

Influence of environmental parameters on toxic cyanobacterial bloom occurrence in a Lake of Bangladesh

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Abstract Increased nutrients have led cyanobacteria to become dominant in many ponds, lakes and reservoirs in many countries of the world. The occurrence and abundance of cyanobacterial population were monitored in a lake (known as Ishakha Lake) at Bangladesh Agricultural University campus, Mymensingh, Bangladesh. The hydrographic parameters such as water temperature, pH, chlorophyll a and nutrients (NO₃–N and PO₄–P) were recorded to find out their relationship with the cyanobacterial bloom formation. During the study period five species of cyanobacteria namely, Microcystis aeruginosa Kütz., M. wesenbergii Kom., M. botrys Teli., M. viridis (A. Br.) Lemm. and Anabaena circinalis Rabenh., were identified and among them M. aeruginosa was the dominant

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species during the bloom period. At the peak period of bloom, the highest cell density of M. aeruginosa was 1550×10^3 cells ml⁻¹ which comprised 97.45 % among the blue-green algae and 96.84 % to the total phytoplankton. The initiation and persistence of natural bloom of cyanobacteria, especially Microcystis spp. was found to be controlled by relatively high temperature (>25.00 °C) and nutrients, especially high NO₃–N (3.80 mg l^{-1}) concentration. Temperature and NO₃-N showed positive correlation with cyanobacterial cells abundance which were r = 0.62 and r = 0.92. Therefore, it could be said that temperature and NO₃-N made a favorable circumstance to form cyanobacterial bloom in as Ishakha Lake. The Enzyme-linked Immunosorbent Assay revealed that the

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concentration of MCs 37,460.00 pg ml^{-1} at the peak period of bloom.

Keywords Environmental factors · Cyanobacteria · Toxic · Bloom · Lake · Bangladesh

1 Introduction

The microscopic planktonic algae play an important role in the environment as critical food for fish and crustaceans. However, in some situations algal blooms can have a negative effect causing economic losses to aquaculture and fisheries (Hallegraeff 1993). Recently, the geographic distributions as well as the intensity of these blooms have increased (Hallegraeff et al. 1988; Han et al. 1992). Cyanobacteria are often reported to be toxic in fresh, brackish and marine waters in many parts of the world (WHO 1984). Blooms have known to occur in waters where the nutrient levels are elevated and often associated with the use of fertilizers. The most bloom forming cyanobacteria are Microcystis, Anabaena, Nodularia, Planktothrix and Aphanizomenon. Such blooms have caused numerous animal poisoning and mortality of wildlife and domestic animals (Carmichael 1992). Fatalities and severe illness resulting from cyanobacterial bloom have been reported in many countries (Carmichael and Falconer 1993).

Excessive phytoplankton growth is a serious problem for aquaculture, because it is known to negatively affect water quality in fish ponds in at least three ways (Smith 1985). First, it can lead to chronic oxygen deficits. Second, dense algal blooms collapse periodically leading to decomposition of the dead algae resulting in fish kills due to anoxia (Boyd et al. 1975; Barica 1975) as well as due to high levels of ammonia (Seymour 1980; Tucker et al. 1984). Third, blue-green algae exude chemicals- geosmin and methyl-isoborneol that taint fish flesh, i.e., causing offflavor of fish (Brown and Boyd 1982; Armstrong et al. 1986).

In Bangladesh, with gradual intensification of aquaculture (with the use of fish-feeds and fertilizers) fish farmers are experiencing many harmful and noxious algal blooms. Furthermore, incidents of hepatitis, liver cancer, asthma, skin rashes and other allergic problems are increasing unexpectedly fast in this densely populated developing country both in rural and urban areas. There are some reports that cyanotoxins have direct or indirect relationship with some of the above diseases (Yu 1989; Turner et al. 1990; Bell and Codd 1994; Ueno et al. 1996). Cyanobacteria forms blooms round the year with the peak abundance in spring and summer in many ponds, lakes and other reservoirs of the country and this bloom might have some relationship with various water quality parameters.

Growth of phytoplankton is one of the most important factors determining the abundance and distribution of microalgae (Admiraal 1977). Growth of phytoplankton in waterbodies is controlled by various environmental factors, such as temperature, pH, light, nutrients, stratification, water turbidity, etc. (Tomas 1978; Uye and Takamatsu 1990). Nielson and Tonseth (1991) suggested that temperature was an important limiting factor in the initiation of blooms of Gyrodinium aureolum Hulburt in north European waters. Nutrients are one of the most important environmental factors that influence algal growth (Okaichi et al. 1989). Ono (1988) found that a nitrogen concentration of 0.3 mg l^{-1} and a phosphorus concentration of $0.03-3.0 \text{ mg l}^{-1}$ were optimal for the growth of *Fibro*capsa japonica Toriumi and Takano. Ecological and physiological parameters of phytoplankton have been suggested to vary from species to species as well as from strain to strain. Honjo (1993) reported five different optimum temperatures for five different strains of Heterosigma akashiwo (Hada). The autecological knowledge of cyanobacteria would be useful in predicting possible danger periods, taking precautions and determining the feasibility of control measures. However, this study was conducted to find out the effect of physic-chemical factors on the bloom formation of cyanobacteria and qualitative and quantitative contribution of different species of cyanobacteria to the bloom in Ishakh Lake.

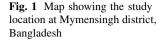
2 Materials and methods

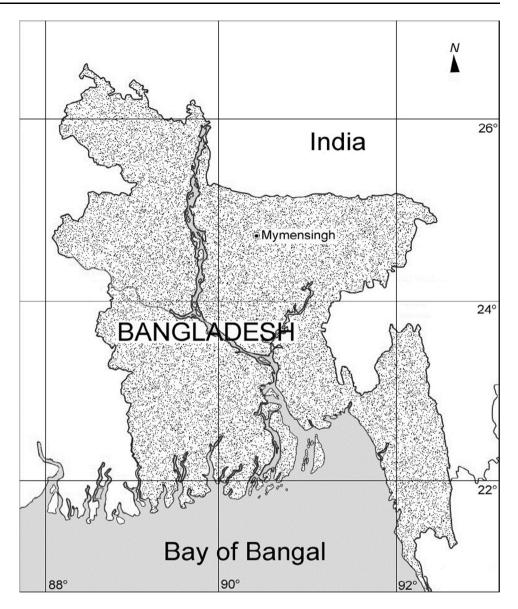
2.1 Study area and sampling

The present study was conducted in a lake at Bangladesh Agricultural University campus, commonly known as Ishakha Lake, Mymensingh, Bangladesh (Fig. 1). Lake water is used for fish culture, bathing and washing clothes. Every year it experiences periodic cyanobacterial bloom. The lake receives kitchen wastes and decomposed organic nutrients through drains of three residential halls of university students situated 20 m from the inlet. Samples were collected directly in one liter plastic bottles from five different randomly selected positions of the pond. Bi-weekly sampling started at the 1st January 2001 and continued up to the initiation of bloom and then daily during the bloom period (22 February–4 March 2001). Just after collection, the samples were preserved in 5 % buffered formalin.

2.2 Analysis of water quality parameters

Surface water temperature and pH were determined using a celsius thermometer and an electronic pH meter (Jenway 3020, Germany), respectively. Nitrate–nitrogen (NO₃–N)





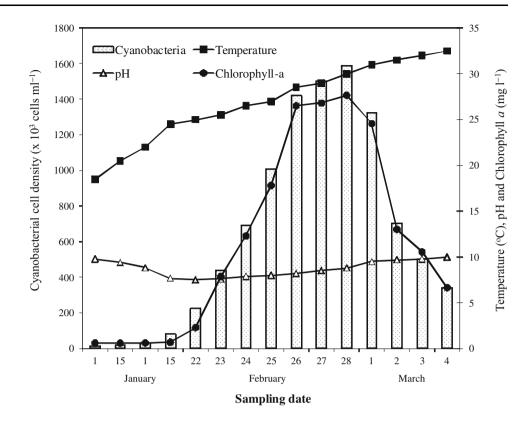
and phosphate–phosphorus (PO₄–P) were measured using a HACH kit (DR/2010, a direct reading spectrophotometer) using high range chemicals (NitraVer 5 Nitrate Reagent Powder Pillows for NO₃–N, and PhosVer 3 Phosphate Reagent Powder Pillows for PO₄–P analysis). Chlorophyll a was determined spectrophotometrically (Milton Roy Spectronic, 1001, Germany) after acetone extraction (APHA 1992). During peak bloom a phytoplankton sample was analyzed for testing microcystins (MCs) concentration following Enzyme-linked Immunosorbent Assay.

2.3 Enzyme-linked Immunosorbent Assay

From each sample, 10 ml water was poured in a 12 ml polyvinyl vial and 1/100 volume of 10 % sodium azide was added and frozen in a deep freezer. The samples

were freeze-thawed twice, and then filtered through glass fiber filters (Whatman GF/C, 25 mm in diameter) and used for enzyme-linked immunosorbent assay (ELISA). The water samples or microcystins LR (MC-LR) standard were mixed with an appropriate dilution of the anti-MC-LR MAb M8H5, and then added to a 96-well microtiter plate coated with MC-LR-bovine serum albumin conjugate. After washing, the bound MAb was detected with horseradish peroxidase-labeled goat anti-mouse IgG (TAGO 4550) plus substrate (0.1 mg ml⁻¹ 3,3'-5,5'-tetramethyl benzidine, 0.005 % H2O2 in 0.1 M acetate buffer pH 5), resulting in an absorption measurement at 450 nm. The concentrations of MCs used when examining the ELISA data were an average of two triplicate estimations expressed as picogram per milliliter (pg ml^{-1}) by MC-LR.

Fig. 2 Influence of temperature and pH on the occurrence and abundance of cyanobacterial cell abundance and content of chlorophyll *a* in Ishakha Lake from 1 January to 4 March 2001



2.4 Phytoplankton study

For species identifications, buffered formalin preserved sample was gently shaken to resuspend all materials and was allowed to settle for 1 min. Then 2–3 drops were removed from the middle of the sample and placed on a glass slide. Taxonomic determination of cyanobacteria was performed with a phase-contrast microscope (Olympus, Japan) at 100–400 X, with brightfield and phase contrast illumination on living materials and on samples preserved with formaldehyde. Identification was done following the morphological characteristics described by Komarek (1958), Skulberg and Skulberg (1985). The quantitative estimation of phytoplankton was done by Sedgewick– Rafter counting chamber (S–R cell) following the method described by Stirling (1985).

3 Results

The temperature increased from 18.50 to 32.50 °C during the study period. The cyanobacterial bloom peaked during high temperature (>29 °C). The average temperature was (28.00–31.50 °C) during peak bloom period (26 February– 1 March). As the temperature continued to increase from 28.60 to 32.50 °C cyanobacteria seemed to decrease rapidly. The pH fluctuation was slight ranging from 7.50 to 10.00 (Fig. 2). Higher cell abundance of cyanobacteria was found when the pH was approximately 8.80. Chlorophyll *a* paralleled the cyanobacterial cell density. The lowest value of chlorophyll *a* (0.60 mg 1^{-1}) was recorded at the beginning of this study. During the bloom period the range of chlorophyll *a* fluctuated from 2.30 to 27.65 mg 1^{-1} (Fig. 2). Nitrate (NO₃–N) concentration ranged from 1.10 to 3.80 mg 1^{-1} , and the higher (2.2–3.8 mg 1^{-1}) concentration was observed during the bloom period. Fluctuation of phosphate (PO₄–P) concentration was small ranging from 0.30 to 1.00 mg 1^{-1} (Fig. 3).

The cyanobacterial cell abundance was low $(15.80 \times 10^3 - 34.20 \times 10^3 \text{ cells ml}^{-1})$ during the 1st sample periods (1 January-15 January). Cell abundance started to increase gradually at the middle of February and cyanobacterial bloom was starting to be visible with naked eyes from 22 February which continued until the first week of March (4 March, 2001). During the initiation of the bloom, the cyanobacterial cell density was 15 times higher $(226.45 \times 10^3 \text{ cells ml}^{-1})$ than the base population (population prior to February 22) (Table 1). The abundance began to increase rapidly from February 22 and peaked within 4 days forming green paint like scum on the surface of the water body. The cell abundance was about seven times higher $(1590.55 \times 10^3$ cells ml⁻¹) than the cell abundance of early bloom. This abundance then began to decline after 28 of February reaching lowest level $(340.00 \times 10^3 \text{ cells ml}^{-1})$ on March 4.

Fig. 3 Influence of NO₃–N and PO₄–P concentration on the occurrence and abundance of cyanobacterial cell abundance in Ishakha Lake during from 1 January to 4 March 2001

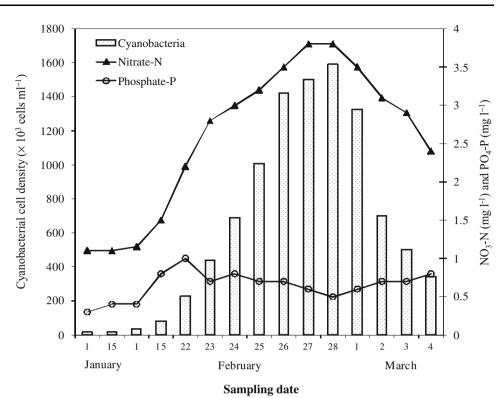


Table 1 Total phytoplankton abundance ($\times 10^3$ cells ml⁻¹), and abundance and percent (%) contribution of Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae during bloom in Ishakha Lake

Day	Total	Cyanophyceae		Chlorophyceae		Bacillariophyceae		Euglenophyceae	
	$(\times 10^3 \text{ cells ml}^{-1})$	$(\times 10^3 \text{ cells ml}^{-1})$	%	$(\times 10^3 \text{ cells ml}^{-1})$	%	$(\times 10^3 \text{ cells ml}^{-1})$	%	$(\times 10^3 \text{ cells ml}^{-1})$	%
01.01.01	35.40	15.80	46.63	16.00	45.20	3.25	9.18	0.35	0.99
15.01.01	36.15	18.75	51.86	15.85	43.85	1.35	3.73	0.20	0.55
01.02.01	70.65	34.25	48.48	34.45	48.76	1.45	2.05	0.50	0.71
15.02.01	116.65	81.35	69.74	33.35	28.60	1.55	1.33	0.40	0.34
22.02.01	236.95	226.45	95.57	10.00	4.22	0.35	0.15	0.15	0.06
23.02.01	450.15	439.00	97.52	10.75	2.39	0.30	0.07	0.10	0.02
24.02.01	703.20	691.15	98.29	11.75	1.67	0.20	0.03	0.10	0.01
25.02.01	1018.15	1006.95	98.90	11.19	1.10	0	0	0	0
26.02.01	1431.10	1420.35	99.24	10.75	0.75	0	0	0	0
27.02.01	1512.95	1502.35	99.30	10.60	0.70	0	0	0	0
28.02.01	1600.65	1590.55	99.37	10.10	0.63	0	0	0	0
01.03.01	1335.60	1325.55	99.25	10.05	0.75	0	0	0	0
02.03.01	745.30	702.25	94.22	43.05	5.78	0	0	0	0
03.03.01	539.25	500.50	92.81	38.75	7.19	0	0	0	0
04.03.01	370.00	340.00	91.90	25.15	6.80	4.85	1.31	0	0

During the peak bloom cyanobacteria contributed 99.37 % of the total phytoplankton population (Table 1). The cell density of other phytoplankton groups, like Chlorophyceae, Bacillariophyceae and Euglenophyceae decreased with increasing cyanobacterial cell density. The cell density of Bacillariophyceae and Euglenophyceae

were absent with very low density of Chlorophyceae when the cell density of cyanobacteria reached 1006.95×10^3 cells ml⁻¹.

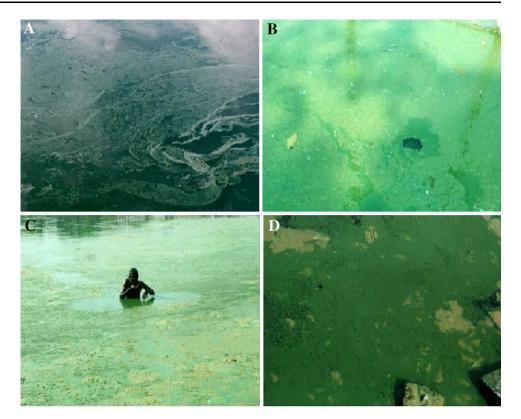
Five species of cyanobacteria *Microcystis aeruginosa*, *M. wesenbergii*, *M. botrys*, *M. viridis* and *Anabaena circinalis* were identified and among them *M. aeruginosa* Table 2 Sampling date, abundance of cyanophyceae $(\times 10^3 \text{ cells ml}^{-1})$, and abundance and percent (%) contribution of different cyanobacterial species during bloom in Ishakha Lake

Sampling date	Cyanophyceae	Dominant				
	$(\times 10^3 \text{ cells ml}^{-1})$	Species	$(\times 10^3 \text{ cells ml}^{-1})$	%		
01.01.01	15.8	M. aeruginosa	6.65	42.09		
		M. wesenbergii	8.15	51.58		
15.01.01	18.75	M. aeruginosa	7.15	38.13		
		M. wesenbergii	10.25	54.66		
01.02.01	34.25	M. aeruginosa	17.1	49.93		
		M. wesenbergii	16.45	48.03		
15.02.01	81.35	M. aeruginosa	40.65	49.97		
		M. wesenbergii	39.05	48.00		
22.02.01	226.45	M. aeruginosa	117.45	51.19		
		M. wesenbergii	90.00	39.74		
		M. botrys	12.00	5.30		
23.02.01	439.00	M. aeruginosa	341.50	77.90		
		M. wesenbergii	86.50	19.70		
		M. botrys	10.00	2.28		
24.02.01	691.15	M. aeruginosa	593.00	85.80		
		M. wesenbergii	80.50	11.65		
		M. viridis	15.50	2.24		
25.02.01	1006.95	M. aeruginosa	928.95	92.25		
		M. wesenbergii	58.00	5.76		
		Anabaena circinalis.	18.00	1.79		
26.02.01	1420.35	M. aeruginosa	1355.00	95.40		
		M. wesenbergii	42.35	2.98		
		A. circinalis.	21.00	1.48		
27.02.01	1502.35	M. aeruginosa	1449.00	96.45		
		M. wesenbergii	35.35	2.35		
		A. circinalis.	16.00	1.07		
28.02.01	1590.55	M. aeruginosa	1550.00	97.45		
		M. wesenbergii	25.55	1.61		
		A. circinalis.	13.00	0.82		
01.03.01	1325.55	M. aeruginosa	1250.00	94.30		
		M. wesenbergii	55.55	4.20		
		M. botrys	16.00	1.20		
02.03.01	702.25	M. aeruginosa	555.00	79.03		
		M. wesenbergii	120.50	17.16		
		M. botrys	25.50	3.63		
03.03.01	500.50	M. aeruginosa	322.50	64.44		
		M. wesenbergii	145.50	29.07		
		M. botrys	30.50	6.10		
04.03.01	340.00	M. aeruginosa	162.50	47.79		
		M. wesenbergii	152.50	44.85		
		M. botrys	25.00	7.35		

was dominant during the bloom period (22 February to 4 March). At the peak of bloom period, the highest cell abundance of *M. aeruginosa* was 1550.00×10^3 cells ml^{-1} which comprised 97.45 % among the cyanobacteria (Table 2). During the bloom period, M. aeruginosa cell abundance was found to be began increasing when the cell density of M. wesenbergii began to decrease.

During the peak bloom planktivorous fish silver carp (Hypophthalmichthys molitrix) was found to be died the morning. The Enzyme-linked Immunosorbent Assay revealed that the concentration of MCs 37,460.00 pg ml⁻¹

Fig. 4 Cyanobacterial bloom at Ishakha Lake; starting of bloom at the middle of March (**a**), blue-green paint scum formation on third week of February (**b**), peak bloom at the end of last week of February (**c**), declining of bloom at the beginning of March (**d**)



at the peak period of bloom when *M. aeruginosa* was 1550.00×10^3 cells ml⁻¹ which comprised 96.84 % of the total phytoplankton.

4 Discussion

The Ishakha Lake, in which this study was carried out, is typical of many other shallow, warmer and eutrophic ponds or lakes in Bangladesh that regularly produce dense bloom of planktonic cyanobacteria from spring to summer. By the last week of February 2001, a typical surface bloom of cyanobacteria started to be observed whose intensity was very high (Fig. 4a, b), then the bloom reached peak by the end of last week of February, and at that time the surface scum of cyanobacteria covered most part of the pond (Fig. 4c). At the beginning of March the bloom was found to be declined (Fig. 4d). The recorded cyanobacterial cell density during the peak bloom was 1590.55×10^3 cells ml⁻¹ in the studied lake. Park et al. (1996) recorded the cell density of Microcystis 6.70×10^5 cells ml⁻¹ during the bloom period in Lake Suwa, Japan which is about two times lower than the population which we found in our present study. M. aeruginosa was the dominant species representing 96.84 % of the total phytoplankton and 97.45 % among Cyanophyceae. This result is similar with the findings of Oudra et al. (1998) who recorded 95.00 % *M. aeruginosa* in a cyanobacterial bloom in eutrophic Lalla Takerkousta reservoir in Morocco.

The initiation and persistence of Microcystis natural bloom in the lake, seemed to be determined by relatively high temperatures (28.00-30.00 °C) in spring season, alkaline pH, high nutrients concentration, especially NO₃-N concentration, and bright sunlight. In our present study, the effect of temperature on the bloom formation of M. aeruginosa agrees fairly well with Eloff (1980) who also found that temperature of 28.80-30.50 °C was optimal for the growth of *M. aeruginosa*. Watanabe and Oishi (1985) also found faster growth of M. aeruginosa at temperature 32.00 °C under culture condition. Alkaline water may have promoted the outbreak of Microcystis bloom. In the present study, the pH ranged from 8.20 to 8.80 during the bloom period. Van der Westhuizen and Eloff (1983) also found highest growth rate of M. aeruginosa in culture media with pH around 9.0 during batch culture.

Cyanobacteria cell abundance increased with the increase in NO_3 –N concentration and dropped with the decline in NO_3 –N. Generally, occasional rainwater during spring months and heavy rainfall in the monsoon months flushes organic materials from the adjacent fallow lands and from the household drains into the lake. Nutrients from decomposition of those organic materials trigger cyanobacterial bloom. During the study period rainfall

occurred only once at the beginning of the spring and earlier than other years. These increased nutrients might have created favorable condition for the out-break of cyanobacteria, mainly *M. aeruginosa* blooms in this lake. The concentration of NO₃-N and cyanobacterial cell abundance showed highly positive correlation (r = 0.92). Similar results were reported by Park et al. (1993) who suggested that increase of NO₃-N concentration favored the growth of Microcystis at Lake Suwa in Japan. Furthermore, toxic Microcystis population collapsed when NO₃-N concentration decreased (Utkilen et al. 1996). Microcystis may not require high concentration of PO₄-P for their growth as in our study where Microcystis bloom occurred in relatively low PO₄-P concentration. Gerloff et al. (1952) also found that Microcystis had a relatively low phosphorus requirement for its growth.

A negative relationship between *M. aeruginosa* and *M.* wesenbergii cell abundance was found which indicating M. aeruginosa might have some growth inhibitory substances and that substances may be suppressed the growth of *M. wesenbergii*. Similarly, Lam and Silvester (1979) also found negative correlation between Cyanophyceae and Chlorophyceae. Similar evidence was observed by Hossain (1989) who described that Anabaena showed more negative correlation with others; Nostoc and Aphanocapsa exhibited more negative correlation with some other genera and species of phytoplankton. However, during the peak bloom of M. aeruginosa, fish mortality occurred which might be related with the toxin produced by M. aeruginosa, or suffocation due low concentration of dissolved oxygen or by gill clogging by the high dense cyanobacteria of lake water, as it is reported elsewhere in the world.

5 Conclusion

It could be said that temperature and NO_3 –N are the most important environmental factors which make favorable condition for bloom formation of cyanobacterial in lake.

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