Bangladesh J. Bot. **46**(1): 43-51, 2017 (March)

BLOOM FORMING PHYTOPLANKTON AND THEIR COMPARATIVE LIMNOLOGY IN WASTEWATER LAGOONS OF BANGLADESH

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Key words: Bloom forming phytoplankton, Limnology, Wastewater lagoons, Bangladesh

Abstract

Fifteen phytoplankton, namely *Arthrospira platensis* Gomont, *Merismopedia glauca* (Ehrenb.) Näg., *Microcystis aeruginosa* Kützing, *Synechocystis salina* Wislouch, *Chlorella vulgaris* Beyerinck, *Coelastrum microporum* Nägelli, *Crucigenia quadrata* Morren, *Euglena* sp., *Euglena acus* (Müller) Ehrenberg, *Trachelomonas volvocina* Ehrenberg, *Phacus acuminatus* var. *granulatus* (Roll) Huber-Pest., *Gomphonema pervulum* (Kützing) H.F. Van Heurck, *Cyclotella comensis* Grunow in Van Heurck, *Cryptomonas erosa* var. *reflexa* M. Marsson and *Rhodomonas lens* Pascher *et* Ruttner have been considered as bloom forming species in the wastewater lagoons of Bangladesh during the sampling period of October, 2009 to July, 2010. Comparative limnological analysis was done in association with different physicochemical variables from the two lagoons. Cluster analysis separated the bloom forming phytoplankton into four groups and SIMPER analysis showed *M. aeruginosa*, *Mer. glauca*, *Euglena* sp. and *A. platensis* were responsible for each of the groupings. Result of PCA and regression analysis showed significant correlation of air temperature, water temperature, NO_3-N and SRP with *A. platensis*, *Mer. glauca* and *Eulgena* sp.

Introduction

Algal blooms occur in aquatic habitats due to the enhancement of nutrients. Limnologists are concerned about blooms of algae in ponds, reservoirs, lagoons, lakes, creeks, streams and rivers because their existence can have ecological, economical, recreational and human health impacts. Mainly, different pigmented species of phytoplankton cause bloom and discoloration of water. Densities of bloom forming phytoplankton may vary from 10×10^6 - 145×10^6 ind/l (Islam and Khondker 1994, Maestrini and Granéli 1991, Reynolds 1972). Blue-green algae often bloom in the lagoons and *Arthrospira platensis* was the most dominant species. Besides, other common blue-green algae in waste treatment lagoons include *Microcystis aerugionosa, M. marginata, Merismopedia glauca* and *Synechocystis aquatilis.* Species of green algae, such as *Chlorella vulgaris, Crucigenia crucifera, Coelastrum microporum* and *Scenedesmus* sp. also form blooms (Onyema 2013, Muthukumar *et al*. 2007).

In Bangladesh, Islam and Morshed (1985) studied the bloom forming algae of the estuarine habitat. Related to the blooms of wastewater lagoons, few studies are available assessing the relationship between bloom forming phytoplankton and nutrient concentrations (Oudra 1990, Pereira *et al*. 2001, Vasconcelos and Pereira 2001, Chindah *et al*. 2007, Onyema 2010, Gani *et al*. 2011, Badr and Moghazy 2013). In sewage lagoons, phytoplankton bloom is desirable as they generate oxygen needed by bacteria for waste stabilization. There was a shift in the algal species present in the lagoons through the seasons, caused by different physicochemical parameters as well as nutrients upload (Gani *et al*. 2011). The present study is conducted at the two lagoons of Pagla Sewage Treatment Plant (PSTP) in Narayanganj, Bangladesh to determine the relationship of bloom forming phytoplankton with other limnological factors.

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Materials and Methods

Samples for the present investigation were collected from the two lagoons (L1 and L2) of PSTP situated near Pagla Bazar in Naraynaganj, Bangladesh. The sampled lagoons L1 (lagoon 1) and L2 (lagoon 10) coordinated by 23°40'54.32" N 90°27'15.02"E and 23°40'42.69"N 90°27'5.53"E, respectively. Details about the study site and sampling procedure were described in Gani *et al*. (2011). Counting of phytoplankton was done with the help of a HBCC (Helber bacterial counting chamber) under a compound microscope (Nikon SE, Japan) at a magnification of ×400. Each sample was counted three times. Different limnological parameters such as air and water temperatures, Secchi depth, TDS (total dissolved solids), conductivity, pH, alkalinity, dissolved oxygen (DO), soluble reactive silicate (SRS), soluble reactive phosphate (SRP) and NO_3-N were determined (Gani *et al*. 2011). In the present investigation, species in a population contributed >10% of the total phytoplankton density were considered as bloom forming.

To perform statistical analyses all data were transformed log (x+1) except for pH, air and water temperatures which were arsine standardized. The hierarchical clustering analysis (Primer v6: Clarke and Gorley 2001), based on Bray-Curtis index was applied to the species of bloom forming phytoplankton for grouping them. Similarity percentage analysis (SIMPER analysis) (Primer v6: Clarke and Gorley 2001) was used to distinguish the phytoplankton species contributing to similarity and dissimilarity between the groups. Principal component analysis (PCA) (Primer v6: Clarke and Gorley 2006) was applied to the different limnological variables to find out significant variables with respect to different sampling periods. Later on, regression analysis (Statistica v8) was performed between bloom forming phytoplankton and PC scores (resulted from PCA) to find out the relationship between individual phytoplankton species and significant limnological variables.

Results and Discussion

In the present investigation, fifteen bloom forming phytoplankton were recorded from the lagoons (L1 and L2) of PSTP. The algal species belonged to five major groups and based on their chlorophyll type, these are categorized as blue-green (Cyanophyceae), green (Chlorophyceae), yellow-brown (Bacillariophyceae), euglenooids (Euglenophyceae) and cryptmonads algae (Cryptophyceae). The class Cyanophyceae and Euglenophyceae consisted of four bloom forming species each, where Bacillariophyceae and Cryptophyceae consisted of two species each and rest three species belonged to Chlorophyceae. In El-Sadat wastewater treatment plant (WWTP) of Egypt, phytoplankton, in general, belonged to Chlorophyta, Euglenophyta, Cyanobacteria and Bacillariophyta (Badr and Moghazy 2013). Oudra (1990) reported cyanobacteria dominated blooms in some wastewater treatment plants (WWTP) of Morocco.

The occurrence of cyanophycean bloom in freshwater consists of both toxin and non toxin producing species (Baker and Humpage 1994). *Arthrospira. platensis* was the most frequent bloom forming species found during five sampling periods in L2 and three sampling periods in L1 while *Microcystis. aeruginosa* was the most dominant bloom forming species contributing the abundance of 99% to the total. This finding was similar to observation of Pereira *et al*. (2001) where *M. aeruginosa* was one of the dominant cyanobacteria in WWTP of Northern Portugal. *M. aeruginosa* considered as toxic cyanobacteria (Hoek *et al*. 1995), occurred in L2 of the present investigation in summer (Table 1). Several studies reveal that *Microcystis* spp. rapidly grow in the water column at the end of spring and continue dominating during summer period (Vasconcelos and Pereira 2001, Reynolds 2006). *A. platensis* a non toxic and edible species populates tropical and subtropical water bodies with high pH (Nyabuto *et al.* 2015). During the present investigation bloom forming incident of the species was observed several times in L1 and L2 within the range of water temperature 23.0 - 31.5 ^{o}C and pH 7.68 - 8.94. During these periods, other limnological variables such as Secchi depth (6.0-23.6 cm), alkalinity (5.05 - 9.10 meq/l), DO (3.54 - 11.50 mg/l), SRP (723.78 -3490.05 µg/l) and SRS (28.19 - 169.48 mg/l) showed wide range of variation. Mean values of conductivity (L1 = 923 and L-2 = 610.6 µS/cm), alkalinity (L1 = 8.10 and L2 = 6.01 meq/l), DO (L1 = 4.9 and L2 = 6.9 mg/l) and SRS (L1 = 37.66 and L2 = 118.49 mg/l) differed greatly in L1 to L2.

Merismopedia. glauca formed bloom during three sampling periods in L2 because in lagoon L2 sedimentation process was not so frequent like L1 supporting the condition as recorded in Izmit Bay (Aktan and Aykulu 2003). During these periods the concentration of NO_3-N (mean value 7.35 mg/l) was the highest in comparison to other Cyanophyceae and relatively low concentration of SRS (mean value 17.06 mg/l) and SRP (mean value 693.47) and high value of Secchi depth (mean value 30.17 cm) was observed. *Synechocystis* was common in some wastewater treatment plants (Oudra 1990). In the present study, *Synechocystis salina* prevailed at lowest value of DO (3.19 mg/l). However, *Chlorella vulgaris* dominated at low DO and relatively high SRS concentration in L1. It was evident that both the species are able to survive under high pollutant load (El-Kassas and Sallam 2014). Among the two other green algae *Crucigenia quadrata* and *Coelastrum microporum* recorded from L2, the later species contributed about 51% to the total abundance.

Among euglenoids, *Euglena* sp. showed four blooms in L2 and one in L1. Except one sampling (Feb B-L2), euglenoid bloom contributed greater than 50% to the total abundance. In the facultative pond of Esmoriz wastewater treatment plant, euglenoids accounted 96% of total phytoplankton density where *Euglena* sp. was dominant (Periera *et al*. 2001). In case of L1 the relative abundance was about 79% and physicochemical data were characterized by higher value of air temperature (L1 = 25.00 and L2 = 19.13 ^{o}C), water temperature (L1 = 22.00 and L2 = 21.38 ^{o}C), Secchi depth (L1 = 34.0 and L2 = 21.5 cm), TDS (L1 = 477.0 and L2 = 408.4 mg/l), conductivity (L1 = 865.0 and L2 = 730.8 µS/cm), alkalinity (L1=7.30 and L2=6.67 meq/l), DO (L1 = 6.30 and L2 = 5.69 mg/l) and SRP (L1 = 2223.33 and L2 = 800.73 µg/l) than the mean value of L2. Other three euglenoids were *Euglena acus* and *Phacus acuminatus* var. *granulatus* recorded from L1 and *Trachelomonas volvocina* recorded form L2. The relative abundance of *E. acus* was about 55% to the total and it was occurred completely anoxic condition (DO=0). Comparatively lower value of Secchi depth (14 cm) and higher value of TDS (591 mg/l) and conductivity (1137 µS/cm) were observed during the euglenoid blooms.

The class Bacillariophyceae, which was another common group in other wastewater lagoons (Oudra 1990, Periera *et al*. 2001 and Badr and Moghazy 2013) represented by *Gomphonema pervulum* and *Cyclotella comensis* contributed greater than 50% abundance to the total and occurred in L1 and L2, respectively. Comparatively high concentration of NO_3-N was accounted during this period and *G. pervulum* was recorded during acidic condition (pH< 7). The last bloom forming phytoplankton group was cryptomonad type where *Cryptomomas erosa* var. *reflexa* and *Rhodomonas lens* were recorded from L1 and characterized by higher concentration of SRP than green and yellow-brown pigmented algae (Table 1).

Cluster analysis performed by Primer was separated the samplings in four groups (Fig. 1). Group A consisted of 2 samples collected in July A and July B from L2 and during these periods, only bloom of *M. aeruginosa* was recorded, Group C consisted of 3 samples collected from L2, during these periods *Mer. glauca* was prominent bloom forming species, Group D consisted of 5 samples collected from L2, during these periods *Euglena* sp. as well as *C. quadrata* and *T. volvocina* was recorded and Group B consisted of 8 samples collected from L1 and L2. Results of SIMPER analysis (Table 2) indicated that *M. aeruginosa*, *Mer. glauca*, *Euglena* sp. and *A. platensis* were the species responsible for groupings of A, C, D and B, respectively.

Table 1. List of bloom forming phytoplankton along with their abundance, contribution (%) and other limnological variables obtained in different sampling periods between October, 2009 and July, 2010 from the lagoons of Pagla sewage treatment plant (PSTP).

Phytoplankton species	Class	Sampling period	Abund. ($\times 10^6$ ind/l)	% of bloom forming species	Air temp. (°C)	Water Temp (°C)	Secchi depth (cm)	TDS (mg/l)	Cond. (µS/cm)	pH	Alka. (meq/l)	DO (mg/l)	SRP (µg/l)	SRS (mg/l)	NO_3N (mg/l)
Arthrospira platensis Gomont	Cyanophyceae	Jun B-L1	10.24	58.99	33	31.5	6	469	960	7.68	8.5	4.57	3490.05	40.99	2.07
		Jul A- L1	10.95	50	29	29	13	472	1022	7.7	9.1	3.54	3257.39	42.8	0.23
		Oct B-L1	13.13	73.68	27	23	28.2	421	787	7.93	6.7	6.61	723.78	29.18	0.23
		Mean-L1	**11.44**	**60.89**	**29.7**	**27.8**	**15.7**	**454**	**923**	**7.77**	**8.1**	**4.9**	**2490.41**	**37.66**	**0.89**
		Std-L1	**1.51**	**11.95**	**3.06**	**4.37**	**11.35**	**28.2**	**121.8**	**0.14**	**1.25**	**1.56**	**1534.36**	**7.40**	**1.06**
		Mar A-L2	21.76	42.95	27	26.5	23.5	370	625	8.49	8.49	6.25	1609.45	28.19	0.48
		Mar B-L2	279.39	95.26	32	26	6	299	592	8.94	6.6	6.1	2067.89	169.48	1.83
		Apr B-L2	198.45	86.51	34	30.5	16	336	666	8.33	5.75	11.5	2377.16	331	0.41
		May A-L2	486.33	73.36	32	31	13	299	592	8.94	5.05	3.94	917.41	35.08	0
		Jun B-L2	37.27	82.77	32	29	18	272	578	8.25	4.15	6.71	3488.25	28.69	0.47
		Mean-L2	**204.64**	**76.17**	**31.40**	**28.60**	**15.30**	**315.2**	**610.6**	**8.59**	**6.01**	**6.90**	**2092.03**	**118.49**	**0.64**
		Std-L2	**191.33**	**20.16**	**2.61**	**2.27**	**6.46**	**38.2**	**35.47**	**0.33**	**1.65**	**2.78**	**954.45**	**133.17**	**0.70**
Euglena sp. Ehrenberg	Euglenophyceae	Feb B-L1	26	78.52	25	22	34	477	865	7.3	7.3	6.3	2223.33	26.21	18.23
		Jan A-L2	169.5	64.83	16	20	20	435	776	7.4	6.4	2.24	912.35	29.92	0
		Feb B-L2	327.75	20.82	22	24	28	423	775	8.5	7.5	8.54	1686.28	31.09	9.02
		Dec A-L2	166	53.06	23	22.5	19	376	669	7.29	6.1	4.88	71.28	28.84	0.59
		Dec B-L2	99	52.38	15.5	19	19	400	703	7.53	6.67	5.69	533.02	21.01	0.68
		Mean-L2	**190.56**	**47.77**	**19.13**	**21.38**	**21.50**	**408.5**	**730.8**	**7.68**	**6.67**	**5.34**	**800.73**	**27.72**	**2.57**
		Std-L2	**97.04**	**18.86**	**3.92**	**2.29**	**4.36**	**26.08**	**53.51**	**0.56**	**0.60**	**2.59**	**683.23**	**4.56**	**4.31**
Microcystis aeruginosa Kützing	Cyanophyceae	Jun B-L2	6.78	15.06	32	29	18	272	578	8.25	4.15	6.71	3488.25	28.69	0.47
		Jul A-L2	7.28	90.21	30	29	19	255	543	8.8	4.05	5.16	3242.57	28.27	0.22
		Jul B-L2	27.72	99	30	29	22	523	510	9	4.3	6.79	3536.31	28.79	0.34
		Mean-L2	**13.93**	**68.09**	**30.67**	**29**	**19.67**	**350.**	**543.7**	**8.68**	**4.17**	**6.22**	**3422.38**	**28.58**	**0.34**
		Std-L2	**11.95**	**46.14**	**1.15**	**0**	**2.08**	**150.1**	**34**	**0.39**	**0.13**	**0.92**	**157.56**	**0.28**	**0.13**
Merismopedia glauca (Ehrenb.) Näg.		Oct B-L2	60.79	52.91	30	25	31.5	317	585	7.82	4.4	9.5	656.63	20.65	3.66
		Nov A-L2	241.15	70.67	33	28	33	273	512	7.33	3.57	8.94	114.76	20.23	9.41
		Nov B-L2	153.06	37.68	19	22.5	26	336	625	7.63	5.27	7.15	1309.01	10.29	8.97
		Mean-L2	**151.67**	**55.09**	**27.33**	**25.17**	**30.17**	**308.7**	**574**	**7.59**	**4.41**	**8.53**	**693.47**	**17.06**	**7.35**
		Std-L2	**90.19**	**14.62**	**7.37**	**2.75**	**3.69**	**32.32**	**57.30**	**0.25**	**0.85**	**1.23**	**597.98**	**5.86**	**3.20**
Cyclotella comensis Grunow in Van Heurck	Bacillariophyceae	Oct B-L2	35.99	31.32	30	25	31.5	317	585	7.82	4.4	9.5	656.63	20.65	3.66
		Nov B-L2	160.85	63.79	19	22.5	26	336	625	7.63	5.27	7.15	1309.01	10.29	8.97
		Mean-L2	**98.42**	**52.56**	**24.50**	**23.75**	**28.75**	**326.5**	**605**	**7.73**	**4.84**	**8.33**	**982.82**	**15.47**	**6.32**
		Std-L2	**88.29**	**30.03**	**7.78**	**1.77**	**3.89**	**13.44**	**28.28**	**0.13**	**0.62**	**1.66**	**461.30**	**7.33**	**3.75**

(Contd.)

Table 1 (Contd.)

Phytoplankton species	Class	Sampling period	Abund. (×10^6 ind/l)	% of bloom forming species	Air temp. (°C)	Water temp. (°C)	Secchi depth (cm)	TDS (mg/l)	Cond. (µS/cm)	pH	Alka. (meq/l)	DO (mg/l)	SRP (µg/l)	SRS (mg/l)	NO_3N (mg/l)
Synechocystis salina Wislouch	Cyanophyceae	Apr B-L1	42.05	20.5	34	31	16	674	1252	7.62	13.9	3.13	2377.16	335.01	0.42
Chlorella vulgaris Beyerinck	Chlorophyceae		40	19.5											
Crucigenia quadrata Morren		Oct B-L2	14.64	12.74	30	25	31.5	317	585	7.82	4.4	9.5	656.63	20.65	3.66
Coelastrum microporum Nägelli		Feb B-L2	798	50.70	22	24	28	423	775	8.5	7.5	8.54	1686.28	31.09	9.02
Euglena acus (Müller) Ehrenberg	Euglenophyceae	May A-L1	53.55	54.31	33	31	14	591	1137	7.65	11.8	0	917.41	49.503	0.94
Phacus acuminatus var. *granulatus* (Roll) Huber-Pest.			14.45	14.66											
Trachelomonas volvocina Ehrenberg		Dec A-L2	11.41	35.70	23	22.5	19	376	669	7.29	6.1	4.88	71.28	28.84	0.59
Cryptomonas erosa var. *reflexa* M. Marsson	Cryptophyceae	Feb A-L2	10.44	25.97	21	20	43	416	754	7.71	7	6.91	1806.54	32.87	0.63
Rhodomonas lens Pascher *et* Ruttner			9.28	23.08											
Gomphonema pervulum (Kützing) H.F. Van Heurck	Bacillariophyceae	Jan A-L1	8.06	85.93	16.5	17.5	20	856	1473	6.78	15.8	2.56	1342.27	31.2	7.57

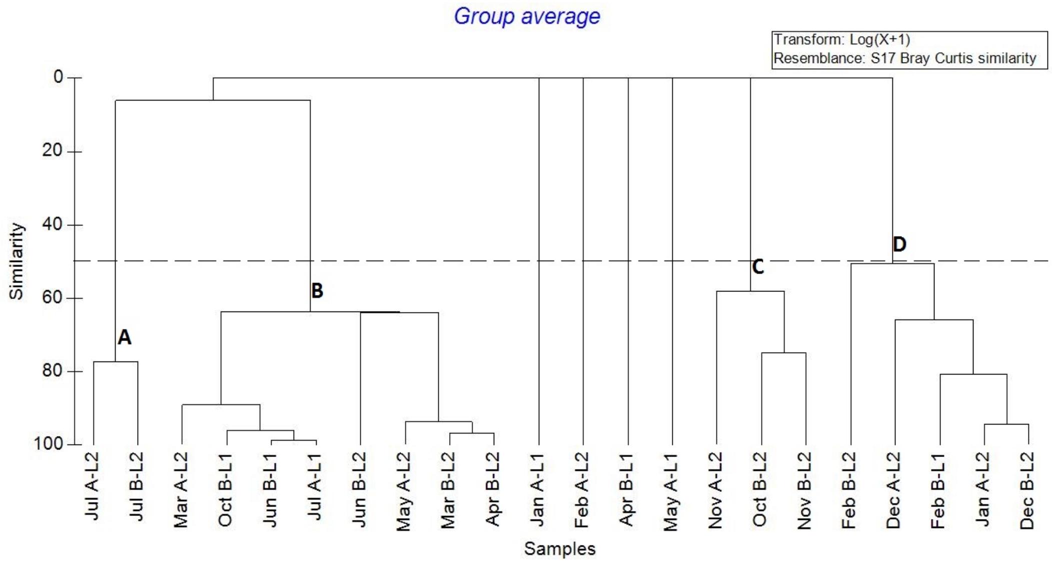

Fig. 1. Bray-Curtis similarity dendrogram of the bloom forming phytoplankton samples of PSTP found during sampling period of October 2010 to July 2011. (Samples of Jan A-L1, Feb A-L2, Apr B-L1 and May A-L1 were cut off for low contributions: <50%).

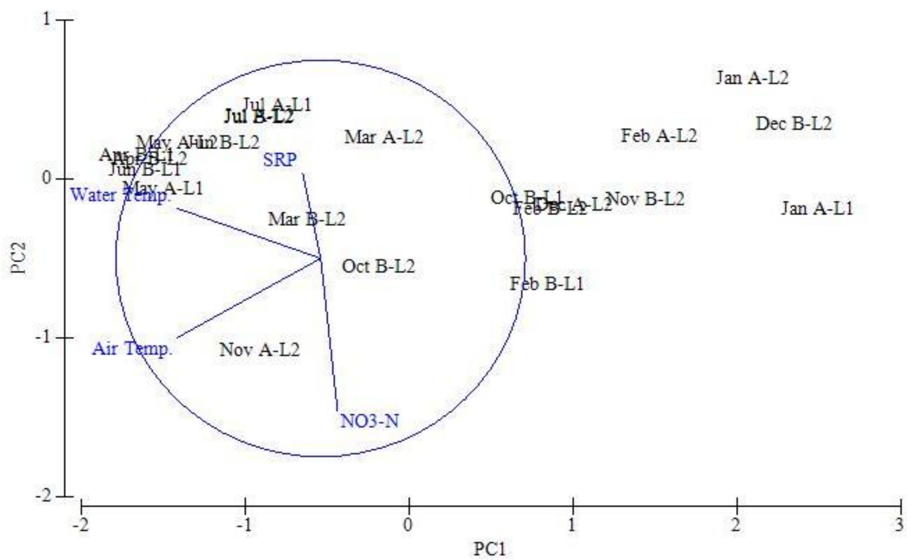

Fig. 2. Principal component analysis (PCA) plot of the first two PC axes on the transformed environmental data (Air temp. = Air temperature, Water temp. = Water temperature, SRP and NO_3-N).

PCA analysis resulted three statistically significant (eigenvalues >1.0) principal components (PCs). The first two PCs explained 91.3% of the total variance (Fig. 2). PC1 correlated negatively to air temperature (r = -0.700) and water temperature (r = -0.704). PC2 correlated negatively with NO_3-N (r = -0.768) and positively with SRP (r =0.432). Regression analysis (Fig. 3) between PC1,

Table 2. Results of SIMPER analysis of the samples of bloom forming phytoplankton from PSTP during the study period (October 2010-July 2011).

Group D - Average similarity: 65.62			
Species	Av. abund	Contrib%	
Euglena sp.	4.79	100.00	
Group B - Average similarity: 73.35			
Species	Av. abund	Contrib%	
Arthrospira platensis	3.93	100.00	
Group A - Average similarity: 77.27			
Species	Av. abund	Contrib%	
Microcystis aeruginosa	2.74	100.00	
Group C - Average similarity: 63.74			
Species	Av. abund	Contrib%	
Merismopedia glauca	4.88	81.67	
Groups D & B - Average dissimilarity = 100.00			
	Group D	Group B	
Species	Av. abund	Av. abund	Contrib%
Euglena sp.	4.79	0.00	46.41
Arthrospira platensis	0.00	3.93	37.66
Groups D & A - Average dissimilarity = 100.00			
	Group D	Group A	
Species	Av. abund	Av. abund	Contrib%
Euglena sp.	4.79	0.00	53.41
Groups B & A - Average dissimilarity = 93.88			
	Group B	Group A	
Species	Av. abund	Av. abund	Contrib%
Arthrospira platensis	3.93	0.00	59.49
Groups D & C - Average dissimilarity = 100.00			
	Group D	Group C	
Species	Av. abund	Av. abund	Contrib%
Merismopedia glauca	0.00	4.88	34.30
Euglena sp.	4.79	0.00	32.08
Groups B & C - Average dissimilarity = 100.00			
	Group B	Group C	
Species	Av. abund	Av. abund	Contrib%
Merismopedia glauca	0.00	4.88	40.76
Arthrospira platensis	3.93	0.00	30.81
Groups A & C - Average dissimilarity = 100.00			
	Group A	Group C	
Species	Av. abund	Av. abund	Contrib%
Merimopedia glauca	0.00	4.88	45.88
Microcystis aeruginosa	2.74	0.00	24.83

*Av. abund = Average abundance and contrib% = Contribution in percentage.

PC2 and abundance of bloom forming species showed that only three bloom forming species correlated with PCs scores. *A. platensis* (r = -0.47; p<0.05) and *Euglena* sp. (r = 57; p<0.05) were correlated with PC1 and that established the impact of air and water temperatures on the abundance of both species.

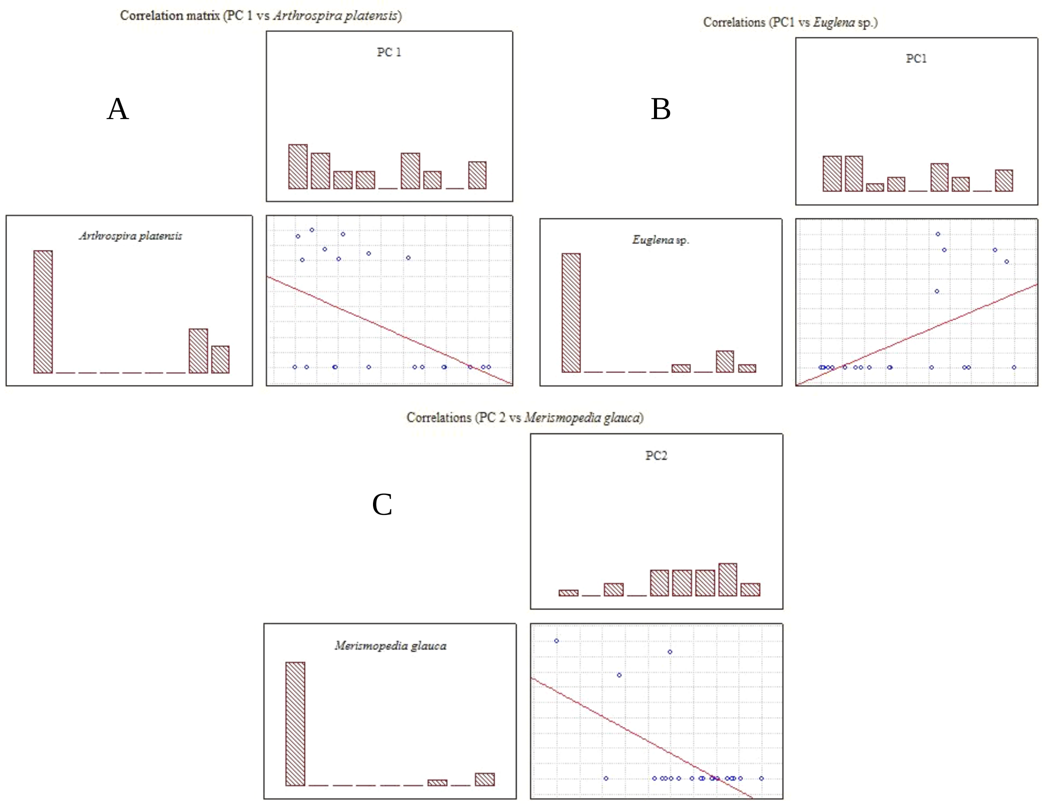

Fig. 3. Correlation matrix between PCs score (PC1 and PC2) and different bloom forming phytoplankton species (abundance of species log(x+1) transformed). (A) PC1 correlated negatively with *Arthrospira platensis* (r = –0.47; p < 0.05), (B) PC1 correlated positively with *Euglena* sp. (r = 57; p < 0.05) and (C) PC2 correlated negatively with *Merismopedia glauca* (r = –0.61; p < 0.05).

In case of *A. platensis* the impact was positive. That means increase of air and water temperatures increase the growth of the species. But in case of *Euglena* sp. the impact was negative. Another bloom forming species *Mer. glauca* (r = –0.61; p < 0.05) correlated negatively with PC2. This also evident there was strong positive impact of NO_3-N on the abundance of *Mer. glauca* meaning increasing tendency of the growth of the species aggregates by NO_3-N concentration. Negative impact of SRP on the growth of *Mer. glauca* was also revealed by regression analysis.

Present investigation revealed that statistically significant correlation was found between four limnological variables of air and water temperatures, NO_3-N and SRP and three bloom forming phytoplankton of *A. platensis*, *Mer. glauca* and *Eulgena* sp.

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(Manuscript received on 2 October, 2016; revised on 13 November, 2016)