VNU Journal of Science, Natural Sciences and Technology 26 (2010) 172-177

Occurrences of *microcystis* spp. and microcystins in some cyanobacterial blooms in freshwater bodies in Vietnam

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Nhận ngày 16 tháng 3 năm 2010

Abstract. This paper reports the analyses of cyanobacteria *Microcystis* spp. and toxin occurrence in bloom samples from some freshwater bodies in Vietnam. Six species of *Microcystis* (*M. aeruginosa*, *M. botrys*, *M. panniformis*, *M. wessenbergii*, *M. flos-aquae* and *M. protocystis*) were identified in 8 bloom samples collected from Lake Tri An (Đồng Nai province), Lake Bien Ho and Lake Duc An (Gia Lai Province), Cua Ngan, Dap Da, Nhu Y, Ho Mung sites (Thua Thien Hue Province), Lake Hoan Kiem (Ha Noi). The results of the quantitative analyses of *Microcystis* spp. and screening of microcystin by ELISA in the water are presented. The cell density of *Microcystis* spp. ranged from $11x10^3$ to $624,5x10^3$ cells/ mL^1 and *M. aeruginosa* as the dominating species. In the water samples, microcystins detected by ELISA varied between 5.854 and 17.966 ng mL^{-1}. The results from toxin analyses showed that there was no correlation between the total biomass of *Microcystis* and microcystins concentration in the same sample.

Keywords: cyanobacteria, microcystins, ELISA, freshwaters, Vietnam.

1. Introduction

Microcystis spp. are planktonic cyanobacteria that distribute all over the world, in freshwater bodies such as ponds, lakes, rivers and even in brackish or saline waters. They occur in dense colonies in eutrophic waters formed blooms [1]. Algal blooms can destroy views, pollute water environment because they may consist of species that can produce toxic substances which can alter the quality of the water.

Microcystins are cyclic heptapeptides, majority produced by *Microcystis* spp. At present, at least 80 microcystin variants have been known [2]. The occurrence of these toxins in supplied drinking waters can cause damage to human and animal health. Microcystins can inhibit protein phosphatases, cause changes in membrane integrity and conductance, and are tumour promoters, in addition to causing major liver damage [3, 4]. Concern about the microcystin health hazard for humans, the World Health Organization (WHO) suggested a guideline level of microcystin-LR at 1μg/L as a safe level in drinking water [5].

Freshwater cyanobacteria blooms are known to have worldwide-occurrence [6]. Nevertheless, studies dealing with toxicology of cyanobacteria in Vietnam are not very abundant. This paper reports the analyses of

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cyanobacteria and toxin occurrence in bloom samples from some freshwater bodies in Vietnam. The toxin contents were determined by ELISA. These results will be the basic knowledge for future studies of potential polluted these toxins in studied area.

2. Materials and methods

Sampling sites: Bloom samples were randomly collected from Lake Tri An (Dong Nai province), Lake Bien Ho and Lake Duc An (Gia Lai Province), Cua Ngan, Dap Da, Nhu Y, Ho Mung sites (Thua Thien Hue Province), Lake Hoan Kiem (Ha Noi). Sampling was carried out on August 20-30, 2008.

Sampling: Qualitative samples were collected by a plankton net and fixed by formal 4% solution. Quantitative samples were collected by a plastic tube, 2 m in length and 10 cm in diameter. Water samples then were mixed in a small bucket. Then the sub-samples of 100 mL were collected and fixed by Lugol acid solution. The ones of 1.5 mL were collected in Eppendorf tubes and were kept at -18 °C for toxin analysis.

Examination of samples: Both live and preserved cyanobacterial samples were examined by light microscopes Olympus BX60 and Olympus DP12 with digital camera. Species were identified and taken photograph. The identification of cyanobacterial species was mainly made with reference to Komárek and Anagnostidis (1999) [7, 8].

Cell counts: direct counts of preserved samples were carried out with Sedgewick Rafter chambers using microscope at 200 x magnification. The *Microcystis* spp. colonies were separated by sonicating in 3 minutes before sediment in chambers.

Microcystins were analyzed by Enzyme - Linked Immunoabsorbent Assay (ELISA) [9]: Microcystin concentrations in bloom samples were analyzed by the ELISA test using Microcystins Plate Kits (Abraxis, USA). The kits were calibrated with a non-toxic microcystin-LR surrogate at levels equivalent to 0.1, 0.4, and 1.6 parts per billion (ppb) (or $\mu g\ L^{-1}$) microcystin-LR. The water samples were sonicated for 3 minutes to lyse the cells, followed by centrifugation for 10 min at 10 000 g. The optical density of the supernatant was measured at 450 nm on a Microreader (Hyperion 3) and the microcystins concentrations ($\mu g\ L^{-1}$) in the samples were determined from the standard competitive curve of microcystin-LR. If the microcystin concentrations in the samples were higher than levels equivalent to the standard calibration (1.6 $\mu g\ L^{-1}$) the samples were diluted until inside the range of the standard curve.

3. Results and discussion

Species composition of microcystis spp. in study waters

In 8 natural bloom samples collected, we identified six species of *Microcystis* including *M. aeruginosa*, *M. botrys*, *M. wesenbergii*, *M. flos-aquae*, *M. panniformis* and *M. protocystis*. Species composition and their occurrences in the studied sites were shown in Table 1.

The species *M. aeruginosa*, *M. botrys* and *M. wesenbergii* were common in all studied sites. They are tropical and subtropical bloom-forming species. *M. panniformis* is tropical species found in all sites except Tri An and Hoan Kiem. *M. flos-aquae* and *M. protocystis* were rare in these materials.

Table 1. Species composition of *Microcystis* spp. and their occurrences in the studied sites

Species	Sampling sites							
	Tri An	Bien Ho	Duc An	Cua Ngan	Nhu Y	Dap Da	Ho Mung	Hoan Kiem
M. aeruginosa	+	+	+	+	+	+	+	+
M. botrys	+	+	+	+	+	+	+	+
M. wesenbergii	+	+	+	+	+	+	+	+
M. flos-aquae	+	–	–	–	+	+	–	–
M. panniformis	–	+	+	+	+	+	+	–
M. protocystis	–	–	–	+	–	–	–	–

Abbreviations: (+) present; (-) not present.

Microcystis biomasses

The results of quantitative analyses of each species and total biomass of 8 bloom samples collected from studies sited from 20-30 Autumn, 2008 were shown in Table 2 and Fig. 1. From Table 2, we found that the biomass of *M. aeruginosa* is the highest in all samples, especially in Nhu Y site with the cells density of $312x10^3$ cells/ mL; then *M. botrys*, with the cells density of $160,5x10^3$ cells/ mL in Nhu Y site. These two species were known can produce microcystins with high concentrations and cause dense blooms in all studied sites. The least amount of *M. protocystis* cells was found in samples collected in Cua Ngan site with only $1x10^3$ cells/ mL. This is also the toxic species with high concentration.

Table 2. Cell density of cyanobacteria in the studied localities

Species	Cell density (x 10^3 cells/mL)							
	Tri An	Bien Ho	Duc An	Cua Ngan	Dap Da	Nhu Y	Ho Mung	Hoan Kiem
M. aeruginosa	108	4,5	10	29	66	312	145	7
M. botrys	10	5	13	151	131	160,5	51,6	7
M. wesenbergii	16	1	3	0,8	4,5	1	1	9
M. flos-aquae	10	0	0	0	11	10,5	0	0
M. panniformis	0	0,5	3,7	96,8	1	140,5	9,6	0
M. protocystis	0	0	0	1	0	0	0	0
Total biomasses of *Microcystis* spp.	144	11	29,7	278,6	213,5	624,5	207,2	23

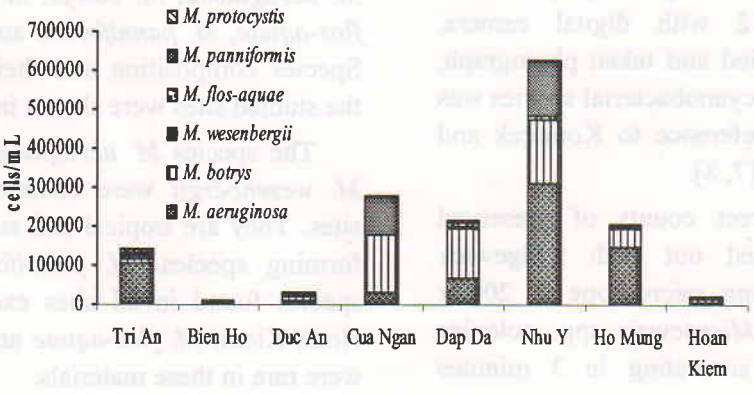

Fig.1. Cell density of *Microcystis* spp. in the studied localities.

Microcystins analyses

The results of ELISA test for microcystins in bloom samples showed that in 8 samples of 8 studied sites, 7 samples were positive about microcystins with rather high concentrations (Table.3; Fig.2). Only the concentration of toxin in Lake Tri An was too low to detect. The toxin concentrations ranged from 17.966 ng/mL in Duc An to 5.854 ng/mL in Nhu Y sample.

Comparing microcystin concentrations and biomasses of *Microcystis* spp. (Table 3), we found that in Duc An site, the concentration of microcystins is highest (17.966 ng/mL) but the biomass is rather low (29,7x10^3 cells/mL). By contract, in Nhu Y, the concentration of microcystins is lowest (5.854 ng/mL) but the biomass of *Microcystis* spp. is highest (624,5x10^3 cells/mL) (Table 3; Fig.2).

Table 3. Microcystins detection by ELISA in natural cyanobacterial blooms in the studied localities

Location	Microcystins concentrations (ng/ mL)	*Microcystis* spp. biomasses (x10^3 cells/mL)
Tri An	0	144
Bien Ho	9.434	11
Đuc An	17.966	29,7
Cua Ngan	17.579	278,6
Đap Đa	14.968	213,5
Nhu Y	5.854	624,5
Ho Mung	13.012	207,2
Hoan Kiem	16.395	23

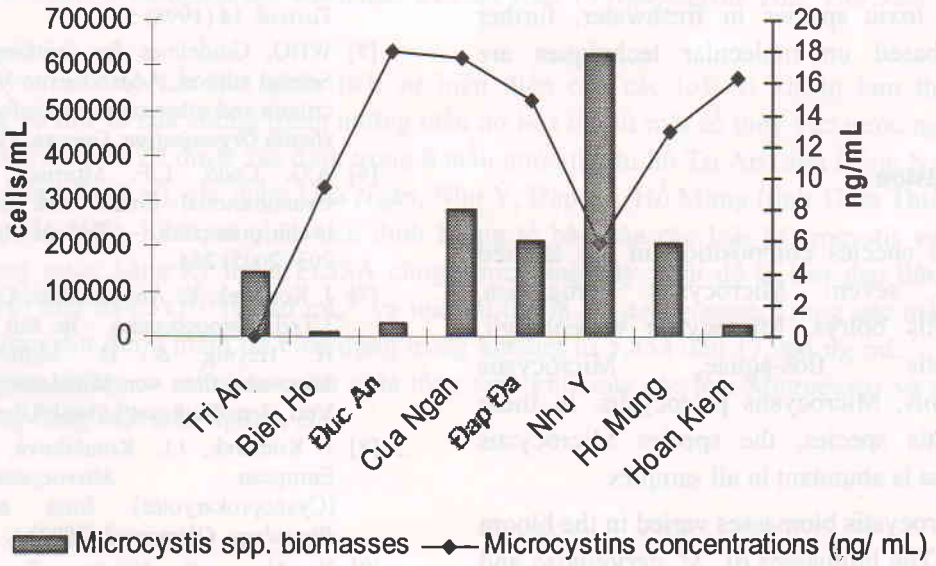

Fig.2. *Microcystis* biomass and microcystins concentrations in water-blooms in the studied localities.

Among six species found in this study, *M.aeruginosa*, *M. botrys*, *M. wesenbergii* and *M. panniformis* are the microcystin-producers [10-14]. The species *M. aeruginosa*, *M. botrys* and *M. wesenbergii* were found in all studied sites. *M. panniformis* were found in Gia Lai and Thua Thien Hue freshwaters. The non-toxic species *M. flos-aquae* occurred with low biomass in Tri An site and *M. protocystis* only occurred in Cua Ngan. Thus, the microcystin producers were abundant in all bloom samples. However, the results from toxin analyses showed that there was no correlation between the total biomass of *Microcystis* and microcystins concentration in the same sample. Microcystin concentrations in waters are not upon the total biomass but the biomass of toxin-producers.

Although many studies demonstrated that toxin-producing ability depend on species, in the same species in populations of cyanobacteria, both microcystin-producing and non-microcystin-producing individuals may coexist [15]. Therefore, in order to affirm potential toxic species in freshwater, further studies based on molecular techniques are needed.

4. Conclusion

- The species composition in the studied sites is seven: Microcystis aeruginosa, Microcystis botrys, Microcystis wesenbergii, Microcystis flos-aquae, Microcystis panniformis, Microcystis protocystis. In these Microcystis species, the species Microcystis aeruginosa is abundant in all samples.

- Microcystis biomasses varied in the bloom samples. The biomasses of *M. aeruginosa* and *M. botrys* were rather high in the waterbodies in Thua Thien Hue, while *M. wesenbergii* was abundant in Lake Hoan Kiem and Duc An.

- The concentrations of microcystin in the samples are high and not correlated to the biomasses of *Microcystis*.

Acknowledgments

This study was supported by a research-grant, funded by the Asia Research Center, Vietnam National University, Hanoi.

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Sự hiện diện của các loài vi khuẩn lam độc hại microcystis spp. và độc tố microcystin trong một số thủy vực nở hoa ở Việt Nam

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Bài báo này trình bày kết quả phân tích sự hiện diện của các loài vi khuẩn lam thuộc chi *Microcystis* spp. và độc tố của chúng trong những mẫu nở hoa thu từ một số thủy vực nước ngọt Việt Nam. Sáu loài *Microcystis* đã được xác định trong 8 mẫu nước thu từ hồ Trị An (tỉnh Đồng Nai), Biển Hồ, hồ Đức An (tỉnh Gia Lai), các điểm Cửa Ngăn, Như Ý, Đập Đá, Hồ Mưng (tỉnh Thừa Thiên Huế), hồ Hoàn Kiếm (Hà Nội). Kết quả phân tích định lượng tế bào của các loài Microcystis và độc tố microcystin trong nước bằng kỹ thuật ELISA cũng được trình bày. Mật độ tế bào dao động trong khoảng từ $11x10^3$ đến $624,5x10^3$ tế bào mL^{-1} và loài ưu thế là *M. aeruginosa*. Trong các mẫu nước, hàm lượng microcystin được thăm dò biến động trong khoảng từ 5,854 đến 17,966 ng mL^{-1}. Kết quả phân tích cho thấy không có mối liên quan giữa tổng sinh khối của các loài *Microcystis* và nồng độ microcystin trong cùng một mẫu nghiên cứu.