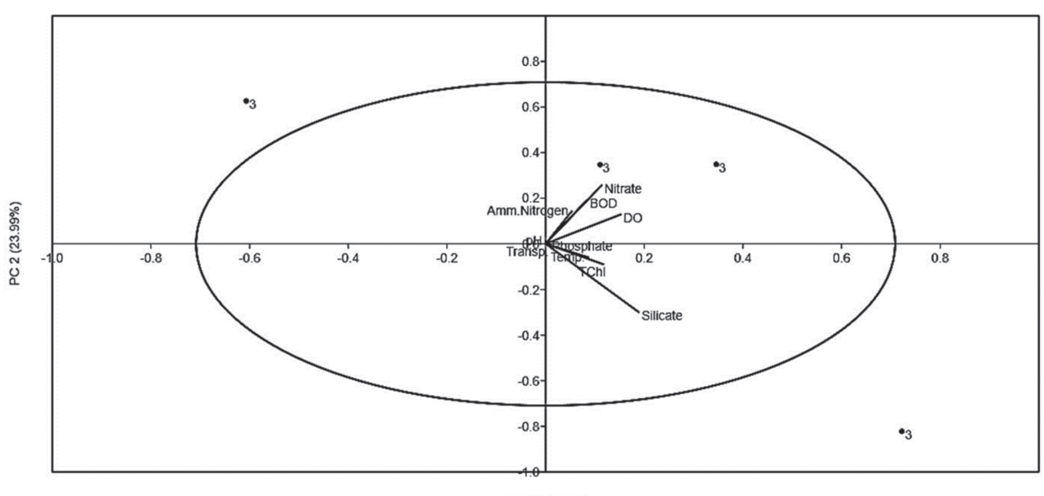

Fig. 8. Principal component analysis (PCA) for PC1 vs. PC2 of Pond-II for winter

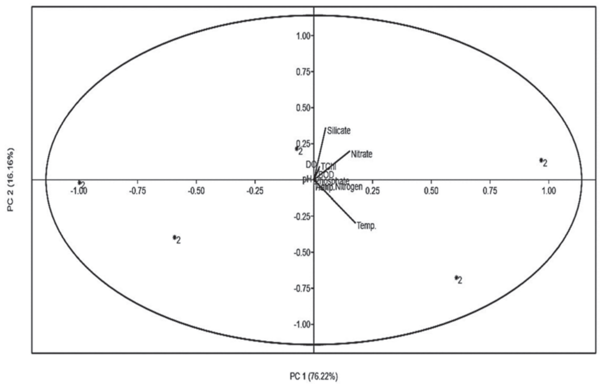

Fig. 9. Principal component analysis (PCA) for PC1 vs. PC2 of Pond-I for monsoon

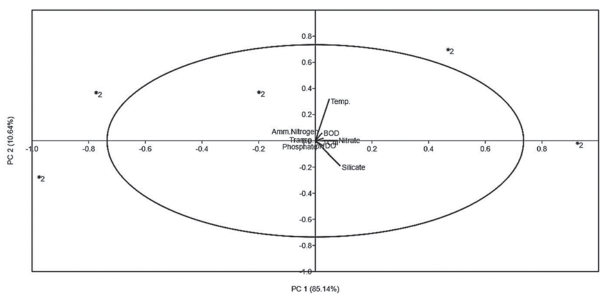

Fig. 10. Principal component analysis (PCA) for PC1 vs. PC2 of Pond-II for monsoon

DISCUSSION

The seasonal changes of solar irradiance, precipitation and biotic factors like, life cycle pattern, grazing effect and overall nutrient concentrations regulate the phytoplankton growth and population pattern, therefore the phytoplankton dynamics of a particular ecological niche. Other analyses retrieved temperature as the main driver for seasonal changes in freshwater eutrophic ecosystem (Singha Roy *et al.*, 2018). Temperature and salinity driven population dynamics have reported from coastal waters of tropical regions (Choudhury and Pal, 2011).

The present investigation revealed the dominance of members of Chlorophycae in Wastewater pond and Cyanophyce in domestic fish pond during winter season. The comparative study also showed less phytoplankton diversity in wastewater pond compared to fishpondas indicated by Shannon's diversity index, while species richness was high resulting in bloom formation of *Kirchneriella lunaris* of Chlorophyceae in wastewater pond in winter. A similar observation was found in earlier study that diversity of phytoplankton was higher during post-monsoon to winter period in the surface water of coastal zone (Hangovan, 1987; De *et al.*, 1994; Choudhury and Pal, 2010). A decreasing tendency of diversity indicates shifting of phytoplankton community from high species richness to bloom formationas reported from different ecological niches (Jacobsen and Simonsen, 1993; Padisak, 1993; Calijuriand Santos, 1996; Kyong andJoo, 1998).

Seasonal fluctuation of phytoplankton biomass appeared to be highly influenced by the nutrient regime as wellas environmental conditions (Singha Roy *et al.*, 2018). In the present investigation the higher pH level of domestic fishpond for using detergent resulted in cyanophycean members to flourish, whereas green algal dominance was recorded in wastewater fed pond with lower pH value. The water transparency was lower in wastewater due to biomass-induced opacity and other suspended particles compared to fishpond and the values were reduced during monsoon period due to excess precipitation. Though more pH showed a tendency towards alkalinity and higher values of nutrient resulted in bloom formation in some cases (WHO, 1992).The present result of transparency data is supported by earlier study also (Dembowska *et al.*, 2018). Besides nitrate and phosphate, silicate also play an important role to increase phytoplankton population in freshwater (Singha Roy *et al.*, 2018). The present study also revealed higher values of silicate throughout the seasons with some increase in diatom population. However, higher level of nutrient parameters may lead to organic water pollution playing an important role for the growth of phytoplankton as observed in Pond-I supported by other researchers also (Lehr *et al.*, 1980; Dzialowski *et al.*, 2005; Singha Roy *et al.*, 2018).

The correlation matrix analysis showed that chlorophyll content had significant positive correlation with temperature, BOD, DO, nitrate and phosphate in study sites and also with temperature and pH level. The surface water eutrophication is identified as the main factor inducing the formation of algal blooms due to the influence of nitrogen, pH and temperature (Rybak and Gabka, 2018).

According to Simeonov *et al.* (2003), PCA is useful tool to determine the variance of a large data set of intercorrelated variables with a smaller set of independent variables. Based on the highly correlated values of the parameter as evident by the PCA, the physico-chemical and nutrient parameters in relation to productivity parameters could be used effectively and efficiently for enhancing the growth of the phytoplankton (Gnanamoorthy *et al.*, 2013). Interestingly, in present case for wastewater fed pondpositive loading was found in which higher values of temperature, BOD, DO, nitrate and phosphate indicated as the controlling factors while for domestic pond temperature and pH were recorded as regulatory parameters in phytoplankton dynamics. In earlier study Singha Roy *et al.* (2018) reported that biomass productivity had correlation with water temperature as evident in our results also for the Pond used for domestic purposes. The PCA plot of monsoon period also indicated same physico-chemical and nutrient parameters controlling the productivity but the values were found comparatively lesser from that of winter season due to higher precipitation followed by dilution factor. No bloom formation was recorded during monsoon period. Phytoplankton productivity decreased and bloom formation hampered due to heavy rainfall as also reported by Affan *et al.*, (2005).

In the present study, significantly higher values of ammonium nitrogen, nitrate, phosphate and silicate as well as GPP were observed in wastewater pond, therefore indicating its high eutrophic condition and winter bloom formation. Comparatively the domestic pond showed moderate nutrient level and found suitable for fish production.

CONCLUSIONS

Elevated nutrient levels mainly nitrate, and phosphate contributes to the growth of phytoplankton resulting in high levels of DO, Chl., GPP and NPP. From the present study, it was found that major controlling factors are temperature, transparency, BOD, DO, nitrate and phosphate in Eutrophic pond while only temperature and pH play important role in mesotrophic pond of tropical country.

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