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Oscillatoria sp. Bloom and the Occurrence of Microcystin in the River Buriganga, Dhaka, Bangladesh

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Abstract: Potentially toxic cyanobacterial blooms are becoming common in the freshwater reservoirs in all regions of Bangladesh. The River Buriganga is a eutrophic urban river in the country and its water is utilized as drinking water supply and other recreational purposes. A bloom of *Oscillatoria* sp. occurred in the river Buriganga during May 2002. Bloom sample was collected and filtered through a glass fiber filter. Methanol-water extract of filtered cells were analyzed by High Performance Liquid Chromatography (HPLC) with UV, detection and Mass Spectrum (MS) detected Microcystin-RR. The concentration of microcystin RR was 0.235 μg L^{-1}. As this river water is supplied to city dweller for drinking and other domestic usage there should have a regular monitoring system for cyanobateria blooms and microcystins to ensure the public health safety. To avoid the microcystins health risk to humans through drinking water, it is advised not to use this river water for drinking or any other domestic purposes until the provisional safety levels of microcystins are reported through the concerned authority.

Key words: *Oscillatoria* sp., cyanobacteria bloom, microcystin, eutrophication

INTRODUCTION

Eutrophication is an increasing problem for aquatic ecosystems in Bangladesh, as in many other countries around the world. Cyanobacterial blooms are very frequent episodes in our reservoirs, most of which are eutrophic or hypereutrophic. This scenario is widespread in all regions of the country, partially as a consequence of favorable climatic conditions, but mainly due to the over-enrichment of rivers, reservoirs and estuaries. The production and release of cyanotoxins is often associated with these cyanobacteria blooms (Ahmed, 2009; Ahmed *et al*., 2008a, b). Most of the toxic cyanobacteria genera have been recognized to produce a range of hepatotoxic toxins- microcystins (Carmichael *et al*., 1988). In recent years, for a variety of reasons, the harmful impact of cyanobacteria on human health has been more reported by Figueiredo *et al*. (2004). The most dramatic example is the human poisoning case attributed to direct exposure to high concentrations of microcystins which occurred in Brazil in 1996 (Jochimsen *et al*., 1998). Concern about the microcystins health risk to humans through drinking water, led the World Health Organization (WHO) to develop and suggest a provisional guideline level of microcystin-LR at 1 μg L^{-1}. Up to now this value has been considered as a safe level in drinking water (Falconer *et al*., 1999).

The supply of clean and safe drinking water is one of the main challenges of public health care in Bangladesh. Traditionally, surface water is the main source of drinking water and consumed without any treatment or after boiling when fuel is available. Resulting, about 4.5 million people suffered from

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watery diarrhea every year, among them a considerable number affected with cholera (Siddique *et al*., 1996). In many of these intensively used eutrophic ponds/lakes cyanobacteria blooms are common and microcystins have been detected occasionally in pond water from several regions, mainly associated with high abundance of *Microcystis* sp. (Ahmed, 2009; Ahmed *et al*., 2008a, b; Aziz, 1974; Welker *et al*., 2005).

Bangladesh has about 700 rivers including tributaries and distributaries. Almost all settlements of the country are established besides river. The Buriganga, a tide-influenced river is passing through West and South of the capital city, Dhaka. About 1.2 million, peoples live in both side of the river and use its water for washing, bathing and other domestic purposes. Water of this river is also supplied to a portion of city dwellers through Chandi Ghat water treatment plant. Recently, there has been an increased use of surface water for human consumption due to arsenic contamination in ground waters. In the case of Dhaka district and perhaps other regions of Bangladesh such a practice could amount of replacing one health hazard with another. Nevertheless, studies dealing with toxins and toxicology of cyanobacteria in Bangladesh waters are not very abundant (Ahmed, 2009; Ahmed *et al*., 2008a, b; Welker *et al*., 2005; Ahmed *et al*., 2007).

This study deals with isolation and characterization of microcystins from a natural bloom of *Oscillatoria* sp. occurring in the river Buriganga, Dhaka.

MATERIALS AND METHODS

The river Buriganga encompasses the West and Southern periphery of Dhaka, the capital city of Bangladesh. It is considered as one of the most polluted urban river in the country. The present study was conducted in the upper reaches of this river (Islambagh zone) from January to December 2002.

Collection and Processing of Samples

Samples of water and phytoplankton were collected fortnightly intervals throughout the study period. The plankton samples were collected using plankton net of 20 µm mesh size and kept in 20 mL glass bottle. All samples were preserved and transported to the laboratory for further analysis. Physico-chemical parameters were recorded by using an ecological HACH fresh water, aquaculture ecological manual, test kit model FF-2. Qualitative and quantitative analysis of phytoplankton were performed using taxonomic keys following Ward and Whipple (1959) and Needham and Needham (1966) methods by Welch (1952).

A bloom of *Oscillatoria* sp. was initiated in the first week of May 2002 and the highest cell density (80% *Oscillatoria*) was recorded on 20 May 2002. Three samples were collected with a plankton net of 20 µm mesh size from different locations of the bloom forming area in the river and the *Oscillatoria* sp. cell density was stated as the average of three counts. A portion of the concentrated samples were filtered through an 0.45 µm glass fiber filter (Whatman GF/C, 47 mm diameter) and dried in an oven at 60-80°C. Dried filters covered with algae cells, were transported to the Institute of Nutrition, University of Jena, Germany for analysis.

Extraction

The algae on the GF/C filter was immersed in 1.0 mL of a mixture of water and methanol (50:50; v:v), sonicated for 20 min and was finally centrifuged (3000 g). The supernatant was filtered through a nylon filter with 0.45 µm pore size.

Chemical Analysis

The HPLC/UV determination of microcystins was carried out following the method by Lawton *et al*. (1994) with some modification (Hummert *et al*., 2001) (C18 column: Phenomenex prodigy, ODS (3), 250×4.6 mm, 5 µm, mobile phases: acetonitrile /water/0.05%TFA). Detection of microcystins was done by an UV detector (Shimadzu SPD-10AV; 238 nm) and a diode array detector (Shimadzu SPD-M6A). The HPLC was coupled by means of an electrospray interface to a single quadrupol mass spectrometer (PE/Sciex API 150EX, Perkin-Elmer,Germany). The detection was carried out in Selected Ion Monitoring (SIM) mode (Hummert *et al.*, 2001).

Microcystins and Nodularin Standards

Standards of Microcystin-RR, Microcystin-LR, Microcystin-YR, Microcystin-LA and nodularin were purchased from Calbiochem/Novabiochem (La Jolla, USA).

Chemicals

HPLC grade acetonitrile and methanol were collected from Baker (Deventer, Netherlands). Water was purified to HPLC grade with a Millipore-Q RG Ultra Pure Water System (Millipore, Milford, USA).

RESULTS

The River Buriganga is increasingly being polluted with city's thousands of industrial units and sewerage wastes. There are three main pollutant discharge routes into the Burigonga; *viz.*, Hazaribugh Tanneries, City drains along the river and Pagla sewerage treatment plant outfall. The physico-chemical parameters of the study area (Station, Islambagh) are shown in Table 1. The details of physico-chemical parameters, phytoplankton abundance and pollution status of the River Buriganga are described by Ahmed *et al.* (2007). Recent investigation also found that the monthly average faecal coliform in the water, zooplankton and phytoplankton sample of Buriganga were 3.99×10^{9}, 4.54×10^{3} and 4.28×10^{2} (cfu L^{-1}), respectively. *Vibrio cholerae* 01 and *V. cholerae* 0139 were isolated from water, zooplankton and phytoplankton samples (Ahmed *et al.*, 2007).

In the original bloom sample the cell density of *Oscillatoria* sp. was 1.6×10^{4} cells L^{-1}. During the bloom the dissolved oxygen, free carbon dioxide, nitrite nitrogen, alkalinity and ammonia, of river water were recorded as 1.5, 63.0, 0.86, 175.0 and 2.05 mg L^{-1}, respectively. The pH was 9.5 and the water temperature was between 28-30°C. The HPLC analysis of *Oscillatoria* sp. bloom extract showed a peak corresponding to the retention time of standard microcystin-RR (Fig. 1). The concentration of microcystin-RR was 0.235 µg L^{-1} and 14.68 pg $cell^{-1}$.

Table 1: Monthly variations of Physico-chemical parameters in the River Buriganga (Station: Islambagh) from January to December 2002

Parameters	Jan.	Feb.	Mar.	Apr.	May.	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Monthly average ±SD
Alkalinity (mg L^{-1})	290	450	380	237	175	180	125	121	110	66	62	250	203.83±17.14
Ammonia (mg L^{-1})	2.3	2.32	2.56	3.22	2.05	1.4	2.3	2.1	1.4	2.6	2.03	2.3	2.19±0.47
Free CO_2 (mg L^{-1})	37	38	174	41.4	63	58	12.4	13.1	24	7.6	6.2	36	2.55±43.48
Chloride (mg L^{-1})	44	112.8	102.3	88.9	72.9	62.9	60.2	12.8	21	18	17	80.5	57.77±33.62
Dissolved O_2 (mg L^{-1})	2.12	1.52	0.25	2.66	1.5	3.5	3.52	4.12	3.08	3.5	4.4	2.12	2.69±1.17
Hardness (mg L^{-1})	160	243	235	225	207	203	200	185	175	185	178	150	195.50±27.64
Nitrite (mg L^{-1})	1.65	0.66	0.52	0.8	0.86	0.04	0.04	0.01	0	0.01	0	0.02	0.38±0.50
pH	9.5	9.5	8.5	9.6	9.5	8.2	8.1	7.6	6.7	8.7	9.1	8.8	8.65±0.85
Tempt. water (°C)	23.58	27.22	32.22	35.55	35.65	34.44	35.5	31.11	26.22	22.66	14.44	18.6	28.09±6.85
Transparency (cm)	15	9.8	6	12	26.5	30.5	62	55	50	63	48	29	33.90±20.05
Total dissolved solids (mg L^{-1})	440	450	661	650	253	240	84	81	66	80	176	350	294.25±207.36
Conductivity (µsec cm^{-1})	885	933	1369	1335	531	515	176.8	175.2	139.8	172.6	360	890	623.35±430.57
Salinity (ppt)	0.5	0.5	0.7	0.6	0.3	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.30±0.20

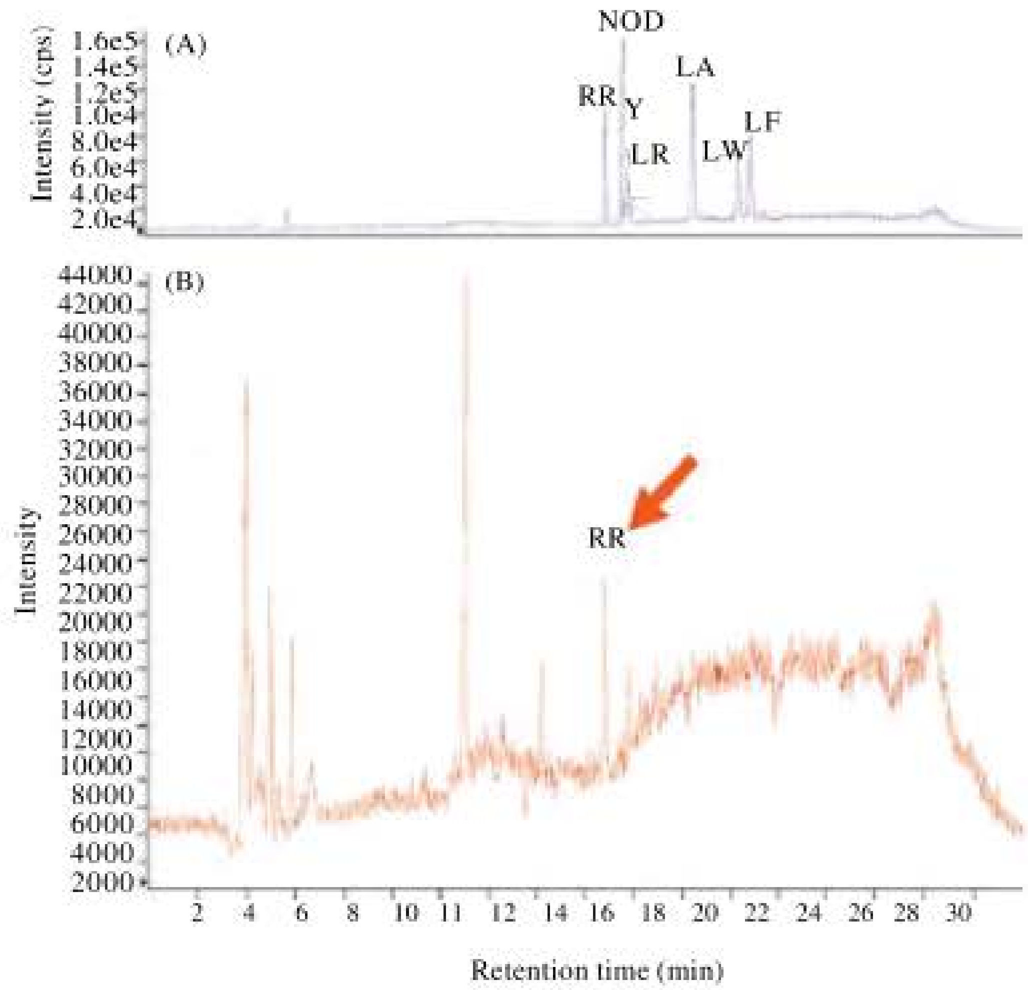

Fig. 1: (A) HPLC separation of standards containing Microcystins and Nodularin: Microcystin-RR, Micricystin-LR, Microcystin-LA, Microcystin-YR, Microcystin-LW, Microcystin-LF. (B) Chromatograms of *Oscillatoria* sp. Blooms extracted with water:methanol (50:50; v:v), UV detection 238 nm wave length

DISCUSSION

The occurrence of cyanobacteria blooms are very common phenomenon in the freshwater environments in Bangladesh. The main factors leading to periodic cyanobacteria proliferations were pointed out as increased dissolved organic nutrients, long sunshine hours and favorable water temperature (Ahmed *et al*., 2007). As in other tropical country, cyanobacteria blooms phenomena will often last year round occurring in many eutrophic lakes and reservoirs in Bangladesh when the climate permits.

The HPLC analysis of *Oscillatoria* sp. extract showed one peak, the retention time of which agreed well with standard Microcystin-RR (Fig. 1). The results of HPLC-MS revealed the identification of microcystin-RR according to its corresponding molecular weight. Further structural variants of microcystins were not detected. The concentration of microcystins detected in the present study was (0.235 $\mu g\ L^{-1}$) less than the previously detected microcystins from a lake and ponds in Bangladesh (Ahmed *et al*., 2008a, b; Welker *et al*., 2005). And that should be due to the differences in location, cell type, cell densities and environmental factors. Welker *et al*. (2005) in a study at three different regions in Bangladesh detected microcystins in 39 ponds, mostly together with varying abundance of potentially microcystin-producing genera such as *Microcystis, Planktothrix* and *Anabaena*. Total microcystin concentrations in their study ranged between <0.1 and up to >1000 $\mu g\ L^{-1}$ and more than half of the positive samples contained high concentrations of more than 10 $\mu g\ L^{-1}$. In Australia, a safety factor for tumor promotion is 1.0 μg microcystins or nodularins L^{-1} (Falconer *et al*., 1999). In Canadian drinking water maximum accepted concentration for MC-LR is 0.5 mg L^{-1} and for other microcystins, 1 $\mu g\ L^{-1}$ of total microcystins (Carmichael, 1995).

The occurrence of *Oscillatoria* sp. blooms in ponds, lakes or rivers that produce hepatotoxic microcystins is a problem, especially if the water is utilized as a drinking supply and/or for recreational purposes. Epidemiological investigations have demonstrated that microcystins cause stomach and intestinal inflammation, liver cancer and disease of the spleen in humans who drink water containing microcystins (McDermott *et al.*, 1998; Ding *et al.*, 2000; Zhou *et al.*, 2002). The Dhaka Water Supply And Sewerage Authority (DWASA) supplied 39.1 million liter of waters per day from the river Buriganga as drinking water to the residents of Dhaka city through its Chandi Ghat water treatment plant.

Recently, it is also evident that microcystins does accumulate in the liver and muscle of fish when they are exposed to toxic cyanobacteria bloom (Magalhaes *et al.*, 2001; Deblois *et al.*, 2008). In tropical and subtropical climates, where blooms can be permanent, chronic year-round exposure is likely to occur, leading to potentially high levels of microcystin contamination. So, the effect of cyanobacteria blooms (microcystins) on human through direct exposure or food chain in Bangladesh waters remains to be identified. Moreover, it is necessary to have regular monitoring on abundance of cyanobacteria in waters for public health safety.

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