Modern analytical methods to study environmental occurrence, fate and chemico-biological interactions of cyanobacterial toxins

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BACKGROUND

• Cyanobacteria are capable of producing a wide range of secondary metabolites, which are often toxic for other organisms, including human beings.

• The most important exposure routes are represented by dermal contact, inhalation or ingestion of contaminated water during recreation in reservoirs with cyanobacteria blooms, and by consumption of drinking water supplied from the reservoirs contaminated with toxic cyanobacteria.

• It is important to study these hazardous cyanobacterial metabolites and to have necessary efficient tools for their environmental research, detection and monitoring, which can provide information about their occurrence, contamination levels and environmental dynamics.

• Main aim of this work is to use combination-approach and knowledge from different fields of study (sampling methods, analytical instrumentation methods, toxicology and environmental chemistry) to obtain new information about cyanobacterial toxins, tools for monitoring of their environmental occurrence, fate, degradation during water treatment processes, and toxicological effects.

AIMS OF STUDIES

MONITORING

Application of passive sampling for monitoring cyanobacterial toxins microcystins (MCs)

THE AIM OF THE STUDY :

- **Monitoring of MCs in drinking water reservoirs and during drinking water treatment**
- **Evaluation of passive sampling (POCIS - based sampler) technique usefulness for monitoring of the most frequently occurring cyanotoxins MCs.**

OCCURRENCE

Occurrence and fate of cyanobacterial toxins microcystins (MCs) in the environment

THE AIM OF THE STUDY :

- **Investigate the occurrence of MCs in biomass of invasive bryozoan *Pectinatella magnifica***
- **Study composition, the quantity, and activity of microbiota of bryozoan colonies**
- **Evaluate *in vitro* toxicity and antimicrobial activity of various extracts prepared from *P. magnifica***

DEGRADATION

THE AIM OF THE STUDY :

- **Evaluate potential of Fe(VI) to treat microcystin-LR (MC-LR) in waters containing carbonate and natural organic matter (i.e., fulvic acid, FA), and in natural lake water.**
- **Determine the kinetics of Fe(VI) reactions with MC-LR and model compounds**
- **Identify oxidation products (OPs) and reaction pathways using (LC-HRMS)**
- **Assess the toxicity of OPs against PP1 assay**

TOXICITY ASSESSMENT

Toxicity assessment of cyanobacterial toxins microcystin-LR (MC-LR) and cylindrospermopsin (CYN)

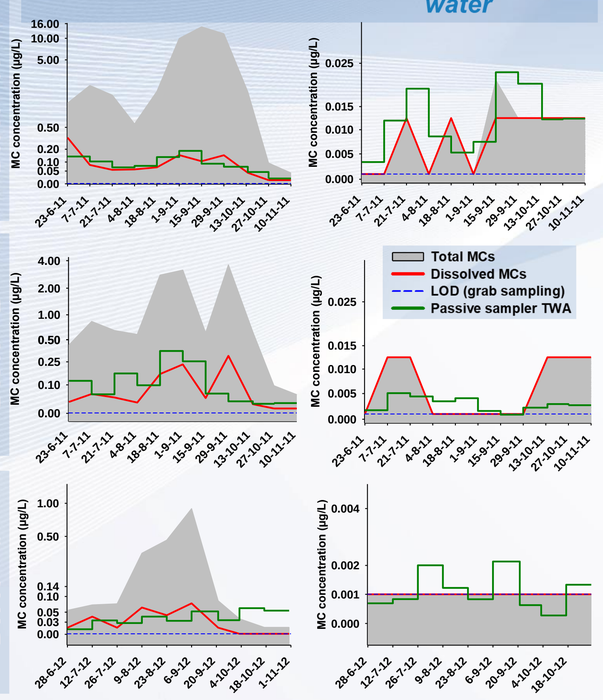
THE AIM OF THE STUDY :

- **Evaluation of hepatotoxic effects in 3D cultures of adult human liver stem cells, including analytical measurement of the concentrations causing toxic effects**

SUMMARY

• **Time-weighted average (TWA) MC concentrations** derived from passive samplers **reflected very well** observed **seasonal dynamics** and **spatial variations of MC concentrations** from grab sample analysis.
• **TWA concentrations** derived from passive sampling **corresponded to absolute concentrations of extracellular MCs**, which represented the fraction of MCs available for sequestration by passive samplers, but **correlated well also with the sum concentration of extracellular and intracellular MCs**.
• Using passive sampling in combination with LC-MS/MS, it was possible to **detect very low MC concentrations** (<1 ng/L).

Water reservoir → DWTP - final treated water

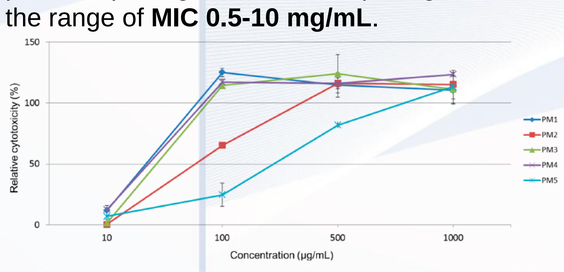


Comparison of microcystin (MC) concentrations (µg/L) determined by passive and grab sampling. *During the summer season (2011 or 2012), MCs were analyzed in three drinking water reservoirs (A-C) and adjacent drinking water treatment plants (DWTPs) by passive sampling (green line, Passive sampler-TWA) and grab sampling (the red line represents concentration of the fraction of dissolved MCs, whereas the gray area indicates the sum of both dissolved and intracellular MC concentrations, Total MCs).*

• **Hepatotoxic cyanotoxins (MCs) were found** (by LC-MS/MS method) in samples**, at low levels ~ng/g d.w.** *P. magnifica.*
• Only **trace concentrations of MCs were** present **in the surrounding water**, and their levels did **not correlate with MC content in bryozoan samples.**
• Findings of MCs in the biomass of *P. magnifica* indicated probably for the first-time possible **biosynthesis of hazardous cyanotoxins by cyanobacterial species colonizing the bryozoan biomass**.

• Extracts tested, except aqueous portion, demonstrated LD_{50} values below 100 µg/mL, which indicates potential toxicity. The water extract of *P. magnifica* with LD_{50} value of 250 µg/mL also showed potentially harmful effects.

• *P. magnifica* seems to be a **promising source of certain antimicrobial agents -** methanolic extract, hexane, and chloroform fractions possessed **selective inhibitory effect** on some potential pathogens and food spoiling bacteria in the range of **MIC 0.5-10 mg/mL**.



Relative cytotoxicity of Pectinatella magnifica extracts in THP-1 cells after 24 h incubation. *PM1-methanolic extract, PM2-hexane portion, PM3-chloroform portion, PM4-ethyl acetate portion, PM5-aqueous portion. The results of LDH release assay are expressed as the means ±SD of three independent experiments, with each condition tested in triplicate.*

Microcystin (MC) detection in P. magnifica colonies and surrounding water

Location, Sampling Date *	MC–RR	MC–YR	MC–LR	MC–RR	MC–YR	MC–LR
	P. magnifica Colony (ng/g d.w.)			Surrounding Water (ng/L)		
Veselí I, 23.7.2015	31.5	6.6	13.9	1.9	<0.6	3.1
Veselí I, 6.8.2015	6.1	<0.6	10.6	38.3	17.9	70.4
Hejtman, 23.7.2015	4.8	<0.6	7.4	6.8	4.5	31.7
Hejtman, 6.8.2015	4.0	<0.6	2.6	121.6	27.5	159.5
Hejtman, 6.8.2015	11.3	6.1	21.9	n.a.	n.a.	n.a.
Hejtman, 9.10.2012	1.7	<0.6	1.4	n.a.	n.a.	n.a.
Hejtman, 31.10.2012	0.7	<0.6	1.4	n.a.	n.a.	n.a.
Hejtman, 9.10.2012	<0.2	<0.6	<0.2	n.a.	n.a.	n.a.
Hejtman, 9.10.2012	<0.2	<0.6	<0.2	n.a.	n.a.	n.a.

* Location were selected from two monitored places in Třeboňsko area (South Bohemia, CZ): a gravel sandpit (Veselí I) and a pond (Hejtman); MC–RR is microcystin RR, MC–YR is microcystin YR, MC–LR is microcystin LR. Values 0.2, 0.6, and 0.2 ng/g d.w. or ng/L, resp. refers to limit of detections of the respective analytical methods (see 4.4), n.a. stands for "not available".

• **Potential of Fe(VI) to effective treat MC-LR** in water containing carbonate ions and fulvic acid in lake water samples **was proved by removal experiment -> 96.2% - 99.0% efficiency,** but higher (> 5.0 mg/L) Fe(VI) dosages would be needed to completely remove MC-LR in lake water compared to deionized water.

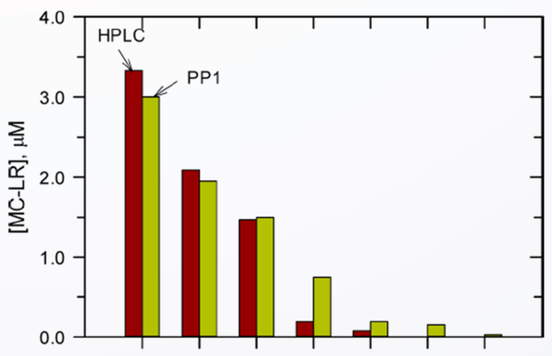
• **Degradation of MC-LR** followed second-order kinetics with the bimolecular **rate constant ($k_{MCLR+Fe(VI)}$) decreasing from 1.3 ± 0.1 × 10^2 M^{-1} s^{-1} at pH 7.5 to 8.1 ± 0.08 M^{-1} s^{-1} at pH 10.0.**

• **OPs were identified and their toxicity assessed by PP1 assay** which proved that the **degradation products of MC-LR did not possess significant biological toxicity.**

Characteristics of MC-LR and oxidized products (OPs)

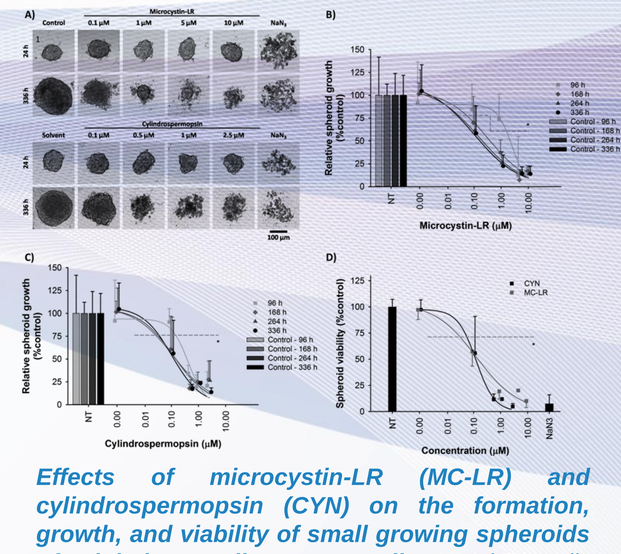
	Mass	Proposed formula	Theoretical m/z[a]	Experimental m/z[b]	Mass error (ppm)
MC-LR	994.5488	$C_{49}H_{74}N_{10}O_{12}$	995.5560	995.5558	-0.2
OP	1042.5335	$C_{49}H_{74}N_{10}O_{15}$	1043.5408	1043.5407	-0.1
OP	1028.5542	$C_{49}H_{76}N_{10}O_{14}$	1029.5615	1029. 5615	0
OP	1026.5386	$C_{49}H_{74}N_{10}O_{14}$	1027.5459	1027.5458	-0.1
OP	1010.5437	$C_{49}H_{74}N_{10}O_{13}$	1011.5501	1011.5506	0.5
OP	794.3923	$C_{34}H_{54}N_{10}O_{12}$	795.3985	795.4001	2
OP	616.3584	$C_{31}H_{48}N_6O_7$	617.3663	617.3663	0

[a] Calculated using ChemDraw 2010. [b] Observed in Orbitrap mass spectrometer



Oxidation of MC-LR by Fe(VI) in buffered deionized water. *The residual concentration of MC-LR was monitored by HPLC (red bars) and PP1 assay (yellow bars). (Experimental conditions: $[MC-LR]_0$ = 3.33 µM; [Fe(VI)] = 66.6 µM; pH 8.0.)*

• **Study represents** probably the **first example of 3D *in vitro* model, utilizing a permanent continuous adult liver stem cell line, which was capable to detect hepatotoxic potential of both MC-LR and CYN** at relatively low concentrations (0.01−0.1 µM).
• Growth and viability of small growing spheroids were inhibited by both (MC-LR and CYN) cyanotoxins (≥0.1 µM) and were associated with blebbing and disintegration at the spheroid surface.
• **Hepatospheroid damage and viability reduction was observed** also in large mature spheroids, with viability **96h-EC_{50} values being 0.04 µM for MC-LR and 0.1 µM for CYN**, and **No Observed Effect Concentrations <0.01 µM.**



Effects of microcystin-LR (MC-LR) and cylindrospermopsin (CYN) on the formation, growth, and viability of small growing spheroids of adult human liver stem cells. *HL1-hT1 cells were seeded into micromolded agarose gel at the initial density 250 cell/spheroid, immediately treated by MC-LR or CYN, and exposed for 336 h. (A) Spheroids were documented periodically by brightfield microscopy (8× magnification, representative images for 24 and 336 h are shown). (B, C) Spheroid size was expressed as a control-normalized change in the spheroid volume. (D) Spheroid viability was evaluated using Alamar Blue assay at the end of exposure. NT-nontreated control (Control), Solvent-solvent control (0.2% MeOH, v/v), NaN3-positive control (1% sodium azide, w/v). Data represent average ± SD values derived from independently repeated experiments, the curves in the graphs depict a fit of 3-parameter sigmoid function. *Data points below the dashed line were significantly lower (Mann−Whitney test, $p < 0.05$) than the solvent control.*

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