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Cyanobacterial toxins profiling in the Subalpine lakes

Harmful Algal Blooms (HABs)

- An algal bloom is a rapid increase in the population of algae in an aquatic system. Typically only one or a few phytoplankton species are involved and some blooms may be recognized by discoloration of the water.
- Algal blooms may also be of concern as some species of algae produce toxins.
- Causes: denaturalization, eutrophication, global changes.

Anabaena lemmermannii

Planktothrix rubescens

Microcystis aeruginosa

Aphanizomenon flos-aquae

Toxic Cyanobacteria in the southern subalpine lakes

First records of Harmful Algal Blooms in the deep southern subalpine lakes.

	Anabaena lemmermannii	*Planktothrix rubescens*	*Microcystis aeruginosa/flos-aquae*	*Aphanizomenon flos-aquae*
Garda	1990		1990-1992	
Iseo	1997	1998	**2006**	
Como	**2006**		1980s and 2000-2003	
Lugano		1980-85	**2006**	1999
Maggiore	**2005**			

Cyanotoxins

Secondary metabolites

Alkaloids

Saxitoxins	>20
Anatoxins	3
Cylindrospermopsins	2

Neurotoxins

Cyclic peptides

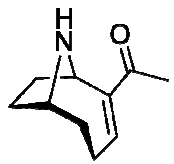
Microcystins (heptapeptides)	>70
Nodularin (pentapeptides)	1

Hepatotoxins
(tumor promotors)

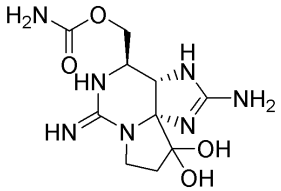
Lipopolysaccharides

(undefined)

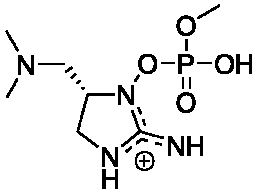
Dermatotoxins



Anatoxin-a

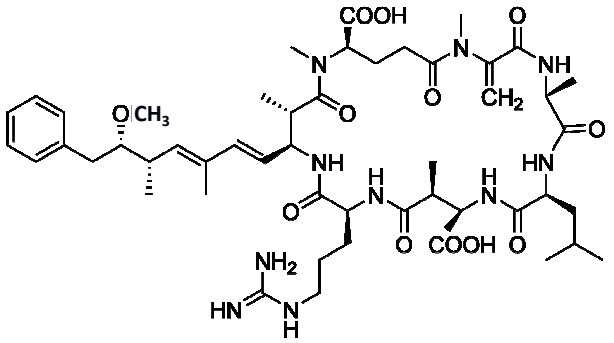


Saxitoxin



Anatoxin-a(S)

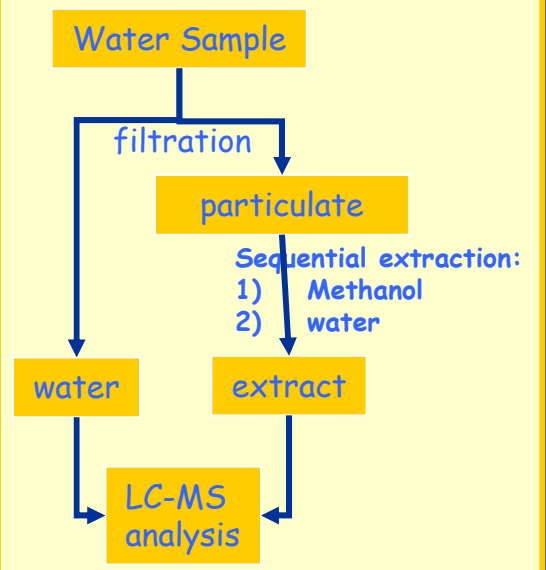
Cylindrospermopsin



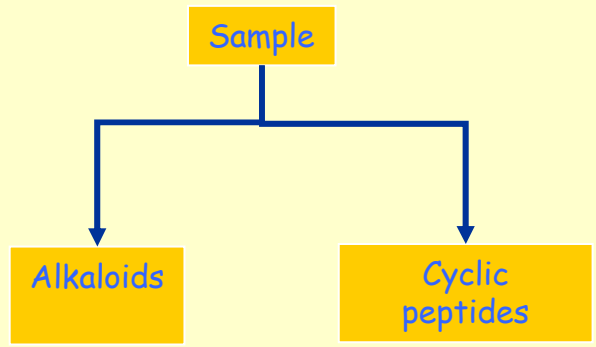
Microcystin-LR

Cyanotoxins extraction and analysis

Sample treatment

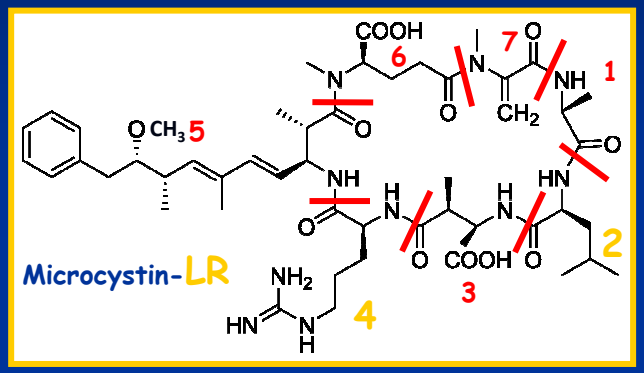


LC-MS Analysis



Waters Acquity UPLC – ABSciex 4000QTRAP

Microcystins Analysis



Commercial standards:

LR
[D-Asp³]LR
RR
[D-Asp³]RR
YR
LA
LF
LW
LY
WR

NOD-R

LC separation – Reverse Phase

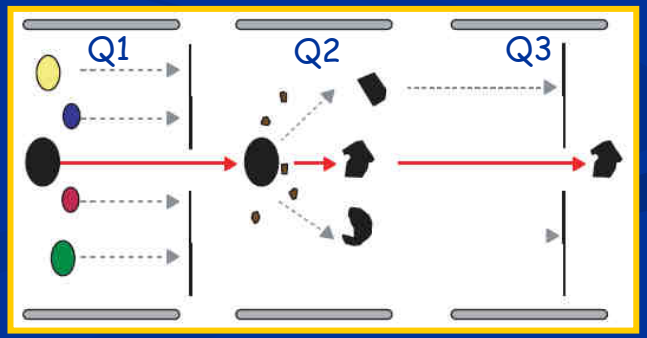
Inj. Volume: 8 µl
Eluents: A= Water, B= ACN (both containing 0.1% FA)
Flow rate: 0.25 ml/min
Gradient: B from 30% (0 min) to 80% (5 min), to 30 % (6 min)
Column: Acquity BEH C18 (1.7 µm) 2.1x50mm, 40°C

MS detection: ESI+, MRM scanning mode

Transition 1 $[M+1]^+/135.1$ (or $[M+2]^{2+}/135.1$)
Transition 2 $[M+1]^+/213.1$ (or $[M+2]^{2+}/213.1$)

General settings:

Ion Spray	5000V
Entrance Pot.	10V
Cell Exit Pot	10V
Interface Heater T	300°C



Product ions **135.1** and **213.1** can be conveniently used for identification (and possibly quantitation) of MC with different AA 2, 3, 4.

List of MC screened:

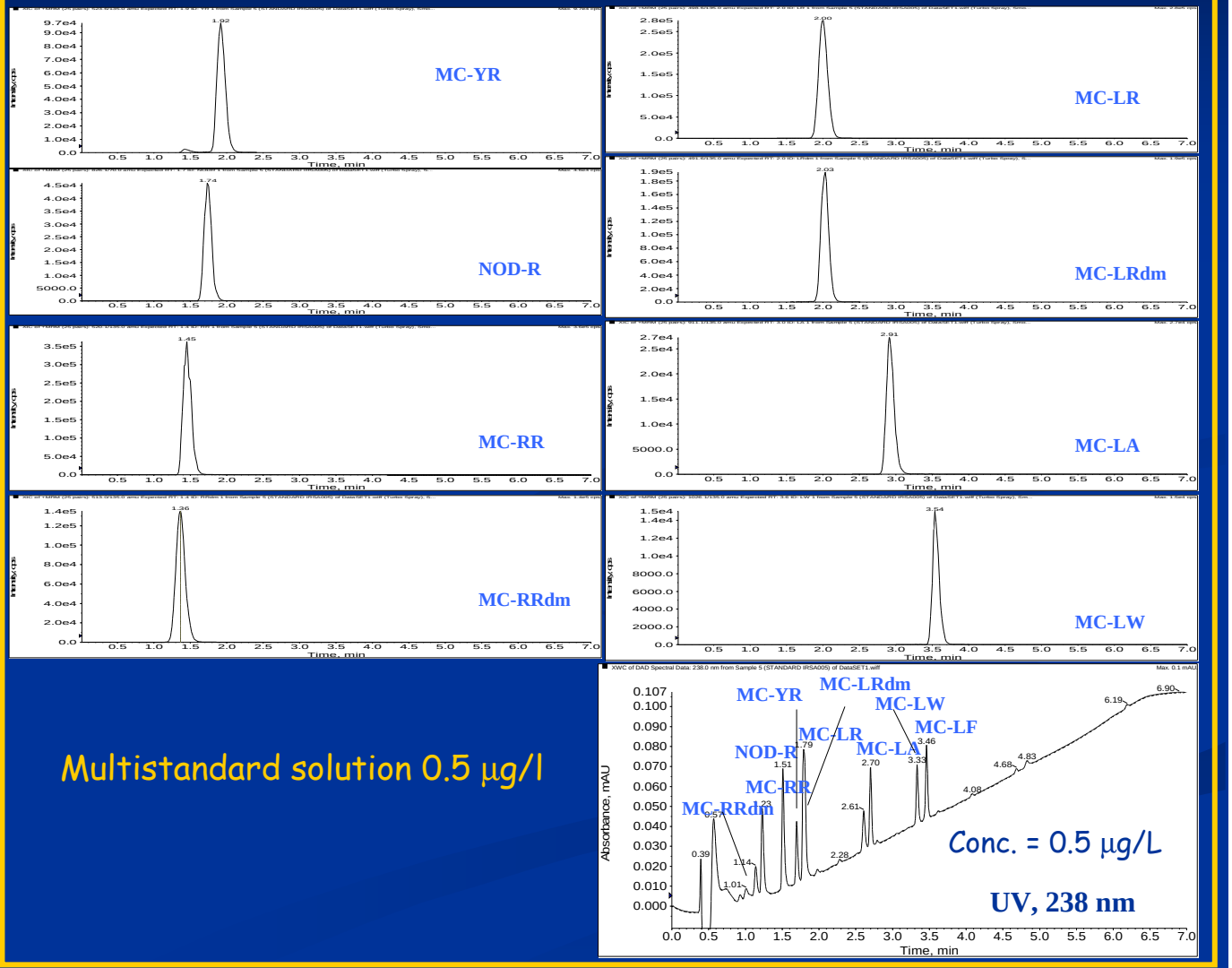
	Molecular ion	DP (V)	CE (V)
LR / [D-Asp³]LR	$[M+2]^{2+}$	50	20
RR / [D-Asp³]RR	$[M+2]^{2+}$	100	43
YR / [D-Asp³]YR	$[M+2]^{2+}$	50	20
LA / [D-Asp³]LA	$[M+1]^+$	70	80
LY / [D-Asp³]LY	$[M+1]^+$	90	100
LW / [D-Asp³]LW	$[M+1]^+$	90	90
LF / [D-Asp³]LF	$[M+1]^+$	90	100
WR / [D-Asp³]WR	$[M+1]^+$	50	20

Microcystins Analysis

Linearity range:
0.1-100 µg/l

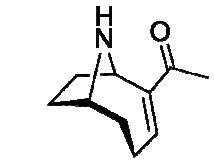
LOQ (µg/l):

LR / [D-Asp3]LR	0.1
RR / [D-Asp3]RR	0.1
YR / [D-Asp3]YR	0.5
LA / [D-Asp3]LA	1.0
LY / [D-Asp3]LY	1.0
LW / [D-Asp3]LW	2.0
LF / [D-Asp3]LF	2.0
WR / [D-Asp^3]WR	2.0
NOD-R	1.0

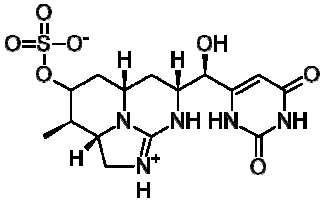


Multistandard solution 0.5 µg/l

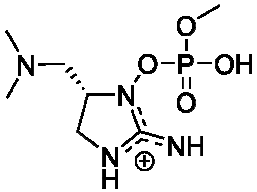
Alkaloids Analysis



Anatoxin-a



Cylindrospermopsin



Anatoxin-a(S)

Commercial standards:

Anatoxin-a
Cylindrospermopsin

LC separation - HILIC

Inj. Volume: 8 µl
Eluents: A= 0.05M NH_4F, B= MeOH
Flow rate: 0.25 ml/min
Gradient: B from 20% (0 min) to 80% (5 min), to 20 % (6 min)
Column: Acquity HILIC (1.7 µm) 2.1x50mm, 30°C

MS detection: ESI+, MRM scanning mode

General settings:		
	Ion Spray	5000V
	Entrance Pot.	10V
	Cell Exit Pot	10V
	Interface Heater T	300°C

List of alkaloids screened:

	Transition 1	Transition 2	DP(V)	CE(V)
Ana-a	**166/149**	**166/91**	**70**	**30**
Dihydro-Ana-a	168/151		70	30
Dihydroxymetoxy-Ana-a	198/181		70	30
Epoxy-Ana-a	182/165		70	30
Hydroxyl-Ana-a	182/165		70	30
HomoAna-a	180/163		70	30
Dihydro-homoAna-a	182/165		70	30
Dihydroxymetoxy-homoAna-a	212/195		70	30
Epoxy-homoAna-a	196/179		70	30
Hydroxy-homoAna-a	196/179			
Cylindrospermopsin	**416/194**	**416/176**	**90**	**53**
Desoxy-cylindrospermopsin	400/194		90	53
Phenylalanine	*166/120*		*70*	*30*

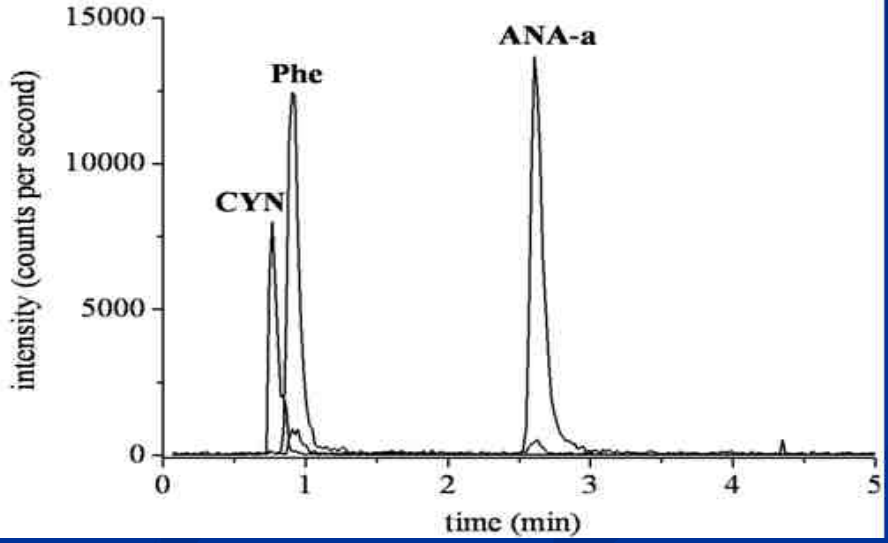
Alkaloids Analysis

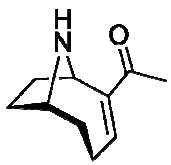
Linearity range:
2 - 100 µg/l

LOQ (µg/l):

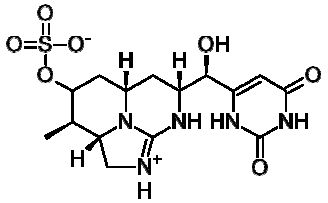
Anatoxin-a	2.0
cylindrospermopsin	4.0

2 standards solution, 10 µg/l



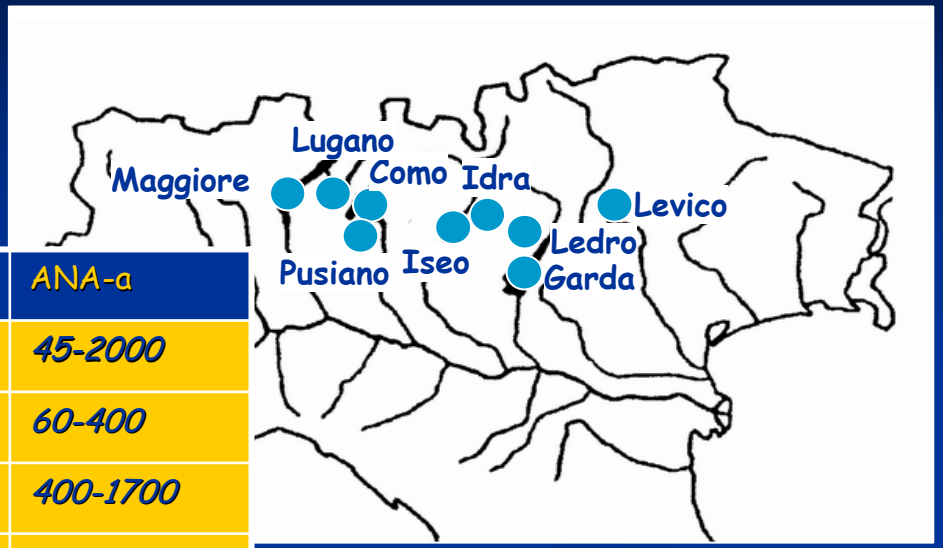


Anatoxin-a



Cylindrospermopsin

Results

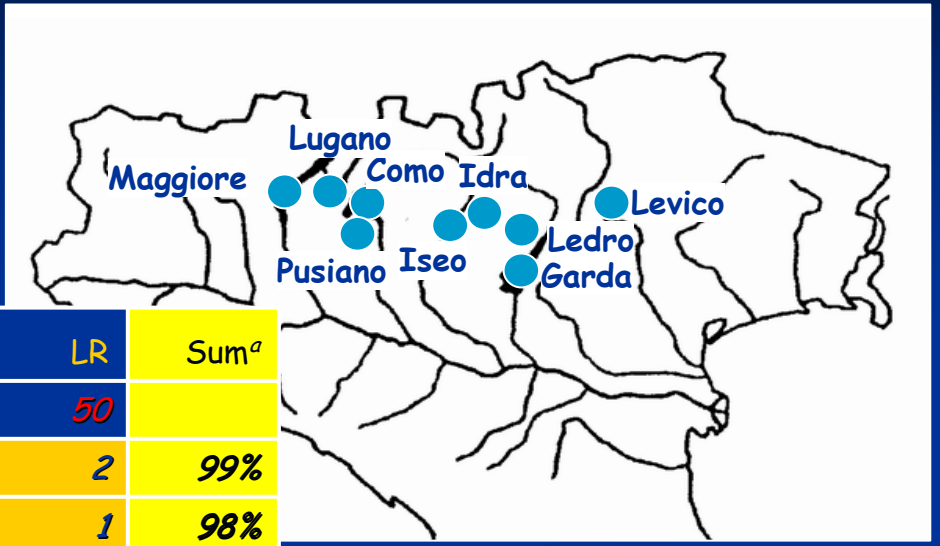


	MC tot	MC tot (LReq)		ANA-a
Garda	*0.3-96*	*0.03-10*		*45-2000*
Iseo	*90-112*	*14-17*		*60-400*
Como	*80-200*	*15-38*		*400-1700*
Lugano	*84-98*	*15-17*		*n.d.*
Maggiore	*26-76*	*12-34*		*120*
Levico	*16-278*	*4.5-78*		*n.d.*
Ledro	*4600*	*375*		*n.d.*
Idro	*15*	*1.4*		*n.d.*
Pusiano	*530-3493*	*70-460*		*n.d.*

Min-max cyanotoxins concentrations (ng/l) in the trophogenic layer (0-20m). Data refer to samplings conducted in June - October 2009.

- MC are widely distributed in the lakes.
- Highest MC concentrations in the most eutrophic lakes.
- Values are always below the WHO limit of **1 µg/l** MC-LR for drinking waters.
- ANA-a is present only in few lakes and is related to the presence of *A. lemmermannii*.

Results



%	[D-Asp^3]RR	YR	[D-Asp^3]LR	LR	Sum^a
LD_{50} in mice	200	100	150	50	
Garda	92	3	3	2	99%
Iseo	64	25	8	1	98%
Como	77	8	4	9	98%
Lugano	75	1	10	8	94%
Maggiore	47	8	6	37	99%
Levico	13	60	27	1	100%
Ledro	97	1	1	-	99%
Idro	95	-	5	-	100%
Pusiano	77	16	5	1	98%

Abundances of individual MC.
Minor variants are LF, LW, [D-Asp^3]YR.

- There is a wide MC diversity among lakes.
- The most toxic variant LR represents a small percentage (with one exception).
- Different lakes have different "toxic potentials".

Thank you for your kind attention

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