Ecotoxicological testing in the studies on the toxicity of cyanobacterial blooms

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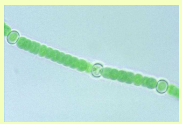
Cyanobacterial blooms, a natural phenomenon occurring in seawater as well as freshwater, have intensified alarmingly in recent years; this is due to eutrophication and overall pollution of waters. The presence of blue-green algae in water may be hazardous to the health of humans and animals alike, particularly in freshwater reservoirs used for the abstraction of drinking water, and in bathing waters. However, cyanobacteria do not always produce toxic substances, therefore at the preliminary stage of waters monitoring it is more important to determine whether they do; the identification of toxins is of secondary importance.



Materials and methods

- the inland water samples from the Pomerania Province and coastal waters from the Gulf of Gdansk collected in the period of June-August 2006 during cyanobacteria blooms,
- cyanobacteria strains isolated from the inland waters of the Pomerania Province and coastal waters from the Gulf of Gdansk. Phytoplankton samples were at first filtered and hence suspension of cyanobacteria was obtained. Subsequently, it was sonificated and disintegrated. Sample obtained this way represent approximate environmental conditions during cyanobacteria blooms, when cyanobacteria die out and toxins are released to water,
- extracts of *Microcistis aeruginosa* and *Nodularia spumigena* grown in BG-11 (fresh water) and BG-11N (brackish water) broth.

Two methods of toxins identification were applied:

- **HPLC** (High Performance Liquid Chromatography) with the use of Photo-Diode Array System (**HPLC-PDA**) which make qualitative and quantitative analysis of cyanotoxins possible. The microcystins (RR and LR) and nodularin were identified by their retention time and characteristic absorption spectrum with maximum at 238 nm,
- **ELISA** (Enzyme-Linked Immunosorbent Assay) test based on the principle of antibody-antibody interaction. Rabbit antibody react with microcystins (Mcyst-RR, YR and LR) and nodularin. Results in this test are defined as toxic equivalent of Mcyst-LR concentration with the detection limit of 0,1µg/l and they indicate all microcystin types.

Two tests were selected for ecotoxicological examination of water:

- THAMNOTOXKIT F™ (test organism - *Thamnocephalus platyurus*) for fresh waters,
- ARTOXKIT M™ (test organism - *Artemia fransiscana*) for brackish and saline waters.

Results

Tab. 1 Ecotoxicity of cyanobacteria species isolated form fresh waters of Pomeranian province THAMNOTOXKIT F (*Thamnocephalus platyurus*)

Species	Strain	Date and place of sampling	Broth	Toxins Methods of isolation – HPLC Toxins concentration	Test results
Chroococales					
Microcystis aeruginosa	MKR 0105	Vistula Bay, 10.10.2005	BG11	mikrocistin 6,9 µg/dm³	Mortality of *T. platyurus* – 0 %
Microcystis aeruginosa	MKR 0205	Vistula Bay, 10.10.2005	BG11	mikrocistin 19,55 µg/dm³	Toxicity effect Mortality of *T. platyurus* – 20 % in the highest concentration
Oscillatoriales					
Planktothrix agardhii	PKLD 0205	Klasztorne Lake, 19.09.2005	BG11, Z8	Toxins not detected	Mortality of *T. platyurus* – 0 %
Oscillatoria sp.	OKRL 0105	KarlikowskieLake 31.08.2005	BG11	mikrocystin Mcst-R 1,68mg/g*	Strong toxicity effect mortality of *T. platyurus* - 82 % in the highest concentration; mortality 53 % in dilution 50 % and Toxicity effect mortality – 23 % in dilution 25 %
Nostocales					
Anabaena sp.	AKLD 0503	Klasztorne Lake 25.07.2003	BG11^{0*}	Toxins not detected	Mortality of *T. platyurus* – 0 %
Anabaena sp.	AKLR 0102	Karlikowskie Lake 25.07.2003	BG11^{0}	Toxins not detected	Mortality of *T. platyurus* – 0 %
Aphanizomenon sp.	AKLD 0104	Klasztorne Lake 31.08.2005	BG11	Toxins not detected	Mortality of *T. platyurus* – 0 %
Calothrix sp.	CJAS 0105	Jasień lake 31.08.2005	BG11^{0}	Toxins not detected	Mortality of *T. platyurus* – 0 %
Cylindrospermopsis raciborskii	CRRU 0105	Rusałka Lake 08.2005	BG11^{0}	Toxins not detected	Mortality of *T. platyurus* – 0 %

Tab. 2 Ecotoxicity of cyanobacteria species isolated from coastal waters of the Gulf of Gdansk ARTOXKIM M (*Artemia fransiscana*)

Species	Strain	Date and place of sampling	Broth	Toxins Methods of isolation – HPLC Toxins concentration	Test results
Oscillatoriales					
Lygbya sp.	LGG 0505	Puck 10.08.2005	BG11	Toxins not detected	Mortality of *A. fransiscana* – 0 %
Phormidium sp.	PGG 0405	Puck 31.05.2005	BG11 PSU7	Toxins not detected	Mortality of *A. fransiscana* – 0 %
Phormidium sp.	PGG 0305	Władysławowo 13.04.2005	BG11	Toxins not detected	Mortality of *A. fransiscana* – 0 %
Phormidium sp.	PGG 0904	Gdynia, 05.08.2004	BG11	Toxins not detected	Mortality of *A. fransiscana* – 0 %
Nostocales					
Anabaena sp.	AGG 0203	Gdynia 04.08.2003	BG11^{0} PSU7	Toxins not detected	Mortality of *A. fransiscana* – 0 %
Nodularia spumigena	NSGG 0205	Gdynia 05.07.2005	BG11^{0} PSU7, Z8xS	nodularin 3,5 µg/mg*	Mortality of *A. fransiscana* – 0 %
Nodularia harveyana	NHGG 0105	Gdynia 21.06.2005	BG11^{0} PSU7, Z8xS	Toxins not detected	Mortality of *A. fransiscana* – 0 %

Tab. 3 Ecotoxicity of cyanobacteria species – samples from fresh waters of Pomeranian province THAMNOTOXKIT F (*Thamnocephalus platyurus*)

Species	Date and place of sampling	Toxins Methods of isolation – HPLC Toxins concentration	Test results
Nostocales, Chroococcales			
Anabaena crassa	Water intake Straszyn (Gdansk) 01.06.06	Mikrocystin Mcst-LR 3,9 µg/dm³	Strong toxicity effect mortality of *T. platyurus* - 100 % in the highest concentration; and toxicity effect mortality – 25 % in dilution 25 %
Anabaena sp.	Water intake Czyzykówko 14.07.06	No data	Significant toxicity effect mortality of *T. platyurus* - 40 % in the highest concentration;
M. aeruginosa, Anabaena grassa, Anabaena flos-aque	Tuchom Lake 04.07.06	Toxins not detected	Mortality of *T. platyurus* – 0 %

Tab. 4 Ecotoxicity of cyanobacterias extracts (*Microcistis aeruginosa* i *Nodularia spungena*) – the sensitivity comparison of both tests: ARTOXKIM M (*Artemia fransiscana*) and THAMNOTOXKIT F (*Thamnocephalus platyurus*)

Species	Samples	Toxins	Toxins concentration	Results of ARTOXKIM M test	Results of THAMNOTOXKIT F test
Nodularia spumigena	Cell extract	nodularin	5,6 µg/ml	Strong toxicity effect 100% mortality of *A. fransiscana* in concentrations of 100%, 50% and 25 % Significant toxicity effect 50% mortality of *A. fransiscana* in concentrations 12,5 %	Strong toxicity effect 100% mortality of *T.platyurus* in concentrations of 100%, 50% and 25 % toxicity effect 20 % mortality of *T.platyurus* in 12,5 % concentrations
Microcistis aeruginosa	Cell extract	mikrocystin Mcst-LR	7,4 µg/ml	Strong toxicity effect 100% mortality of *A. fransiscana* in all concentrations used (100% - 6,25%)	Significant toxicity effect mortality of *T. platyurus* - 43 % in the highest concentration
Nodularia spumigena	Broth extract	nodularin	0,1 µg/ml	Significant toxicity effect 57% mortality of *A. fransiscana* in the the highest concentrations toxicity effect 20 % mortality of *A. fransiscana* in 12,5 % concentrations	Mortality of *T. platyurus* – 0 %
Microcistis aeruginosa	Broth extract	mikrocystin Mcst-LR	0,1 µg/ml	Strong toxicity effect 100% mortality of *A. fransiscana* in concentrations of 100%, 50% and 25 %	Mortality of *T. platyurus* – 0 %

Tab. 5 Ecotoxicity of cyanobacteria species – samples from coastal water of the Gulf of Gdansk ARTOXKIM M (*Artemia fransiscana*)

Species	Date and place of sampling	Toxins Methods of isolation Toxins concentration		Test results
		HPLC	ELISA	
Nostocales				
Nodularia spumigena	Sopot 25.07.06	Nodularin 0,4 µg/ml		Strong toxicity effect mortality of *A. fransiscana* - 100 % in the highest concentration
Anabaena sp.	Gdynia 09.08.06		Mikrocystin Mcst-LR	Mortality of *A. fransiscana* – 0 %

Conclusions

1. The obtained results prove that tests THAMNOTOXKIT and ARTOXKIT are useful tools in measuring ecotoxicity in monitoring programs.
2. Considerable sensitivity of the two chosen tests was confirmed by ecotoxicological examinations of cell extracts, isolated strains and uppermost the environmental samples:
   - ecotoxicological research on cell extracts showed that mortality of marine crustacean *Artemia fransiscana* was 100% at concentration of Mcst-LR of 1,85µg/ml. However, fresh-water crustacean *Thamnocephalus platyurus* proved to be more resistant as only 43% of them died at concentration of Mcst-LR 7,4 µg/ml. This dissimilarity was not observed in case of nodularin. Both species reacted with 100% mortality at nodularin concentration of 1,4µg/ml. Yet, at lower concentration (0,7µg/ml) fresh-water crustacean *Thamnocephalus platyurus* turned out to be again less sensitive with mortality of 20%. At the same nodularin concentration 50% of *Artemia fransiscana* died (tab. 4).
   - considering the environmental samples, 100% of *Artemia fransiscana* died at nodularin concentration of 0,4 µg/ml, while Mcst-LR concentration of 3,9 µg/l caused 100% morthality of *Thamnocephalus platyurus* and what is more Mcst-LR toxicity properties were still observed at 0.975 µg/l concentration with *Thamnocephalus platyurus* mortality of 25% (tab. 3 and tab. 5).
   - examination of cell extracts and natural environment samples enabled to state that differences between the two species' response to cyanotoxin exposion are significant, but it is possible that they may be caused by other environmental factors, not cyanotoxins. The problem is going to be worked on.
3. Due to high toxicity of microcystin Mcst-LR WHO decreed a recommendation that acceptable concentration of this toxin in drinking water should be less than 1µg/l. Another suggestion (still under preparation) states that concentration of Mcst-LR in bathing water should be below 5µg/l Though the performed examinations affirm that the two test organisms (*Thamnocephalus platyurus, Artemia fransiscana*) do not react with mortality at such low concentration of hepatotoxins, results are still deficient and insufficient data don not confirm conclusion with satisfying reliability.

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