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Anabaena sp. bloom and the occurrence of microcystin-LR from a eutrophic pond in Bangladesh

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Abstract

A bloom of *Anabaena* sp. occurred in a freshwater pond in Brahmanbaria. 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, MS and MS-MS detection detected three variants of microcystins viz, [D-Asp³, Dha⁷] Microcystin-LR, [Dha⁷] Microcystin-LR and Microcystin-LR. The total concentration of microcystins was 4.0 µg l⁻¹, well above the WHO provisional guideline value for drinking water. So, the cyanotoxin risk assessment has become important to protect public health in Bangladesh where surface water is used as drinking-water source.

Keywords: *Anabaena*, cyanobacteria bloom, microcystin, Bangladesh.

1. Introduction

The rapid increase of human population and consequent intensification of agricultural and industrial activities along with deficient water management have led to the enhancement of eutrophication in superficial freshwater bodies used for domestic purposes and as drinking water sources [1]. Eutrophication of freshwaters and appearance of cyanobacteria blooms have become now a serious problem in Bangladesh. Pond water is directly used intensively or extensively for personal hygiene, washing of clothes and dishes, bathing of cattle, cooking rice and aquaculture. In many of these intensively used eutrophic ponds cyanobacteria blooms are common, and microcystins have been detected occasionally in pond water from several regions, mainly associated with high abundance of *Microcystis* sp. [1-6]. The occurrence of microcystins in freshwaters may invite another scourge like arsenic for public health in Bangladesh.

This paper describes the first report of microcystin-LR from a natural bloom of *Anabaena* sp. occurring in a freshwater pond.

2. Materials and Methods

The study pond was 0.74 ha in size and located in Brahmanbaria district (23°58' N latitude 91°06' E longitude) 98 km east from Dhaka. *Anabaena* sp. bloom was initiated in the first week of April, 2008 and the highest cell density (90% *Anabaena*) was recorded on 14 April 2008. A portion of the concentrated samples were filtered through a 0.45 µm glass fiber filter (Whatman GF/C, 47 mm diameter) and dried in an oven at 60-80 °C.

2.1 Extraction

The GF/C filters were extracted with 2.0 ml of mixture water and methanol (50:50; v:v) per filter by ice-cooled sonication for 4 min with an ultrasonic probe GM 70 (Bandelin, Berlin, Germany) and subsequent treatment for 15 min in an ultrasonic bath. Extracts were centrifuged (10000 g, 15 min) and the supernatants were filtered using 0.22 µm nylon syringe filters (Roth, Karlsruhe, Germany). The extracts were directly subjected to the liquid chromatography.

2.2 Chemical analysis

The HPLC/UV determination of microcystins was carried out following the methods of Lawton *et al.* (1994) [7] with some modifications [8] C18 column: Phenomenex prodigy, ODS (3), 250 x 4.6 mm, 5 µm, mobil phases: acetonitrile /water/0.05% TFA). Detection of microcystins was done by the use of an UV detector (Shimadzu SPD-10AV; λ=238 nm).

HPLC/MS and HPLC/MS-MS analysis were applied to ensure the identity of the toxin peaks in the chromatograms. Liquid Chromatography was performed with a PE Series 200 Quaternary Pump and a PE Series 200 autosampler (Perkin-Elmer, Shelton, CT, USA). The chromatographic separation was carried out on a reversed-phase column (Luna C18(2), 5 μm 250 x 4.6 mm I.D., Phenomenex, Torrance) using gradient elution (0 min 20% B, 15-17 min 90% B, 18-30 min 20% B) with a flow rate of 1 mL min⁻¹, throughout. Mobile phases consisted of 5 mM ammonium formate and 53 mM formic acid in (A) water and (B) water-acetonitrile (10:90, v:v), respectively. The HPLC was coupled by means of an electrospray interface to a single quadrupole mass spectrometer (API 150, PE Sciex Instruments, Canada) and additionally to a triple quadrupole mass spectrometer (API 365, PE Sciex Instruments, Canada). The detection was carried out in selected ion monitoring (SIM) mode using LC/MS and multiple reactions monitoring mode (MRM) using LC/MS-MS [9].

2.3 Quantification

Since reference materials for desmethylated microcystins are not available commercially, determination of the concentrations of desmethyl-MCs, [D-Asp³, Dha⁷] MC-LR, and [Dha⁷] MC-LR, was performed using the standard calibration curves of MC-LR. This approach should be kept in mind looking at the toxin values given in this paper.

2.4 Chemicals

Reference standards of Microcystin-RR, -LR, -YR, -LA, LF and -LW were purchased from Calbiochem/Novabiochem (La Jolla, CA, USA). Acetonitrile and methanol obtained from VWR (Leuven, Belgium) were HPLC grade. Water was purified to HPLC grade quality with a Millipore-Q RG Ultra Pure Water System (Millipore, Milford, USA). All chemicals were at least analytical grade.

3. Results and Discussion

The density of *Anabaena* sp. was recorded as 3.5 X 10⁶ individuals l⁻¹ in the original bloom sample. During the bloom

the dissolved oxygen, free carbon dioxide and nitrite nitrogen of pond water were recorded as 4.2, 16.0 and 0.66 mg l⁻¹ respectively. The pH was 8.8 and the water temperature was between 24-28 °C. The main factors leading to periodic cyanobacteria proliferations were pointed out as increased dissolved organic nutrients, long sunshine hours and favorable water temperature [3, 10]. As in other tropical country, cyanobacteria blooms phenomena will often last year round occurring in many eutrophic lakes and ponds in Bangladesh when the climate permits. HPLC analysis of *Anabaena* sp. extract showed three peaks, the retention time of which agreed well with standard [D-Asp³, Dha⁷] Microcystin-LR, [Dha⁷] Microcystin-LR and Microcystin-LR. (Fig. 1). The results of HPLC-MS revealed the identification of three variants of microcystin-LR (Fig. 2), according to their corresponding molecular weight: [D-Asp³, Dha⁷] Microcystin-LR (at m/z 967.5 [M+H]⁺), [Dha⁷] Microcystin-LR (at m/z 981.6 [M+2H]²⁺) and MC-LR (at m/z 995.5 [M+H]⁺). In *Anabaena* sp. sample the amount of dmdm- MC-LR was the highest (2.02 $\mu\text{g l}^{-1}$) followed by dm-MC-LR (1.29 $\mu\text{g l}^{-1}$) and MC-LR (0.69 $\mu\text{g l}^{-1}$). The concentration of total microcystin MC-LR (4.0 $\mu\text{g l}^{-1}$) is much above the WHO provisional guideline value of 1 $\mu\text{g l}^{-1}$ MC-LR for drinking water [11]. A child of 10 kg body weight will already be exposed to the TDI (tolerable daily intake) through consumption of 100 ml of water containing 4.0 $\mu\text{g l}^{-1}$ MC-LR (as proposed by WHO 4 ml of water containing 100 $\mu\text{g l}^{-1}$ of MC-LR) [12]. The occurrence of *Anabaena* sp. blooms in lakes/ponds that produce hepatotoxic microcystin-LR is a problem, especially if the water is utilized as a drinking supply and/or for aquaculture. It is evident that, MC-LR is a potent cancer promoter in mammals [13]. Thus, chronic exposure to low concentrations of microcystins in drinking water can be a serious problem to public health, contributing to promotion of cancer in humans. Epidemiological studies have already related the presence of microcystins in drinking water to an increase in the incidence of colorectal cancer [14] and primary liver cancer [15, 16].

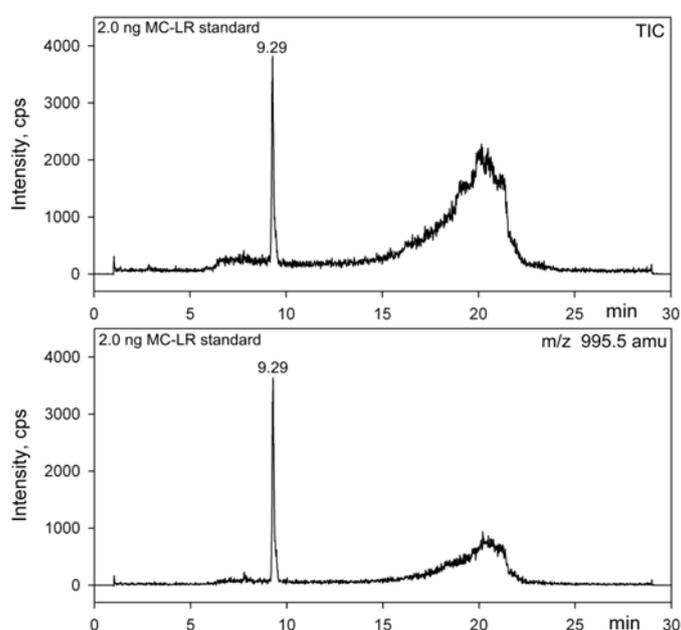


Fig 1: LC-MS total ion chromatogram (TIC) and monitoring of [M+H]⁺ + 995.5 amu (m/z) of a microcystin-LR reference standard solution, 2 ng on column.

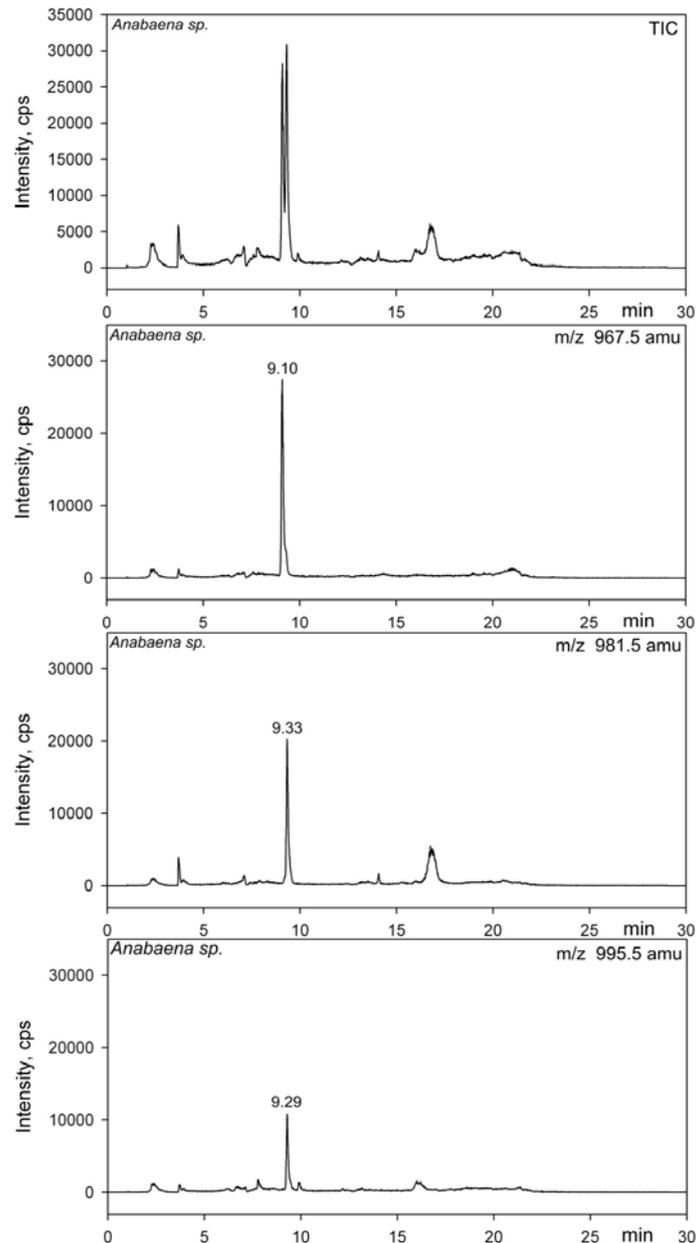


Fig 2: LC-MS in multiple ion monitoring mode (MIM) of extracts of the filter retentate from a water sample containing *Anabaena sp.*

Accumulation of microcystin in fish is a potentially important route of exposure for humans. The freshwater fish *Oreochromis niloticus* accumulates microcystins in the guts, liver and kidneys [17, 18] but there are fish that can also accumulate these toxins in the muscle tissue, posing high risks to humans that consume contaminated fish [19]. So, the effect of cyanobacteria blooms (microcystins) on aquatic animals and human through direct exposure or food chain in Bangladesh waters remains to be identified.

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