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Studies on Occurrence of Microcystein in the Water of Lake Surha, Ballia, Uttar Pradesh, India.

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Abstract:
Four different sites of Lake Suraha, Ballia were selected for study. The water was sampled for cyanobacterial diversity and physicochemical properties. Out of 13 cyanobacterial species isolated in four were the isolates of Microcystis aeruginosa. The Kaithawali site of lake was densely populated by cyanobacterial bloom, whereas, least population was recorded at Basantpur. Dominating blooms of M. aeruginosa were recorded at Kaithawali followed by Bhikpur and Rajpur sites. The water of Kaithawali and Rajpur site was more alkaline compared to Bhikpur and Basantpur. Freer CO_2 was recorded in morning samples compared to evening samples. Total alkalinity ranged between 240 and 198 mg l^{-1}. In general, the DO ranged between 15.4-22mg l^{-1} in evening samples and 8.7-13.1 mg l^{-1} in morning samples. The BOD values of the samples were estimated after 5 days of incubation and it ranged between 10.8-20 mg l^{-1}. The growth of M. aeruginosa isolates was recorded in vitro in B-12 medium. The exponential phase lasts up to day 14 after inoculation followed by stationary phase. Rapid growth of M. aeruginosa isolate SPM15 was recorded compared to other isolates, however, no significant (P = 0.05) difference in growth of the isolates was recorded on M-12 media. On the basis of growth rate and preliminary toxin analysis isolate SPM15 was selected for further study. The toxin in crude water or methanol extracts of M. aeruginosa isolates was preliminarily characterized by estimating zone of inhibition of Pseudomonas solanacearum and Xanthomonas compestris on nutrient agar by sensitivity disc method. All the isolated inhibited the growth; however, maximum inhibition was recorded by isolate SPM 15. In general, methanol extracts have more pronounced effect. The concentration of microcystin in the different isolates of M. aeruginosa was evaluated by comparing the peaks with the standard. The result shows that isolate SPM 15 was highly toxic and contains maximum microcystin ca. 3200 μg g^{-1} dry weight followed by .SPM7 (965 μg), SPM 19 (438 μg) and SPM 9 (257 μg). The relationship between the amount of microcystin and biomass of M. aeruginosa cells in different growth conditions (Temperature, pH, nitrate and phosphate) was estimated. In general, a significant (P=0.05) positive correlation was observed in the microcystin concentration and population of M. aeruginosa.

Keywords: *Cyanobacterial diversity, Microcystis aeruginosa, Microcystein, Lake Surha, Cyanotoxin*

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Introduction

Cyanobacteria or blue green algae are frequent components of many fresh, brackish and marine ecosystems. The cyanobacteria provide an extraordinarily wide-ranging contribution to human affairs in everyday life (Tiffany, 1958) and are of economic importance (Mann and Carr, 1992). Both the beneficial and detrimental features of the cyanobacteria are of considerable significance. They are important primary producers and their general nutritive value is high. The nitrogen-fixing species contribute globally to soil and water fertility (Rai, 1990). The use of cyanobacteria in food production and in solar energy conversion holds promising potential for the future (Skulberg, 1995). Sometimes, they produce massive growth as a consequence of nutrient enrichment of natural water due to agricultural fertilizer run off or from domestic and industrial effluents. This massive growth of cyanobacterial strains are referred as cyanobacterial blooms. Death or illness of animals and birds after oral intake of cells or the free toxins has been documented well. Most of the reports of hazardous effect of these blue green algae are from tropical countries but very little attention has been paid in India, where cyanobacterial strains grow luxuriantly in most of the ponds, pools and ditches. Francis (1878) first recognized hazardous effect of cyanobacterial bloom and scums. Since then reports of toxic cyanobacteria are available from at least 44 countries (Carmichael, 1986; Codd, 1998) In many cases the environmental

samples examined for toxicity consist almost, or entirely of one cyanobacterial genus or species (Skulberg *et al.,* 1993). These toxins pose a challenge for water management (Dietrich and Hoeger, 2005). Microcystines from *Microcyustis aeruginosa* is known as "fast death factor" (Sivonen, 1990a, b) and commonly known as "very fast death factor" (VFDF)(Sivonen, 1990 a, b). A large number of reports on the effect of cyanobacterial blooms on human health are available, among these the Palm Island of Australia and Caruaru syndrome of Brazil is important (Hawkins *et al.*, 1985; Pouria *et al.*, 1998; Hilborn *et al.*, 2005). Several cases of contact irritation have been reported among swimmers. Phenolic bislactones, aplysia-toxin and debroma-aplysia toxin produced by *Lyngbyamajuscula¸ Schizothrix calcicola* and *Oscillatoria nigroviridis* are the responsible substances (Moore, 1984; Fujiki *et al*., 1990). Keeping these points in view, the present work focus on collection of the cyanobacterial strains from different sites of the famous Suraha Lake used for commercial production of fishes, evaluation of presence of toxin in the samples

Materials and Methods

Location, sampling and experimental organism

The cyanobacterial and water samples were collected from 4 different sites of lakeSurha, Ballia (Table 1). The samples were collected from the surface water bloom (0 to 0.3m depth) and concentrated by plankton net of 22 µm. The phytolanktons were brought to laboratory, microscopically identified. The cyanobacterial species were purified and cultured on B-12 media (Nakagawa *et al.*, 1987) having following composition (in milligrams per liter of deionized distilled water): $NaNO_3$, 100; K_2HPO_4, 10; $MgSO_4$ $7H_20$, 75; $CaC1_2$ $2H_20$, 40; Na_2CO_3, 20; ferric citrate, 6 (autoclaved separately); disodium EDTA. $2H_2O$, 1; and vitamin B_{12}, 0.1. The pH was adjusted to 9.0. Single cell isolation of the growing isolates was performed by streaking the colonies on agar plates. Identified and purified isolates of *Microcystis aeruginosa* were maintained on same medium.

Analysis of water

The Physico-chemical properties of the lake water were analyzed as per the methods described by APHA *et al.* (1998). The water samples were collected in the 1 litre screw cap storage bottle (Schotts, Germany) and brought to laboratory for chemical analysis.

A. Temperature and pH

The temperature of water was measured at sampling site with the help of Celsius thermometer. pH of the sample was measured by glass electrode pH meter (Tanco, India Ltd.)

Alkalinity

Total alkalinity was measured following the method described by Saxena (1990). In brief, two or three drops of phenolphthalein indicator (Merck, India) was mixed with 100 ml of the sample in a 250 ml conical flask (Schotts, Germany), If the sample turns pink or red, then the phenolphthalein alkalinity was determined by titrating the sample with N/50 sulphuric acid until the pink colour just disappears. The amount of sulphuric acid was noted (p). If no colour was produced, the phenolphthalein alkalinity is zero. In both the cases few drops of methyl orange indicator was added to the sample (yellow colour appears) and again titrated with N/50 sulphuric acid until the first perceptible colour change towards orange is observed. The reading was noted (t). Total alkalinity was calculated by given formula:

$$\text{Phenolphthalein alkalinity as } CaCO_3 \text{ (mg ml}^{-1}) = \frac{p \times 1000}{S}$$

$$\text{Total alkalinity as } CaCO_3 \text{ (mg ml}^{-1}) = \frac{t \times 1000}{S}$$

Where p = volume of titrant (sulphuric acid) used against phenolphthalein indicator; t = volume of titrant (sulphuric acid) used against the two titrations (ml); S= volume of sample (ml)

Free CO_2

Free Carbon di oxide is calculated at the site quickly with all precaution to avoid it's volatality. Analysis was done by volumetric titration of 250 ml sample with N/44 NaOH using phenopthaline as indicator. The value was calculated as follows:

$$\text{Free } CO_2 = \frac{(V_1 \times N)\ NaOH \times 1000 \times 44}{\text{Volume of water sample}}$$

Where V_1= volume of NaOH and N= Normality of NaOH

Dissolved Oxygen

Dissolved Oxygen was estimated using Winkler's Method. In brief, to fix the DO in sample, at the collection site, 2 ml of $MnSO_4$ solution and 2 ml of alkaline-iodide-azide solution was added in the water sample collected in BOD bottle with proper precaution to avoid any water bubble,. The contents were mixed by inverting the bottle several times. A brown floc was formed in the bottle then 1 ml of H_2SO_4 (18 M) was added to the solution without splashing, to disappear the floc, now the liquid in the bottle had a yellowish-brown colour. The dissolved oxygen in the sample was now "fixed. The fixed sample was brought to laboratory, few drops of indicator starch solution was added to develop a blue colour. The sample was titrated against sodium thiosulphate solution (0.025 mol l^{-1}) until the blue colour disappears. (For details of reagent see Appendix I). The DO is calculated using the formula:

$$DO = \frac{V_1 \times N \times 8 \times 1000}{V_2 - V_3}$$

Where V_1 = volume of titrant (ml); N = Normality of titrant 90.025); V_2 = Volume of sampling bottle after placing the stopper; V_3 = Volume of MnSO4 solution and alkaline-iodide-azide

BOD

BOD of the water sample was estimated following the method given below. The sample was diluted with equal volume of the dilution water (See Appendix II). Two sets of BOD bottles (200 ml) were filled with the diluted sample and 1 ml of allothio urea was added to both the samples. The dissolved oxygen in one set was determined immediately, whereas the other set was incubated for 5 days in a BOD incubator (Tanco, India) at 20 ^oC. After 5 days of incubation the samples were assayed for DO content. The BOD was calculated using the formula:

BOD_5 (mg l^{-1}) = (D_0-D_5) x Dilution factor

Where D_0= initial DO at day 0 and D_5 is DO at day 5

Microcystis aeruginosa

Microcystis aeruginosa isolates were grown and maintained on B-12 medium with combined nitrogen source. Solid media was prepared by mixing separately autoclaved agar (Hi-Media) solutions when cooled to pouring temperature, to give a final agar-agar concentration of 0.8% (w/v). The cultures were grown and maintained at 28±2 ^oC in the culture room illuminated with day light fluorescent lamps giving an average light intensity of 70 μE $m^{-2}s^{-1}$ on the surface of vessels with a 14:10 light dark rhythm. Liquid cultures were shaken manually twice a day.

The clonal and axenically growing culture of *M. aeruginosa* strains were derived from exponentially growing culture. The exponentially growing suspension was homogenized with sterile glass beads. Resulting homogeneate of single cells of *Microcystis aeruginosa* were suitably diluated and spread on agar plates. After an incubation of 4-5 days (28 ± 2 ^oC; light intensity of 70 μE $m^{-2}s^{-1}$), the micro-colony developing from single cell were picked by glass capillaries, inoculated to B12 broth and allowed to grow under standard growth conditions. Axenic nature of the cultures were rigorously tested both microscopically (Nikon Optiphot, Japan) using phase contrast objectives and oil immersion as well as plating the cultures on agar media.

Growth Measurement

Growth of homogeneous cultures was measured turbidometrically in a spectrophotometer (Systronics, India). The known volume of B-12 broth (20 ml) was taken in specially designed conical flask with side arm to which a known amount of inoculum (1 ml) was added. The initial absorbency of the culture was recorded at the beginning of growth at a wave length of 663 nm. The flask were incubated (28 ± 2 ^oC; light intensity of 70 μE $m^{-2}s^{-1}$) and absorbancy was recorded at regular intervals.
The growth rate was calculated as Guillard (1973):

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

where μ is the growth rate and x_2 and x_1 represents the celldensities at two times t_2 and t_1.

Generation Time

Generation time (GT) is the time of cells to divide (in days) and is calculated from growth rate.

$$GT = 06931/\mu$$

Effect of Environmental Factors on Growth

The effect of environmental factor on growth and toxicity of *M. aeruginosa* was studied following the methods described by Watanabe and Oishi(1985). Effect of temperature an pH was studied by altering the incubation temperature (20, 25 and 30 ^oC) and pH of media by adding predetermined amount of N/10 HCl (for pH 7 and 8) and N/10 NaOH (for pH 10). To evaluate the effect of light, the light intensity was altered to 3.5, 7, 35 and 70 μE $m^{-2}s^{-1}$ by using variable number of day light fluorescent lamps. The phosphate and nitrogen deficiency experiments were performed. For phosphate deficiency experiments, the amount of K_2HPO_4, was reduced to 1/10 or 1/20 of the original level. For nitrogen deficient conditions, sodium nitrate was reduced to 1/10 or 1/20 of the original medium concentration. The biomass (14 days) was assayed for toxin.

Toxin analysis

The *M. aeruginosa* biomass from exponential phase cultures of different experiments as described above or from the collection site was collected through centrifugation (1000 *g*). The cells were lyophilized and stored at -20 ^oC before the extraction procedure.

(i) Water extracts

Water extracts were made by resuspending 70 mg offreeze-dried cells in 3 ml of distilled water. After careful mixing, the sample was kept at room temperature for 30min. The suspension was centrifuged at 2500 *g* for 6 min. The supernatant fluid was withdrawn with a pipette, and 100 μl of the aqueous extract was transferred to each well in the agar diffusion tests.

(ii) Organic extracts

The organic extractions were made using methanol and ethylacetate in acid, neutral and alkaline conditions. The most pronounced antimicrobial effects were obtained with methanol-acetic acid extracts, and this method of extraction was used in the studies with the strains of *M. aeruginosa.* After removing the supernatant fluid from the water extraction, 3 ml of methanol and 100 μl acetic acid were added. The sample was mixed and shaken for 10 seconds and was then left with the extraction fluid for 10 min. The

sample was centrifuged at 2500 *g* for 6 min and the supernatant fluid then transferred to a clean glass-stoppered tube. The organic solvent was evaporated to dryness under a stream of N_2 gas at 40 °C and a Rotary evaporating unit (Remi, India). The dry residue was resuspended in 1 ml methanol the final concentration was equivalent to 50 mg offreeze-dried cyanobacteria.

To test the toxicity of water or organic extracts, sterile filter discs with a diameter of12.7 mm (Millipore) were soaked in the extract solutions obtained in previous experiment and kept overnight at room temperature to evaporate the methanol. Control Filter discs were soaked in sterilized distilled water or 75 µl methanol and evaporated.

Results

Location and sampling

Thirteen different cyanobacterial species were isolated in the period from July to September (Table 1). The Kaithawali site of lake was densely populated by cyanobacterial bloom, whereas, least population was recorded at Basantpur site. Dominating blooms of *M. aeruginosa* was recorded at Kaithawali followed by Bhikpur and Rajpur sites (Table 2).

Table 1: Location and Sampling of Cyanobacteria from Lake Suraha

S.No.	Location	Total Number of Cyanobacterial spp.	Number of *Microcystis aeruginosa* isolates
1.	Bhikpur	9	**1**
2.	Basantpur	7	**-**
3.	Kaithawali	10	**2**
4.	**Rajpur**	**9**	**1**

Table 2: Cyanobacterial Diversity at experimental sites in Suraha

S.No.	Species	Bhikpur	Basantpur	Kaithawali	Rajpur
1.	*Anabaena* spp.	++	+	+	+
2.	*Nostoc* spp.	+	+	++	++
3.	*Oscillatoria* spp.	+	-	+	++
4.	*Microcystis* spp.	+	-	+++	+
5.	*Cylindrospermum* spp.	-	+	+	+
6.	*Rivularia* spp.	-	-	+	+
7.	*Gloeocapsa* spp.	+	+	-	-
8.	*Aphanothece* spp.	+	++	+	+
9.	*Aulosira* spp.	+	+	+	+
10.	*Anabaenopsis* spp.	-	-	+	-
11.	*Lyngbya* spp.	+	-	+	+
12.	*Calothrix* sp.	-	+	-	-
13.	***Gloeotrichia* sp.**	**+**	**-**	**-**	**-**

Analysis of water

The physicochemical properties of lake water at different experimental sites were studied. The mean temperature ranged between 23-24 °C (8 a.m.) and 31-32 °C (4 p.m.). The pH of water was alkaline and ranged between 8.3 to 8.6. The water of Kaithawali and Rajpur site was more alkaline compared to Bhikpur and Basantpur site (Table 6). More free CO_2 was recorded in morning samples compared to evening samples. For example, the level of free CO_2 was 24 mg l^{-1} in the morning and 15 mg l^{-1} in the evening at Basantpur site. Total alkalinity ranged between 240 and 198 mg l^{-1}. The level of dissolved oxygen (DO) in water was analyzed and higher level was recorded in evening samples compared to morning samples. In general, the DO ranged between 15.4-22mg l^{-1} in evening samples and 8.7-13.1 mg l^{-1} in morning samples. The BOD vales of the samples was estimated after 5 days of incubation and it ranged between 10.8-20 mg l^{-1}. The BOD was more (20 mg l^{-1}) at Kaithawali site compared to other sites (Table 3).

Table 3: Physicochemical Properties of Water

S.No.	*Physicochemical properties of water	Bhikpur		Basantpur		Kaithawali		Rajpur	
		8 A.M.	4 P.M.	8 A.M.	4 P.M.	8 A.M.	4 P.M.	8 A.M.	**4 P.M.**
1.	Temperature (°C)	24	32	24	32	23	31	23	**32**
2.	pH	8.3	-	8.4	-	8.6		8.6	**-**
3.	Free CO_2 (mg l^{-1})	20.0	15	24	15	18	14	22	**16**
4.	Total Alkalinity (mg l^{-1})	210	-	198	-	240	-	215	
5.	Bicarbonate Alkalinity (mg l^{-1})	122	-	165	-	98	-	107	**-**
6.	DO (mg l^{-1})	8.7	15.4	10.1	16.3	11	19.5	13.1	**22**

7.	BOD (mg l^{-1})	12	-	16	-	20	-	10.8	-

** Values are mean of 12 samples taken at a regular interval of 7 days. - = not recorded*

Growth measurement

The growth of *M. aeruginosa* isolates was recorded *in vitro* in B-12 medium. The exponential phase lasts up to day 14 after inoculation followed by stationary phase. Rapid growth of *M. aeruginosa* isolate SPM15 was recorded compared to other isolates, however, no significant (P = 0.05) difference in growthof the isolates was recorded on M-12 media (Table 4). On the basis of growth rate and preliminary toxin analysis isolate SPM15 was selected for further study.

Table 4: The growth of *Microcystis aeruginosa* isolates cultured in B-12 medium

Days	Population of *Microcystis aeruginosa* isolates (log cells ml^{-1} culture)			
	SPM 7	SPM9	SPM15	SPM19
0	1.5±.02	1.5±.009	1.5±.011	1.5±.01
2	1.54±.01	1.64±.014	2.14±.016	2.28±.016
4	1.74±.014	1.97±.013	3.51±.012	4.66±.012
6	2.38±.02	2.71±.016	5.02±.01	5.76±.017
8	3.15±.011	3.51±.02	6±.018	6.46±.009
10	3.92±.016	4.12±.015	6.6±.011	6.76±.02
12	4.49±.01	4.55±.014	6.77±.012	6.86±.012
14	5.2±.013	5.36±.017	6.8±.013	6.76±.018
16	5.32±.011	5.44±.01	6.69±.009	6.47±.016
18	5.42±.015	5.4±.019	6.46±.015	6.09±.012
20	5.42±.016	5.4±.02	6.2±.012	5.52±.011

Effect of environmental factors on growth

Effect of temperature, pH, light nitrate and phosphate on growth of *M. aeruginosa* SPM 15 was studied. Significantly (P=0.05) higher growth rates were observed at 30°C than at 25 and 20°C (Fig. 1). For example, the population of *M. aeruginosa* SPM 15 was 6.14, 5.47 and 5.08 log cells ml^{-1} at 14th day of incubation (Fig. 1). The pH of B-12 medium had a marked effect on the growth. A significantly (P= 0.05) higher growth rate was recorded when the pH of media was changed from 7 to 9 thereafter, the growth decreased non significantly. Maximum growth was obtained at pH 9. For example, at pH 9 the population was 4.62, 6.6 and 6.8 on 4, 10 and 14 days of incubation at pH 9 respectively, whereas, it was 4.66, 6.4 and 6.6 at pH 10 and 2.28, 5.18 and 5.08 log cells ml^{-1} at 4, 10 and 14 days, respectively at pH 7 (Fig. 2). The effect of light intensity on growth was recorded on 4 light intensities *i.e.*, 3. 5, 7.0, 35 and 70 µE m^{-2} s^{-1}. The growth was decreased significantly (P = 0.01) at lower light intensities *i.e.* 3.5 and 7.0 µE m^{-2} s^{-1} compared to 37.0 and 70 µE m^{-2} s^{-1}. For example, the population was 5.2, 5.36, 6.76 and 6.8 log cells ml^{-1} on day 14 at 3.5, 7.0, 37.0 and 70 µE m^{-2} s^{-1}, respectively (Fig. 3).

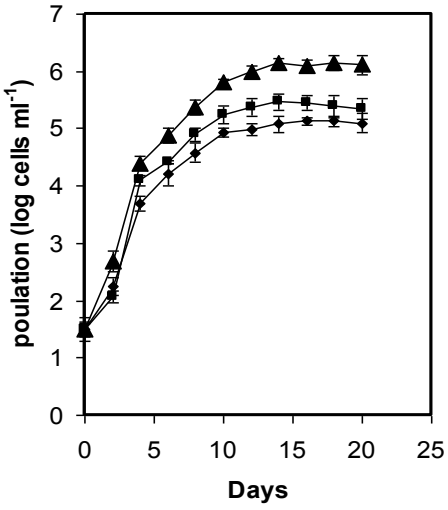

Fig. 1: Effect of Temperature on Growth of *Microcystis aeruginosa*. Symbols represent ◆ = 20 °C, ■ = 25 °C and ▲ = 30 °C. Data are mean of six replicates; | = S.D.

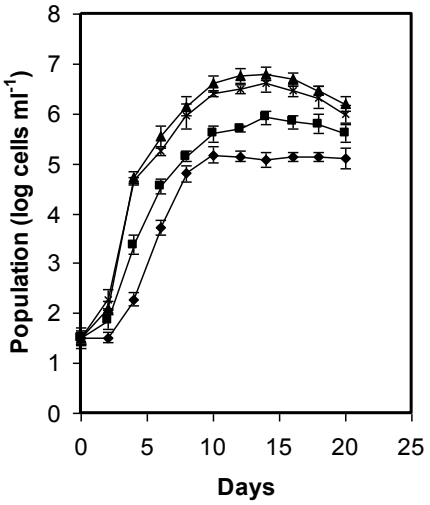

Fig. 2: Effect of pH on Growth of *Microcystis aeruginosa*. Symbols represent pH; ◆ = 7, ■ = 8 and ▲ = 9 and ✕ = 10. Data are mean of six replicates; | = S.D.

Figures 4 and 5 show the growth curves of *M. aeruginosa* SPM 15 grown under nutrient-deficient conditions. As B-12 medium contains rather high amounts of nitrogen and phosphorus, these nutrients had to be reduced to 1/10 and 1/20 their original levels to provide nutrient-deficient conditions. In the nitrate deficiency experiment, higher growth rates were observed for cells grown

under standard nitrogen conditions, whereas the cells grown in either 1/10 or 1/20 nitrogen medium showed retarded growth from day 4 (Fig. 4). For example, on day ten, 5.13, 5.33 and 6.47 log cells ml-1 were obtained in the cultures grown at 1/20, 1/10 and standard nitrate conditions. Similar growth pattern was observed in the phosphate deficiency experiment; however, in phosphate deficiency marked difference in growth rate was observed at all the concentrations. Maximum growth rate was observed at standard concentration followed by 1/10 and 1/20 (Fig. 5). The exponential phase biomass (from day 14 cultures) of *M. aeruginosa* SPM 15 obtained from the experiments; temperature, pH, light, nitrate and phosphate deficiency were freeze dried and used for microcystin estimation.

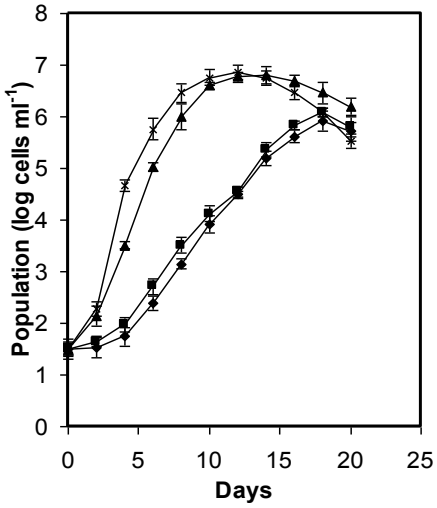

Fig.3: Effect of light on Growth of *Microcystis aeruginosa*. Symbols represent light intensity μ E m^{-2}; ◆ = 3.5, ■ = 7.0 and ▲= 37.0 and × = 70. Data are mean of six replicates; | = S.D.

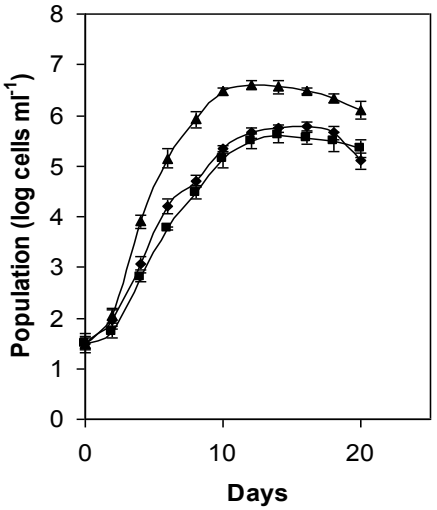

Fig. 4: Effect of nitrate on Growth of *Microcystis aeruginosa*. Symbols represent nitrate concentration; ◆ = 1/10, ■ = 1/20 and ▲= normal as recommended in media. Data are mean of six replicates; | = S.D.

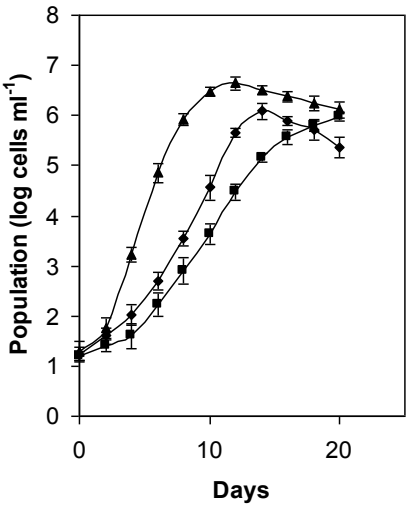

Fig.5: Effect of phosphate on Growth of *Microcystis aeruginosa*. Symbols represent nitrate concentration; ◆ = 1/10, ■ = 1/20 and ▲= normal as recommended in media. Data are mean of six replicates; | = S.D.

Discussion

Few of the phytoplankton blooms collected from Lake Suraha in this investigation were toxic and *M. aeruginosa* was the most dominant species. One out of 4 isolates was found to be toxic. *M. aeruginosa* is the most common toxic cyanobacterium. Scott (1986) reported that the toxicity of water-blooms was always associated with *M. aeruginosa* forma *aeruginosa* and that nontoxic strains of *M. aeruginosa* forma *aeruginosa* were not common in such blooms. The toxicity of the cells in culture varies according to the growth phase. Carmichael and Gorham (1981) and Scott (1986) demonstrated that there were toxic and nontoxic blooms and the phytoplankton assemblages as well as their toxicity varied greatly depending on where and when the blooms were collected. These parameters changed even on a daily basis. No special morphological difference was seen among the isolated toxic and nontoxic *M. aeruginosa* strains. Moreover, morphological changes of the cells occurred while they were maintained in culture in our laboratory. Therefore, it is not possible to study toxic *Microcystis* spp. on the basis of their morphological characteristics alone. Establishment of axenic strains of *Microcystis* is necessary for physiological, biochemical and genetic studies. The toxicity of *M. aeruginosa* cells was variable depending on the growth phase. Hughes *et al.*,(1958) reported that the toxicity of the *M. aeruginosa* NRC-1 strain was greatest in the mid-log phase (under normal growth conditions) or in the late-log phase (under the slow growth condition). Watanabe *et al.* (1992) found that the toxicity of *M. aeruginosa* cells was the highest at the beginning of the stationary phase but low in the mid-log phase. In our experiments, the greatest toxicity of isolate SPM15 cells was observed in

the mid-log to early stationary phase. Considering these results, the toxicity of toxic *Microcystis* cells is variable, dependent upon the strains used and the culture conditions. This observation agrees with those of Hughes *et al.*,(1958) and Falconer *et al.*,(1981).

The effects of environmental factors on microcystin concentrations have been extensively studied and microcystin production is usually highest under optimal growth conditions (Sivonen and Jones, 1999). The toxicity of *M. aeruginosa* depends on the growth phase, light intensity, temperature and nutrients (Watanabe and Oishi 1985; Westhuizen *et al.*,1986; Codd and Poon, 1988; Ohtake *et al.*,1989). Case studies of the population dynamics of microcystin-producing cyanobacteria in relation to microcystin concentrations in water bodies have shown that changes in microcystin concentrations cannot be explained by changes in population density alone (Fastner*et al.*, 2002; Jähnichen*et al.*, 2001; Welker *et al.*, 2001).

Environmental factors may affect microcystin concentrations in two principal ways: by regulating the abundance of microcystin-producing strains within a population and by regulating microcystin production by the toxigenic strains. In culture experiments with single strains of different cyanobacteria, effects of photosynthetically active radiation (light intensities) (Sivonen, 1990a, b; Utkilen and Gjølme, 1992; Böttcher *et al.*, 2001;Hesse and Kohl, 2001), pH (Jähnichen *et al.*, 2001), iron (Utkilen and Gjølme, 1995), phosphorus (Oh*et al.*, 2000) and nitrogen (Orr and Jones, 1998; Long *et al.*, 2001), as well as interactive effects of phosphorus and nitrogen (Vezie*et al.*, 2002), on microcystin content have been found. In present investigation microcystin production was found to be positively correlated with growth rate under nitrogen and phosphorus limitation. Similar findings have been reported by (Orr and Jones, 1998; Oh*et al.*, 2000; Long *et al.*, 2001), while such correlations were not reported for the other growth factors. Overall, there is no uniform pattern in the regulation mechanism of microcystin content and production that can be concluded from these studies. In fact, inconsistent results for effects of the same parameter were reported. As for light, it was found that microcystin content was enhanced with increasing irradiance (Utkilen and Gjølme, 1992) and with decreasing irradiance (Sivonen, 1990a, b) and that it was not affected by light (Böttcher*et al.*, 2001). However, the findings suggest that microcystin content was enhanced in the cells grown at increased irradiance. These apparent contradictions may be due to a great extent to differences in methodology, since different species and strains with different microcystin variants were studied in batch, semi-batch, or continuous cultures.

Conclusion

The results are an indicative of the presence of microcystin, a cyanotoxin from M. aeruginosa in the lake Surha of Ballia. However, their toxicity varied greatly depending on different environmental and growth parameters. These toxins may affect the fishes and other aquatic animals in lake. A detailed study is required to anyluyse3 the effect of cyanotixin on aquatic community of lake surha

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