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BLOOM FORMING CYANOBACTERIA: A THREAT TO AQUACULTURE PRACTICE IN INDIA

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In recent years, due to increase in eutrophication of water bodies, which involves enrichment of plant nutrients, specially phosphorus components, there has been a major shift in phytoplankton community leading towards 'bloom forming' toxic cyanobacteria. These species are capable of producing potentially lethal toxins and have been implicated in numerous instances of fish kills and deaths of domestic livestock. In Darbhanga, three such cases were recorded. Studies revealed a thick cyanobacterial bloom dominated by *Microcystis aeruginosa* (7.6 x 10^2 units/ml) and *Anabaena*. Other toxic cyanobacterial genera included *Nostoc, Oscillatoria, Gleotrichia* and *Aphanizomenon*. Bioassay test was conducted on *Channa punctatus*. The aliquot was prepared from the cellular sediments collected from thick bloom situation of the respective ponds by lysing the cell walls. All the fish died within 18 hours indicating the sample to be positive. In moribund fish, the symptoms characterising neurotoxic effects of algal poisoning were well marked. In India, the literature, of late, suggests that the physical and chemical qualities of inland water is rapidly changing towards eutrophication due to pollution. As a consequence frequency of the 'toxic-algal-blooms' can be expected to increase posing a threat to aquaculture practices.

INTRODUCTION

Since, 1940s -'50s, the incidence of cyanobacterial bloom has been on increase. This may, at the outset, be attributed to eutrophication of water bodies due to increased urbanization of catchments, domestic and municipal sewage disposal, leaching of fertilizers and other forms of cultural eutropohication (Boney, 1975).

The planktonic cyanobacteria share some properties that are unique to the group. One of these is possession of specialized intracellular gas filled vacuoles adding buoyancy to the cell (Fritsch, 1945). In mass production of the constituent organisms gives rise to the formation of buoyant scum that secondarily concentrates towards the shore under the influence of wind. This causes a rapid change in colouration of the water bodies drawing the attention of even casual observers and earning a name in the collective term "Water-bloom". Some of the members of this group have gained recognition for producing potentially lethal toxins and are implicated in numerous instances of fish kill and death of

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domestic livestock (Gorham, *et al.*, 1964; Collins, 1978; Carmichael, 1981) and can develop nuisance blooms in response to nutrient enrichment or eutrophication (Smith *et al*, 1987).

Numerous cyanobacterial blooms in small and large water bodies have been reported (Karl, 1970; Edler *et al.*, 1982; Moita *et al.*, 1984; Skulberg *et al.*, 1984). In 1951, the toxicity of living cells of *Microcystis aeruginosa*, when injected intraperitonially into carp, was demonstrated and the lethal dose was calculated to be 100 mg net weight/kg body weight of fish (Gentile, 1971). Populationdensity of 5 x 10^5 cells/ml of *Aphanizomenon flos aquae* was reported to kill varieties of fish with variable survival rates between 15 minutes to 240 minutes (Gentile, 1971; Carmichael *et al.*, 1975). Isolated reports on poisoning/mass mortality in swine (Beasley *et al.*, 1983); dairy cows (Galey *et al.*, 1987); shellfish (Frage, 1984) and fishes (Parker, 1981; WHO, 1984) are also available.

In India, it is often referred that algae reported to be toxic in foreign texts do not elaborate toxins in our inland waters. It is also perceived as a rare event. Steinberg and Hartmann (1988) have opined that nutritional enrichment of water bodies, especially phosphorus components, has resulted in significant shift in phytoplankton community towards cyanobacteria specially toxic genera. This provides ample evidence to answer the prevalent misconception in our inland water context. Practically, it is the micronutrient environment that decides the onset of the toxigenic potentiality of a given species.

In parts of North Bihar, mass mortality of freshwater fish in which tonnes of fishes die within hours, is a common feature during summer and monsoon months. The phenomenon is locally called as 'UJAH'. There such consecutive cases recorded during 1995, 1996 and 1997 (referred to as case I, II and III, respectively) in three different water bodies were investigated. Scores of people using the pond water for routine purposes developed skin irritations and erythematous lesions. Consequently, a study was undertaken to determine the role of cyanobacteria in causing mass mortality of fishes.

MATERIAL AND METHODS

In most cases, grab samples were taken from 'thick-algal-bloom' situation. However, the samples also represented 'bloom' and 'non-bloom' situations of the respective ponds. Sterile 250 ml polythene bottles were used for collection and samples were stored at 4°C prior to analysis.

A Sedgwick-Rafter counting cell was used for counting the cells following the methods described by APHA-AWWA-WPCF (1980). The samples were treated with a drop of formalin and stirred well. Per cent incidence of different genera was determined by counting a total of 100 cells/colony (in triplicate) in one 'strip'.

In each case, aliquot for bioassy was prepared using 25 ml of cellular sediments obtained from the representative samples collected from relatively fresh thick bloom areas of the respective ponds. The aliquot was prepared by lysing the cell-walls to free intracellular components including toxins. Lysing was acomplished by three successive freeze-thawing.

The bioassay test was conducted on *Channa punctatus* (weighting 10-12 g) separately. The test group of 10 fish were administered with 10 units (through insulin syringe) of the aliquot. The control fish were injected with similar amount of 0.9% normal saline.

RESULTS AND DISCUSSION

Morphometric and bathometric features of the ponds (Table 1) indicate that the size of the pond has practically no impact on the incidence of cyanobacterial blooms.

Table 1. Morphometric and bathometric features of the **Gangasagar, Harahi and K. M. Tank of Darbhanga**

Features	Ganga Sagar	Harahi	K. M. Tank
	Case I	Case II	Case III
Maximum effective length	614 m	316 m	32 m
Maximum effective width	302 m	316 m	28 m
Maximum depth	5.80 m	570 m	4.80 m
Depth (mean)	2.50 m	2.10 m	2.00 m
Surface area (approx.)	20 ha	11.6 ha	2.2 ha

The blue-green algae, most commonly found included *Microcystis, Anabaena, Oscillatoria, Aphanizomenon, Synechoccus, Gleotrichia, Oformosa, Nostoc* and *Lyngbya.* All these genera represented by almost 40 species have earlier been reported form ponds of Darbhanga (Ahmad and Siddiqui, 1990). Out of the 50 genera consisting almost 250 species of freshwater blue-green alage, only six *viz., Microcystis, Anabaena, Aphanizomenon, Oscilatoria, Nostoc* and *Gleotrichia* have been considered toxic (Gentile, 1971; Collins, 1978; Christensen, 1980).

Records on the per cent incidence of the five genera of toxic blue-green algae (Table 2) indicate the dominance of *Microcystis* represented by *Microcystis aeruginosa* followed by *Anabaena, Oscillatoria, Nostoc* and *Gleotrichia.* A record incidence of *Microcystis* (86.0%) with cellular density being 7.6 x 10^2 units/ml was encountered in case II which was followed by case I (80.0%) with cellular density 7.4 x 10^2 units/ml (Table 3). In case III, however, per cent incidence of both *Microcystis* and *Anabaena* was comparable.

Table 2. Percent incidence of toxic cyanobacterial genera in ponds of Darbhanga

	CASE I				CASE II				CASE III			
	TB*	B*	NB*	AV*	TB	B	NB	AV	TB	B	N B	AV
Microcystis (*Microcystis aeruginosa*)	80	76	32	62.6	86	62	56	68.0	46	32	30	36.0
Anabaena sp.	13	08	30	13.6	10	26	20	18.6	30	40	36	35.3
Oscillatoria sp.	-	05	10	5.0	-	04	12	5.3	10	12	16	12.6
Nostoc sp.	02	03	20	8.3	-	04	08	4.0	10	08	09	9.0
Gleoctrichia sp.	-	-	-	-	02	-	-	0.6	02	07	08	5.6
Others	05	08	08	7.0	02	04	04	3.3	02	01	01	1.3

*TB= Thick bloom, *B = Bloom, *NB = Non bloom, *AV = Average

Table 3. Cellular density of *Microcystis aeruginosa* in thick bloom, bloom and non bloom situation in ponds of Darbhanga

Water body	Population density x 10^2 (units/ml)	
Ganga Sagar	Thick bloom	7.40
CASEI	Bloom	6.00
	Non-bloom	3.50
Harahi	Thick bloom	7.60
CASE II	Bloom	5.15
	Non-bloom	4.87
K.M. Tank	Thick bloom	4.17
CASE III	Bloom	3.35
	Non-bloom	3.00

In 'bloom' situation of case III, the per cent incidence of *Anabaena* was even higher (40.0%) than *Microcystis* (30.0%) showing cellular density of 3.35 x 10^2 units/ml (Tables 2 and 3). In 'non-bloom' situation cellular density of *Microcystis* varied between 3.0 x 10^2 units/ml to 3.35 x 10^2 units/ml. A record of 5 x 10^2 cells or even higher cellular counts presented by various authors (Gentile, 1971) could not be obtained. However, in present case, the cellular density represents *Microcystis* colony expressed in units/ml following APHA-AWWA-WPCF (1980) and not in terms of cells as reported in previous cases.

Bioassay experiments in all the respective cases were conducted separately maintaining similar experimental conditions. Comparison of results indicated that in each

case fish were able to maintain their normal profile till 2-4 hours following toxin administration, thereby becoming listless with increase in time. Higher frequency of surfacing in order to gulp air was well marked indicating onset of the toxic effect (after 4-6 hours) on respiratory system as indicated by Repavich *et al.* (1990). With lapse of 10-12 hours, gradual loss in balance and uncoordinated body movement was distinct with increase in mucus over body surface.

The first death, in response to the toxic effect, was recorded at 16 hours in case III. However, no fish could bear the toxic insult over 18-20 hours. Repavich *et al.* (1990) has opined that the algal toxin samples used for bioassay are considered to be positive if the test animal dies within 24 hours with symptoms characterising algal poisoning.

Moribund anatomy of the test fish revealed pale gill filaments distended with mucin. The liver, in comparison to control, appeared to be enlarged but apparent sign of engorgement could be marked only in a few fish (cases I and II) indicating haemorrhagic necrosis. Intestinal petechiae were frequently observed.

The blue-green algal toxins have been categorized as neurotoxins, hepatotoxins and neuromuscular blocking agents (Carmichael *et al.*, 1985; Carmichael, 1986a). Of these, neurotoxins, normally produced by *Anabaena* and *Aphanizomenon* genera, have been described as fast acting agents causing respiratory distress leading to death in mice within minutes to hours of ingestion (Carmichael, 1986b). The toxic component of the toxin, tentatively an alkaloid, has often been referred to as very fast death factor (VFDF).

Microcystis aeruginosa has been reported as most frequent offender (Gentile, 1971) in which production of toxin and its subsequent accumulation as endotoxin are dependent upon the developmental stage of the cell/colony. Obviously, the amount of the toxin likely to be liberated in the water system by *Microcystis* bloom will also vary. Carmichael *et al.* (1975) has described the *Microcystis* toxin as 'hepatotoxic' and two factors designated as Microcystic FDF (Fast Death Factor) and SDF (Slow Death Factor) have been identified. At least in case of FDF, the toxicity has been found to be associated with the peptide component of the toxin. It has been described that the liver engorgement associated with intestinal petechiae characterise *Microcystis* poisoning (Repavich *et al.*, 1990).

Since the present aliquots prepared for bioassay were obtained from natural population, it contained almost all the five genera of toxic blue-green algae in variable frequency of occurrence (Table 2). Thus, a mixed pattern of pathoanatomical manifestations characterising neurotoxic as well as hepatotoxic effects, in present case, needs no further explanation.

Infrequent occurrence of toxic algae in freshwater bodies and their incapability of producing potential lethal toxins thus appears to be more apparent than real. Plenty of reports, however, are available on the quality (both physical and chemical) of the water bodies in India which are undergoing eutrophication mainly due to industrial and domestic sewage. Most of our small water bodies have become stagnant or compact systems due to non-existence of drainage and proper care. As a consequence, the frequency of toxic-algal-bloom can be expected to increase. The water bodies, once yielding aquatic gold are gradually changing into chemical pools harbouring undesired biomass like toxic cyanobacterial forms.

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