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Eawag:
Swiss Federal Institute of Aquatic Science and Technology

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aquatic research

Cyanobacterial Toxins

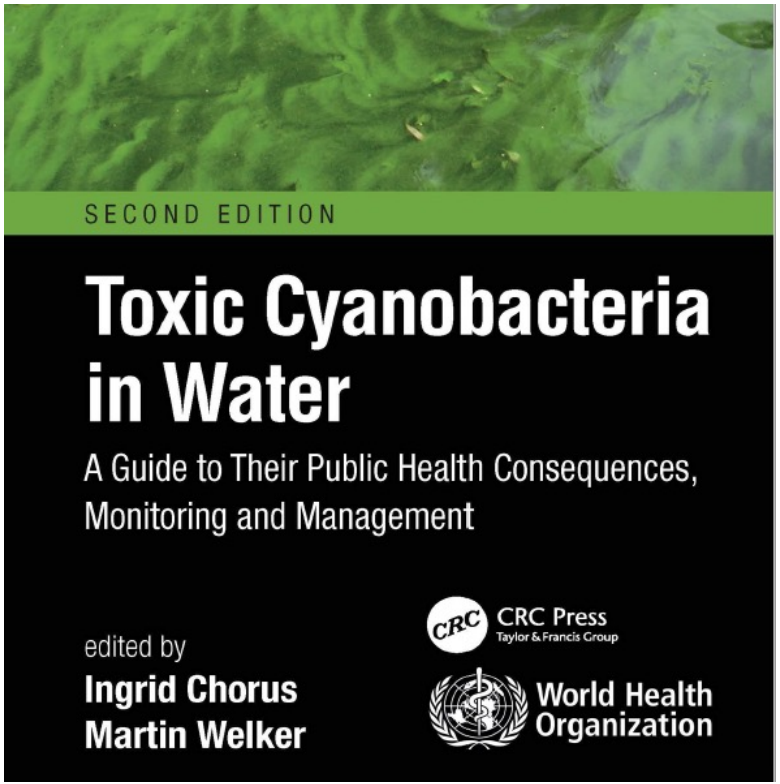
LC-MS analytics and rapid tests

Cercl'eau Tagung 14-15 June 2022

1. WHO Toxins
2. Analytical methods
3. Additional bioactive metabolites

WHO Reference book (2021)

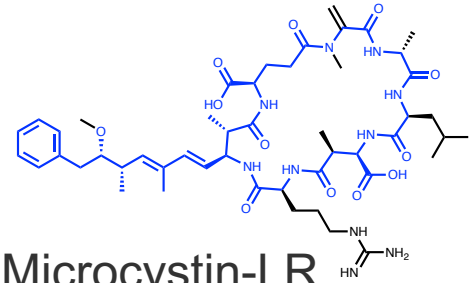
available online



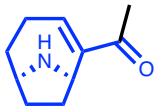
- Occurrence and exposure
- Risk assessment
- Bloom monitoring program

→ **calibrate each system**

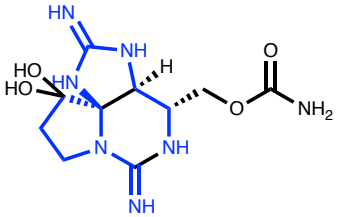
“WHO Toxins” in water quality guidelines



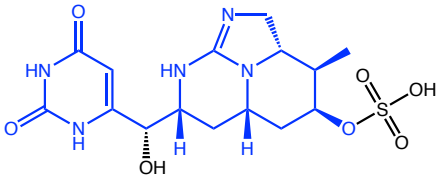
Microcystin-LR



Anatoxin-a



Saxitoxin



Cylindrospermopsin

WHO (2021)	**CHRONIC**: provisional lifetime **drinking-water** guideline value (µg/l)	**ACUTE**: provisional short-term **drinking-water** guideline value (µg/l)	**recreational water** guideline value (µg/l)
Microcystin-LR	1	12	24
Anatoxin-a		30	60
Saxitoxin		3	30
Cylindrospermopsin	0.7	3	6

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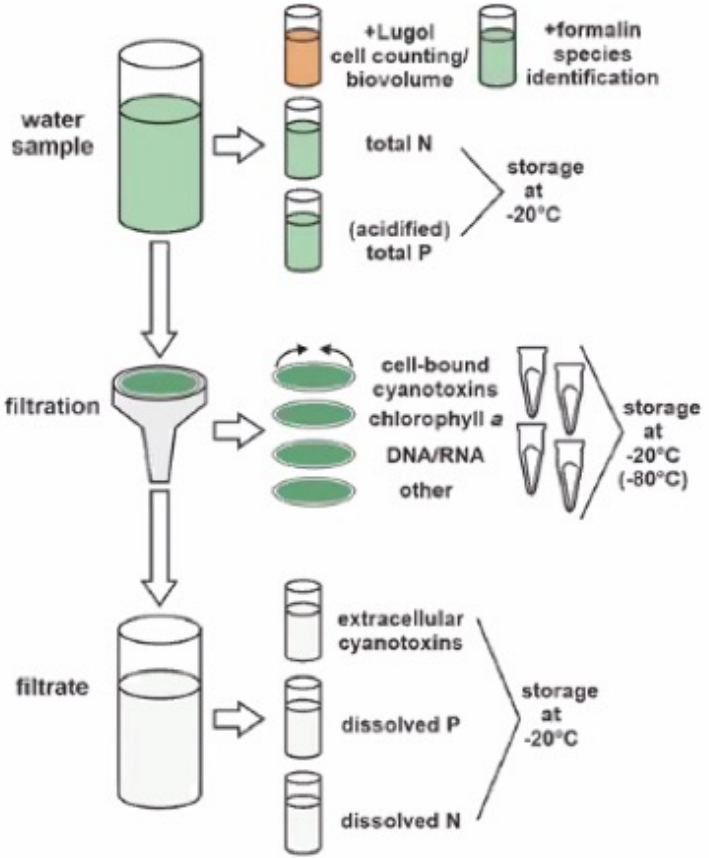
Analytical methods

Microscopy
→ cyanobacteria genus/species

Genetics
→ toxin-producing genes (e.g., McyE)

Chemical analysis
→ toxin concentrations, »fingerprints»
- «rapid» tests
- LC-HRMS/MS



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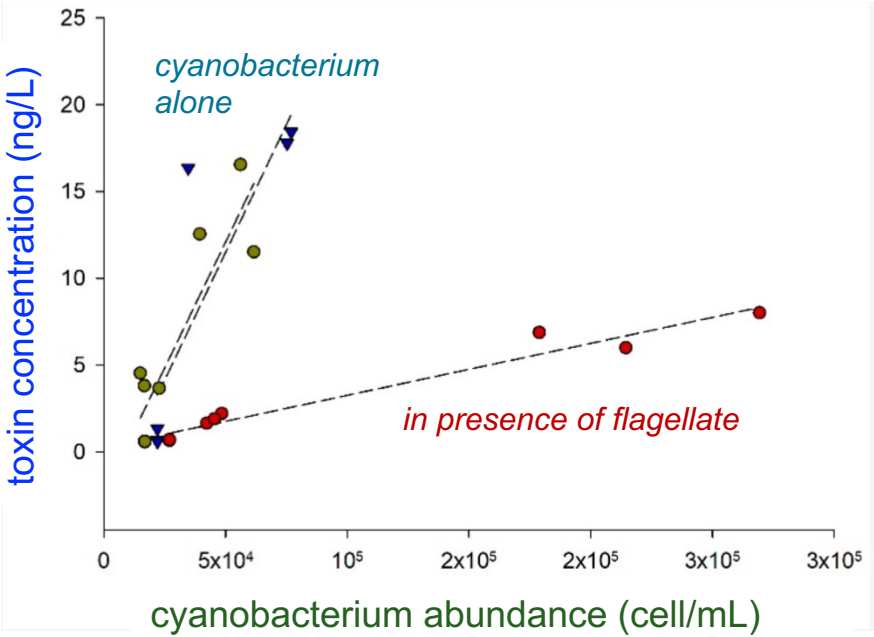


Production of Cyanotoxins by *Microcystis aeruginosa* Mediates Interactions with the Mixotrophic Flagellate *Cryptomonas*. DeVaul, Hendricks, White. *Toxins, 11(4), 223; https://doi.org/10.3390/toxins11040223.* ***2019***

Analytical methods

	Quantitative	Training/cost	Compound specific	Sum of comp. class
Lateral Flow test	no (range)	novice / $$	no	yes
ELISA	semi-quant	intermediate / $$	no	yes
LC-MS/MS	yes	expert / $$$	yes	no

"No analytical procedure is superior to others *per se* – rather, it is the **context and purpose** that renders an individual method appropriate – or inappropriate." *from WHO guidelines 2021*

Lateral Flow Tests

(35-40 CHF / test)

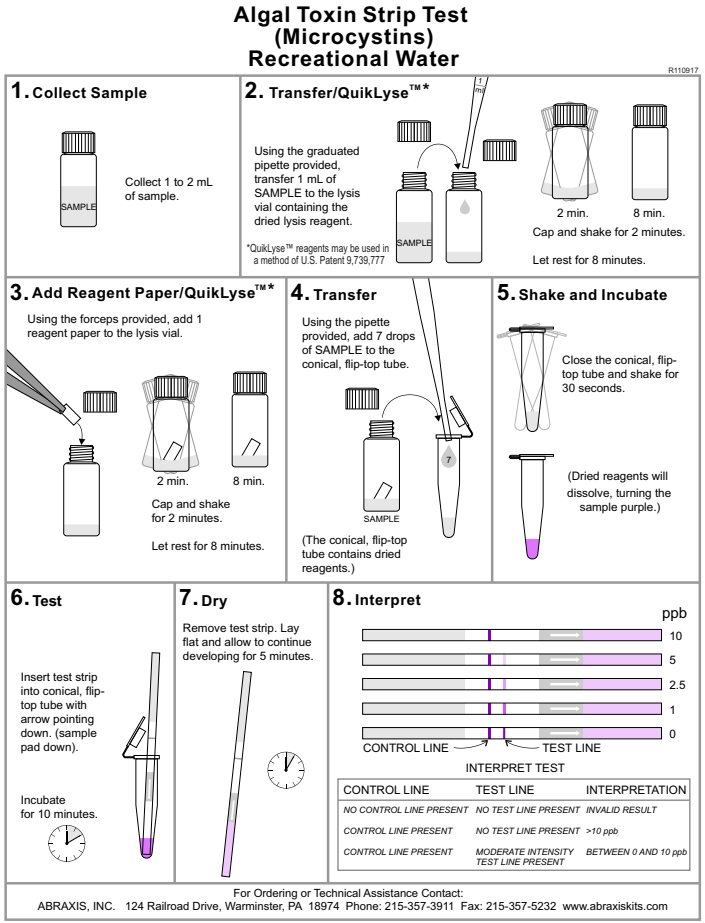
available for

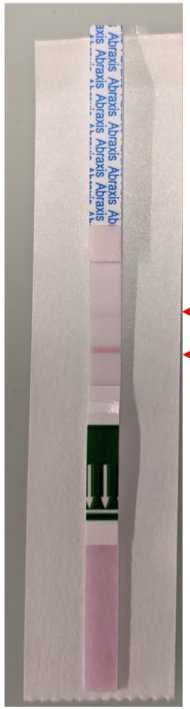
- microcystins
- anatoxins
- cylindrospermopsins

- ❑ immunochromatographic test
- ❑ qualitative only (!)
- ❑ not compound specific

→ recommended for event screening used in the field at potentially high concentrations, guideling sampling

https://abraxis.eurofins-technologies.com





ELISA test - plate reader assay

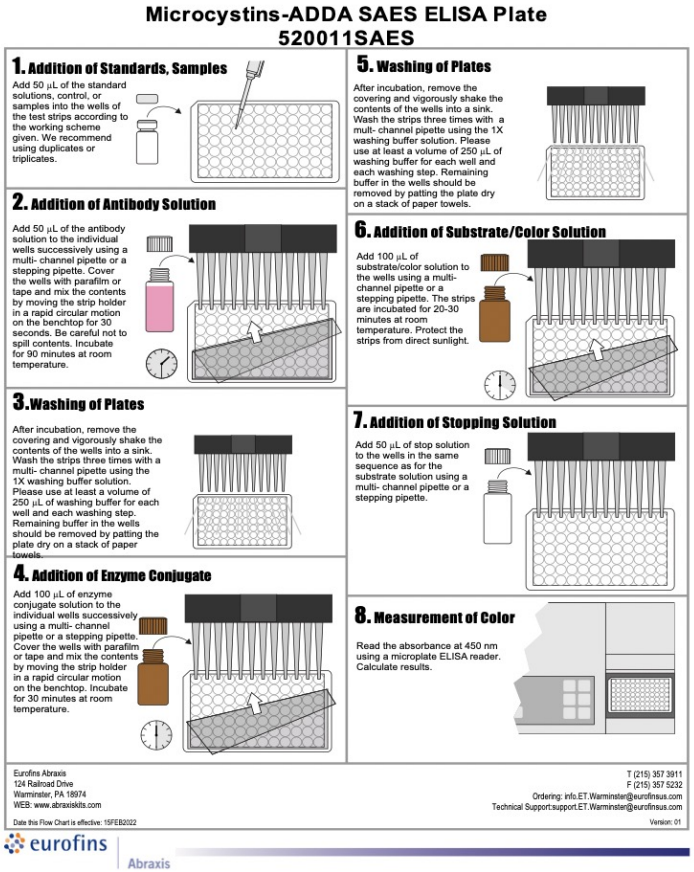
(600-700 CHF / 96 well-plate)

<u>available for</u>

- microcystins
- anatoxins
- cylindrospermopsins

- ❑ enzyme-linked immunosorbent assay
- ❑ semi-quantitative
- ❑ <u>not</u> compound specific

→ recommended for routine, rapid screening to trigger LC-MS/MS analysis



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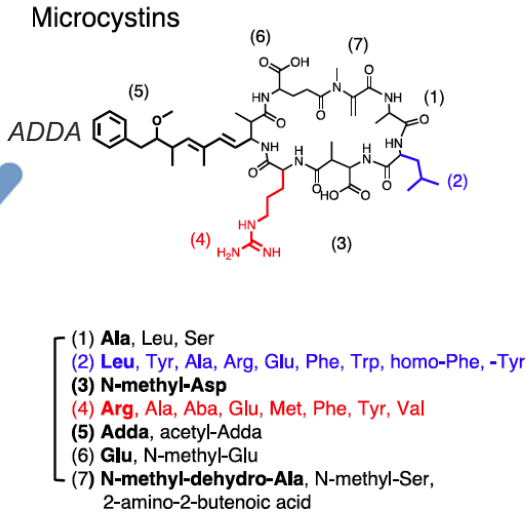

- ❑ enzyme-linked immunosorbent assay
- ❑ semi-quantitative
- ❑ not compound specific

→ recommended for routine, rapid screening to trigger LC-MS/MS analysis

- ❑ 310 known microcystins
- ❑ ~ 12 reference standards

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ELISA test - plate reader assay

(600-700 CHF / 96 well-plate)

<u>available for</u>

- microcystins
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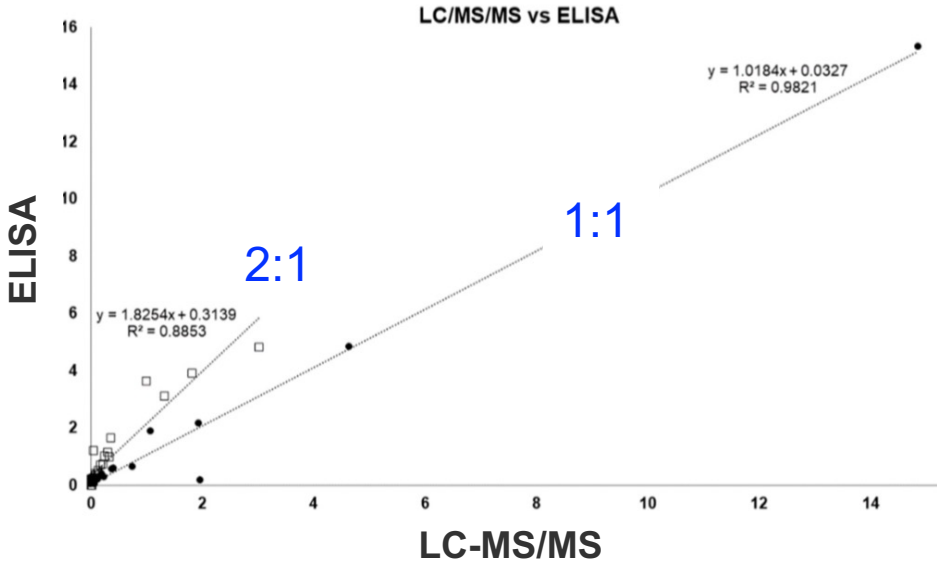

https://abraxis.eurofins-technologies.com

Comparative Analysis of Microcystin Prevalence in Michigan Lakes by Online Concentration LC/MS/MS and ELISA. T*oxins*, *11*(1), 13; https://doi.org/10.3390/toxins11010013, **2019**.

LC-MS/MS analysis

(150-300 CHF / sample)

available for targeted analysis of

- microcystins (approx. 12 congeners)
- anatoxins
- cylindrospermopsins
- saxitoxins

❑ mass-to-charge ratio of ionized toxin
❑ quantitative but requires reference standard
❑ individual compounds

→ recommended to evalute specific presence of toxins
→ for quantitiative analysis for risk assessment

quantification in the low ng/L range

Quantification of multi-class cyanopeptides in Swiss Lakes with automated extraction, enrichment and analysis by online-SPE HPLC-HRMS/MS. Jones and Janssen, *CHIMIA*,76, No 1/2, p. 133-144, **2022**.
https://doi.org/10.2533/chimia.2022.133

1. WHO Toxins
2. Analytical methods
3. **Additional bioactive metabolites**

2010 known secondary metabolites of cyanobacteria

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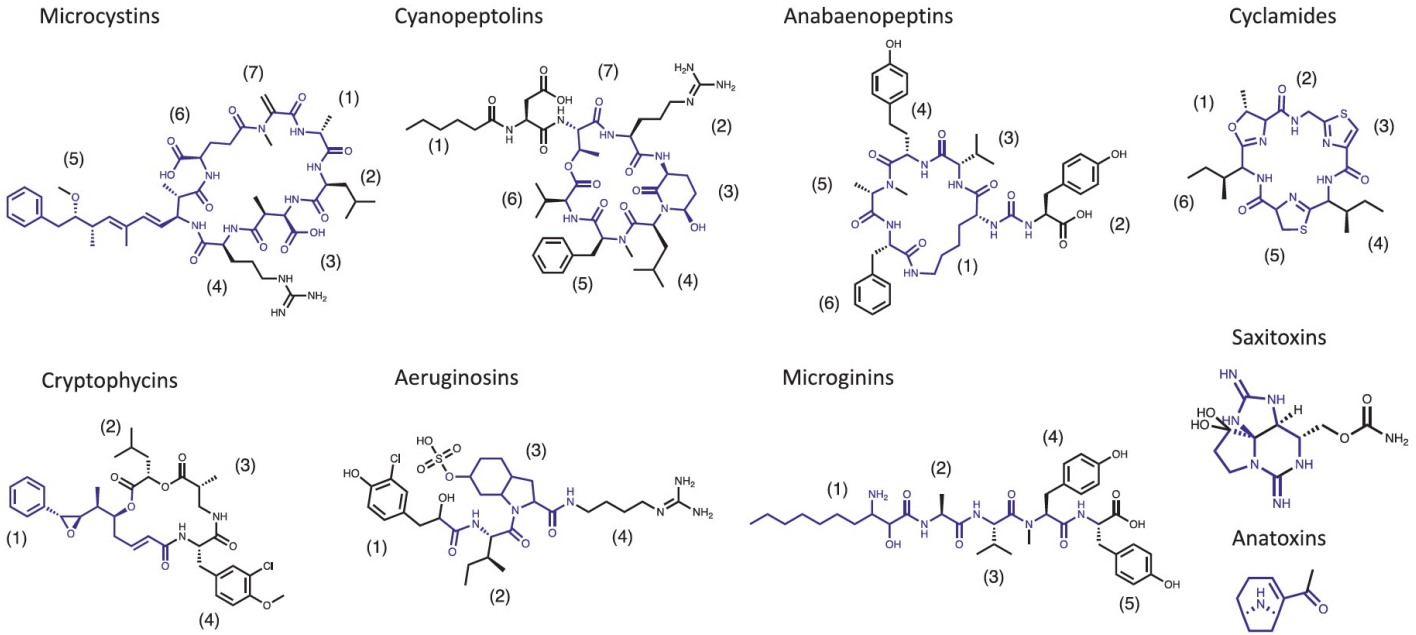
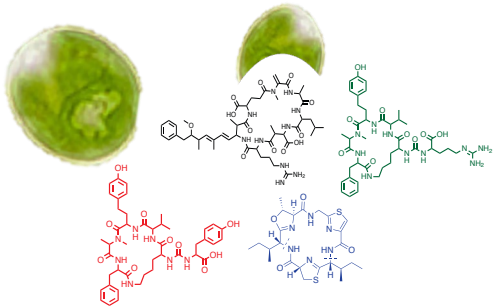


CyanoMetDB secondary metabolites

CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria.
Jones and Janssen at al. *Water Research*, 197, 117017 **2021**. https://doi.org/10.1016/j.watres.2021.117017

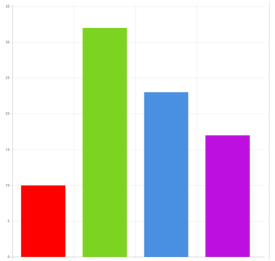
Reason why we are interested to measure other metabolites

1. **Co-production**
   of toxins and other secondary metabolites by one cyanobacteral species.



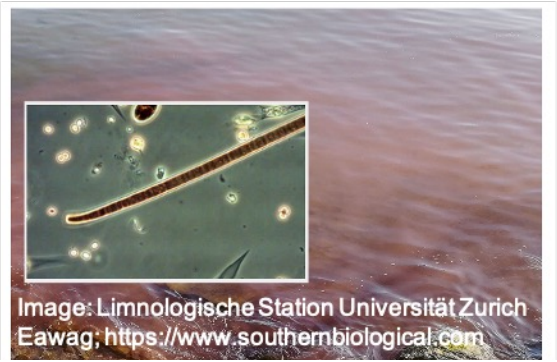
2. **Toxic effects**
   other metabolites contribute to toxicity (mixtures)

3. **Monitoring**
   other metabolites can be present in high concentrations → better for monitoring



Additional bioactive metabolites

Planktothrix rubenscens
Lake Zürich



Metabolites
[D-Asp^3,E-Dhb^7]MC-RR

- ❑ **no** WHO toxins
- ❑ non-target microcystin dominates

reference standards (> 95% purity)

Microcystin LR
Microcystin LY
Microcystin LF
Microcystin LA
Microcystin LW
Microcystin YR
Microcystin RR
Nodularin

[D-Asp3,E-Dhb7]MC-RR
D-Asp3-MC-LR

Additional bioactive metabolites

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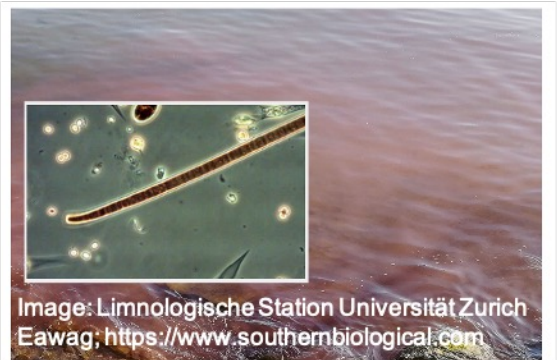

Metabolites

[D-Asp^3,E-Dhb^7]MC-RR
Anabaenopeptin B
Anabaenopeptin A
Anabaenopeptin F
Oscillamide Y

- ❑ **no** WHO toxins
- ❑ non-target microcystin dominates
- ❑ higher concentrations of **other bioactive metabolites**

reference standards (> 95% purity)

Microcystin LR
Microcystin LY
Microcystin LF
Microcystin LA
Microcystin LW
Microcystin YR
Microcystin RR
Nodularin

[D-Asp3,E-Dhb7]MC-RR
D-Asp3-MC-LR

bioreagents (> 90% purity)

Anabaenopeptin A
Anabaenopeptin B
Oscillamide Y
Anabaenopeptin NZ857
Cyanopeptolin A
Cyanopeptolin D
Aerucyclamide A
Aeruginosin 98B

Suspect screening for toxins & metabolites

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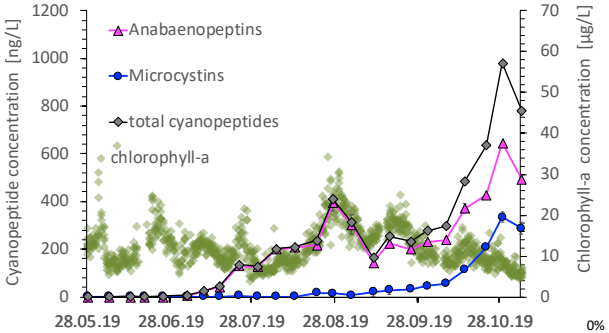
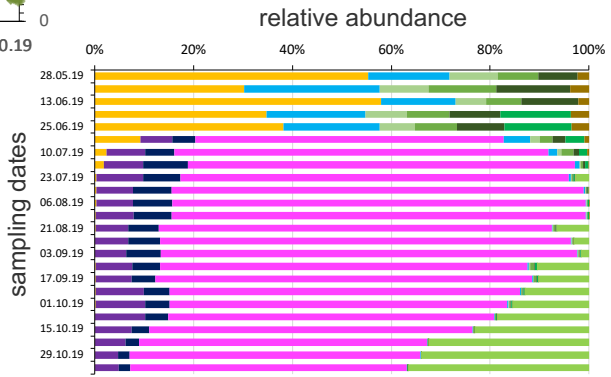
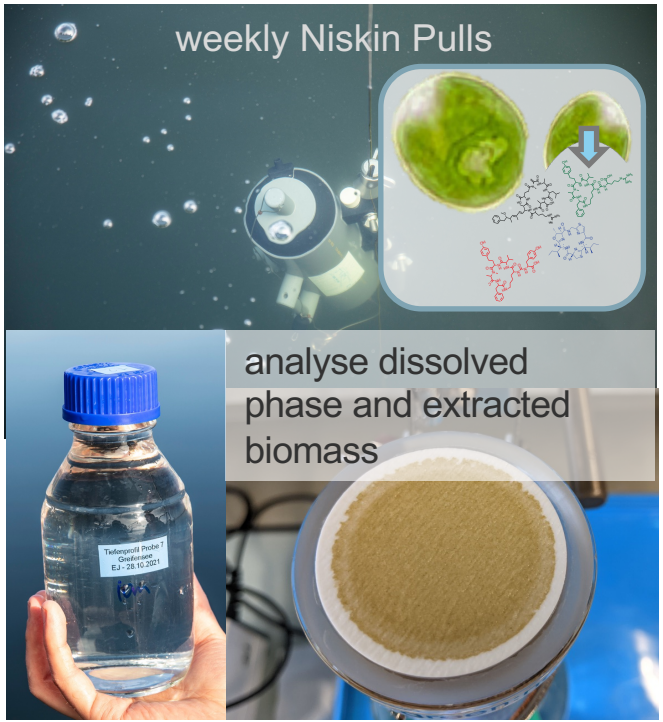
Investigate evolution of toxin concentrations and profiles



time series



toxin profiles



Checklist for analysis of toxins

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"No analytical procedure is superior to others *per se* – rather, it is the context and purpose that renders an individual method appropriate – or inappropriate." *from WHO guidelines 2021*

- ❑ What toxins need to be analysed ?
  - WHO toxins or selected toxins/metabolites known to occur in the respective water?
  - toxins/metabolites with highest concentrations or known risks?
  → literature information about local situation or pre-screening study to prioritise substances
- ❑ Which type of sample is of interest ?
  - water, cells, sediment, tissue
- ❑ Is a quantitative results (always) required ?
  If yes, which detection limit
- ❑ What analytical infrastructure and expertise is available ?
  - in-house and external
  - required training
- ❑ What is the available capacity for sampling and analyis ?
  - sampling, storage, analysis
  - number of samples to be analysed in what time frame
- ❑ What data will be assessed and how will it be reported ?

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