POTENTIAL INTOXICATION BY TOXIC CYANOBACTERIA AND THEIR TOXINS IN SOME VIETNAMESE FRESH WATERBODIES

Dang Hoang Phuoc Hien[1], Dang Thi Thom[1], Nguyen Sy Nguyen[1], Duong Thi Thuy[1], Dang Dinh Kim[1], Jens Dahlmann[2]

[1]Institute of Environmental Technology, VAST

[2]Friedrich-Schiller University, Jena, Germany

ABSTARCT

12 toxic cyanobacterial strains were isolated from HaNoi lakes and HaNoi adjacent area fishponds and cultivated in laboratory conditions.

The toxicity and toxins of these isolates and natural cyanobacterial bloom samples were investigated using Artemia salina bioasssay and HPLC method. Almost all investigated samples showed their toxicity for Artemia salina with the LC50 values varied from 7.11 to 96.51mg.mL-1. The toxicity of the samples was confirmed by HPLC analysis: they contain different MCs with the total MCs content varied from 0.002 to 3.583 mg.g-1 D.W

INTRODUCTION

Eutrophication in freshwaters is caused by increases in levels of nutrients, usually phosphorus and nitrogen compounds from untreated wastewaters of municipal, industrial, agricultural and aquacultural sources, and in its turn can result in visible cyanobacterial mass occurrences (blooms). Many cyanobacteria produce toxins that are responsible for acute and chronic human and animal health problems and the impairments of water supply and water-based activities. The most studied cyanotoxins are microcystins (MCs) - the cyclic heptapeptide toxins that show tumor promoting activity through inhibition of PP 1 and 2A.

In Vietnam, surveys on ecology and occurrence of toxic cyanobacterial blooms in freshwater environment showed that they have been very frequent in both highly eutrophicated lakes and waterbodies where water is considered to be of good or acceptable quality for aquaculture. The bloom forming genera often are Microcystis, Anabaena, Oscillatoria, Aphanizomenon and Gomphosphaeria. The most frequent species recorded is Microcystis aeruginosa. (Fig.2).

MATERIALS AND METHODS

- Natural cyanobacterial bloom samples from different lakes, fishponds and isolates.
- *Artemia salina* bioassay, HPLC/MS

RESULTS AND DISCUSSION

Toxicity test by Artemia salina bioassay

Screening for the toxicity of natural cyanobacterial bloom samples and isolates by *Artemia salina* bioassay showed that the LC50 values varied widely from 7.1 to 96.5 mg.mL-1 (Tab.1) while Fig. 2 describes relationship of *Artemia*'s death rate and microalgal concentration.

However, the investigated samples tend to divide clearly into 2 groups according to LC50 values : 1st group includes the samples with high LC50 values (LC50 > 20 mg.mL-1 - low toxicity), accounting for 36% (14/39 samples) and the 2nd group with relatively low LC50 value samples (LC50 values < 15 mg.mL-1 - high toxicity) accounting for 64 % (25/39 samples). It's interesting to note that in almost all samples of first group MCs were not detected (n.d) by HPLC/MS while samples of 2nd group contain different MCs with total MCs content varied from 0.004 to 3.583 mg.g-1 D.W

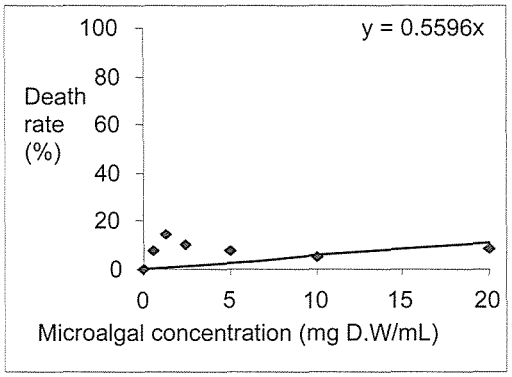
Table 1. LC50 values and total MCs concentration of the investigated samples

N°	Samples	Sampling date	LC_{50}(mg.mL^{-1})	Total MCs mg.g^{-1} DW
1	D6DB-BN	19.7.01	96.5065	Nd
2	D10 DB-BN	19.7.01	71.1642	Nd
3	D8 DB-BN	19.7.01	69.8714	Nd
4	D8 DB-BN	18.6.01	63.1792	Nd
5	*M. aeruginosa TC3*	isolate	58.9275	Nd
6	B10 TL-TT-HN	21.8.01	57.4317	0.014
7	D7 DB-BN	18.6.01	52.8374	Nd
8	D5 DB-BN	18.6.01	34.5232	0.006
9	*O. limosa*	isolate	32.6800	0.002
10	B8 TL-TT-HN	21.8.01	31.7642	
11	D10 DB-BN	17.1.02	28.2406	nd
12	D12 DB-BN	isolate	24.9401	nd
13	D10 DB-BN	20.9.02	21.1721	nd
14	KTX6 DB-BN	17.1.02	20.1102	nd
15	B9 TL-TT-HN	6.9.01	15.874	0.046
16	M.aeruginosaHK4	isolate	14.5476	0.041
17	*M.aeruginosa* TC1	isolate	13.6200	2.163
18	Parbung Canal	16.06.01	13.4383	0.004
19	D4 DB-BN	20.9.02	13.0921	0.269
20	*M.aeruginosa* HK1	isolate	12.364	0.060
21	KTX2 DB-BN	25.9.01	11.6637	0.165
22	C18 DB-BN	3010.01	10.5621	0.329
23	D8 DB-BN	17.1.02	10.5296	0.945
24	KTX2 DB-BN	30.8.01	9.7656	1.308
25	C20 DB-BN	30.10.01	9.7532	0.332
26	**A. spiroides**	isolate	9.7435	0.018
27	*M.aeruginosa* SVDB1	isolate	9.7112	nd
28	KTX7 DB-BN	17.1.02	8.8756	nd
29	SV DB-BN	18.6.01	8.5869	0.195
30	HK- Bloom	2002	8.5008	-
31	Hai Phong	05.01	8.4256	0.7032
32	SV DB-BN	19.07.01	8.2761	1.3352
33	C18 DB-BN	26.12.01	8.2273	1.4760
34	C16 DB-BN	30.10.01	8.1074	1.095
35	YenXa-NamDinh	25.8.01	7.9916	0.9
36	*M.aeruginosa* TC2	isolate	7.6578	0.007
37	*M.aeruginosa* TL	isolate	7.4133	1.929
38	C27 DB-BN	30.10.01	7.2849	3.583
39	C25 DB-BN	26.12.01	7.1119	0.150

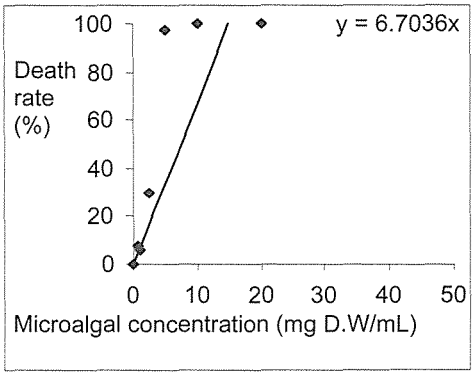
Toxin analysis by HPLC/MS

Toxin analysis of all investigated samples showed that the toxin composition of these samples was various: 6 MCs were identified with different concentrations (Tab 2 and Fig. 3). Among them, the most common MCs are: MC-RR, -YR , -LR and - LA. The percentage of samples containing these MCs was 56 %, 46%, 58,5% and 41 %, respectively. Two others MCs: MC-LW and - LF are rarer (only 7.3% of samples contain MC-LW and 14.6% of samples contain MC-LF) but they are in high concentration in some samples, for example, in *Microcystis aeruginosa* TL the content of MC-LW and - LF were 0.394 and 0.420 mg.g-1 D.W, respectively. Some samples contain only 1 MC (for example, *Oscillatoria limosa* isolate and bloom sample from fishpond D5-DB-BN) while others contain up to 4 or 5 MCs. MCs content of *Oscillatoria limosa* and *Anabaena spiroides* isolates is low. Total MCs concentration of these samples was 0.002 and 0.018 mg.g-1 D.W, respectively. It's known that besides MCs, many *Oscillatoria* and *Anabaena* strains produce neurotoxins like anatoxin-a and/or

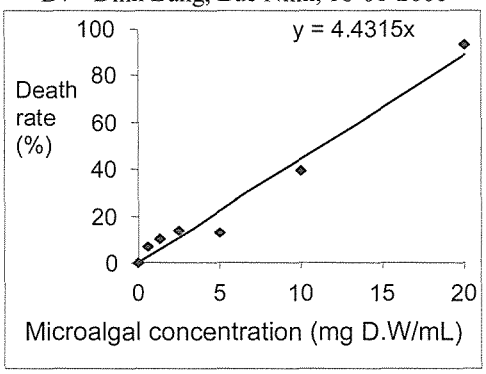
homoanatoxin - a (for *Oscillatoria*) and anatoxin - a, anatoxin - a(S) and saxitoxins (for *Anabaena*) (K. Sivonen and G. Jones ; 1999). Results of HPLC analysis also revealed that some of the investigated samples (15 / 41 samples) accounting for 36%, do not contain MCs.



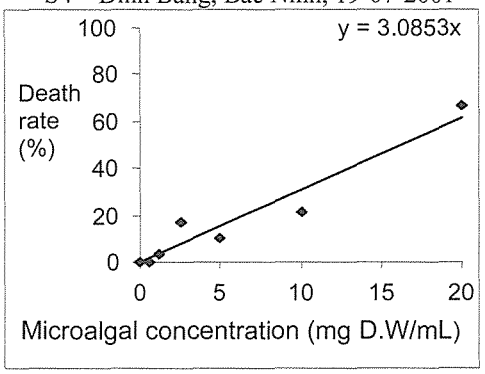
Bloom sample from fishpond
D7 - Dinh Bang, Bac Ninh, 18-06-2001



Bloom sample from fishpond
SV - Dinh Bang, Bac Ninh, 19-07-2001

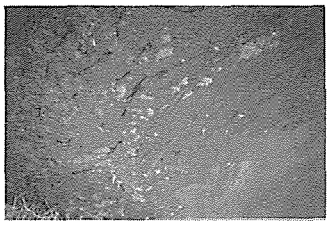


M. aeruginosa TC2 – isolated
from Thanh Cong lake

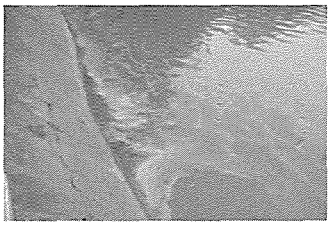


M. aeruginosa HK1 – isolated
from Hoan Kiem lake

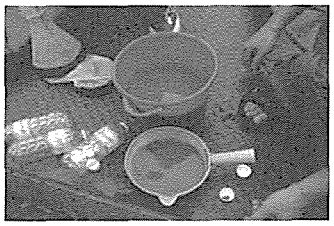
Fig 1. Regression lines discribing relationship of Artemia's death rate and microalgal concentration



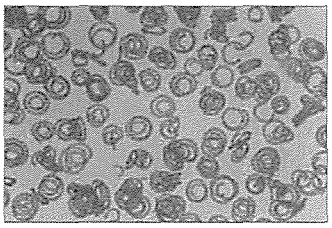
DinhBang (BacNinh) fishpond



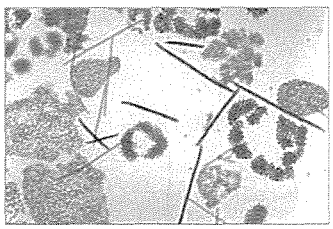
HoanKiem lake (HaNoi)



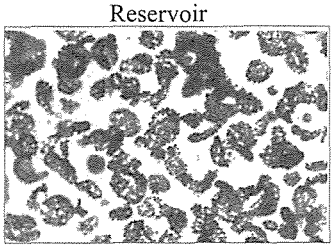
Sampling Bloom from HoaBinh Reservoir



Anabaena spiroides is
dominant, DB - BN fishpond



Mixture of different *Microcystis*
and *Oscillatoria* strains
(C5 DB - BN fishpond)



Microcystis aeruginosa is
dominant, DB - BN fishpond

Fig 2. Natural cyanobacterial blooms and phytoplankton populations in the moment of cyanobacterial bloom

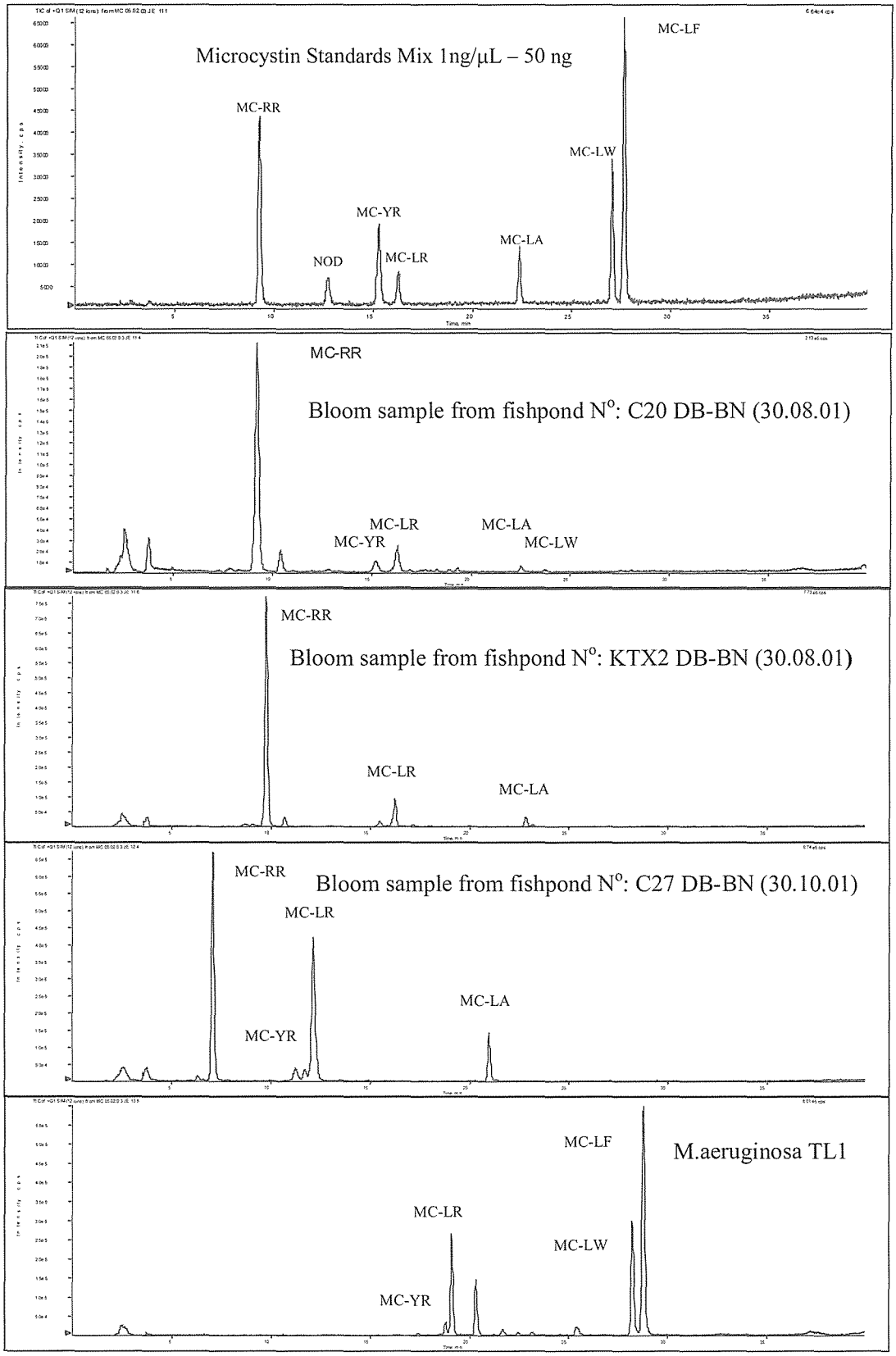


Fig 3. HPLC/MS chromatograms

Table 2. MCs concentration of the investigated samples determined by HPLC/MS

N°	Sampling		Microcystins concentration (mg. g^{-1}D.W)						
	Site	Date	MC-RR	MC-YR	MC-LR	MC-LA	MC-LW	MC-LF	Total
1	D7 DB -BN	18.06. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
2	D8 DB -BN	18.06. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
3	D10 DB -BN	18.06. 01	0.0067	n.d.	0.0136	0.0040	n.d.	n.d.	0.0243
4	SV DB -BN	18.06. 01	0.0751	0.0068	0.0852	0.0250	n.d.	0.0026	0.1722
5	D6 DB -BN	19.07. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
6	D8 DB-BN	19.07. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
7	D10 DB-BN	19.07. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
8	E16 DB-BN	19.07.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
9	B8 TL-TT-HN	21.9.01	0.0150	0.0084	0.0277	nd	nd	nd	0.0512
10	B10 TL -TT, HN	21.08. 01	0.0064	0.0030	n.d.	0.0047	n.d.	n.d.	0.0141
11	Hai Phong	05.01	0.4943	0.0712	0.1377	nd	nd	nd	0.7032
12	YenXa-Nam§inh	25.08 .01	0.7033	0.0662	0.1119	0.0188	n.d.	n.d.	0.9002
13	C20 DB-BN	30.08.01	0.286	0.0290	0.1364	0.0181	0.0036	n.d.	0.4731
14	D12 DB-BN	30.08. 01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
15	KTX2 DB-BN	30.08. 01	0.7110	0.0357	0.4435	0.1182	n.d.	n.d.	1.3084
16	B9 TL- TT HaNoi	06.09. 01	0.0045	?	0.0193	0.0250	n.d.	0.0013	0.0501
17	KTX2 DB-BN	25.09 .01	0.0975	0.0167	0.0484	n.d.	n.d.	0.0024	0.1866
18	C16 DB-BN	30.10. 01	0.5528	0.0176	0.4603	0.0627	0.0014	n.d.	1.0948
19	C18 DB-BN	30.10. 01	0.2051	0.0132	0.1063	0.0043	n.d.	n.d.	0.3289
20	C20 DB-BN	30.10. 01	0.2100	0.0153	0.1037	0.0034	n.d.	n.d.	0.3324
21	C23 DB-BN	30.10. 01	0.3263	0.0350	0.2216	0.0376	n.d.	0.0013	0.6218
22	C27 DB-BN	30.10. 01	0.6202	0.0769	2.412.9	0.4734	n.d.	n.d.	3.5834
23	C18 DB-BN	26.12. 01	0.6885	0.1573	0.5950	0.0352	n.d.	n.d.	1.4760
24	C25 DB-BN	26.12. 01	0.0296	0.0071	1.4327	0.0221	n.d.	n.d.	1.4915
25	D8-DB-BN	17.01 .02	0.1397	nd	0.8053	nd	nd	nd	0.9450
26	D10 DB-BN	17.01. 02	n.d.	n.d.	n.d	n.d	n.d.	n.d.	nd
27	KTX6 DB-BN	17.01. 02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
28	KTX7 DB-BN	17.01. 02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
29	*M.aeruginosa.* HK1	1999	0.0521	0.0081	n.d.	n.d	n.d.	n.d.	0.0602
31	*M.aeruginosa* TC1	1999	1..9419	0.2160	0.0054	n.d.	n.d.	n.d.	2.1633
32	*M.aeruginosa* TC2	1998	0.0044	n.d.	0.0014	0.0011	n.d.	n.d.	0.0069
33	*M.aeruginosa* TC3	1999	n.d.	n.d.	n.d.	n.d.	n.d.	n.d	nd
35	*M.aeruginosa* HK4	1999	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
37	*M.aeruginosa* SVDB1	2001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
38	*M.aeruginosa* TL	2001	n.d.	0.0054	1.1106	n.d.	0.3938	0.4195	1.9293
39	*O. limosa*	2001	n.d.	n.d.	0.0020	n.d.	n.d.	n.d.	0.0020
40	D5 DB-BN	18.6.01	n.d.	n.d.	0.0047	n.d.	n.d.	n.d.	0.0047
41	Parbung Canal	18.6.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
44	D4 DB-BN	20.9.02	0.0873	0.0151	0.1789	0.0306	n.d.	0.0013	0.3132
45	D10 DB-BN	20.9.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	nd
46	A.spiroides	2000	0.0020	n.d.	0.0148	0.0012	n.d.	n.d.	0.018
Samples which contain MC (%)			56	46	58.5	41	7.3	14.6	63

CONCLUSION

The toxicity for *Artemia salina* and high content of MCs in investigated natural bloom and isolated samples from different Vietnam lakes and fishponds show the potential intoxication by toxic cyanobacteria and their toxins in these waterbodies, especially, during bloom collapse when toxins may release from cells into water environment.

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