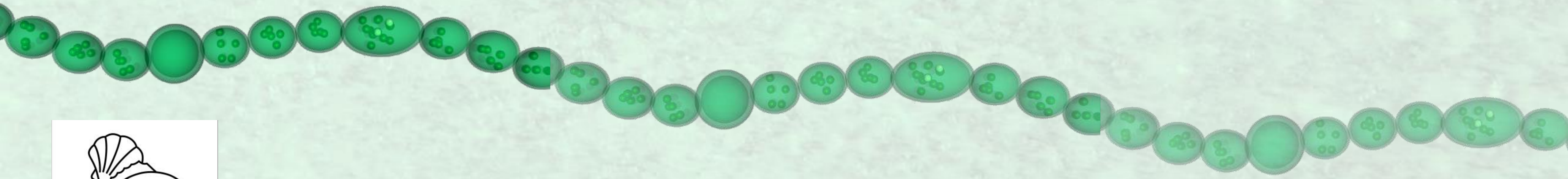
The efficiency of chlorine-based treatments on Microcystis aeruginosa cultures by untargeted LC-HRMS

Luciana Tartaglione

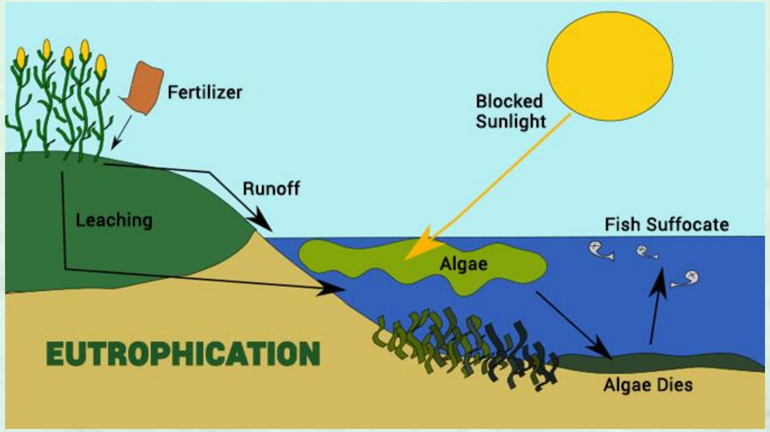
University of Naples Federico II, Italy



Aquatic Toxins Symposium - 10-11 June 2024 - Kaiserin-Friedrich-Hörsaal Robert-Koch-Platz 7, 10115, Berlin

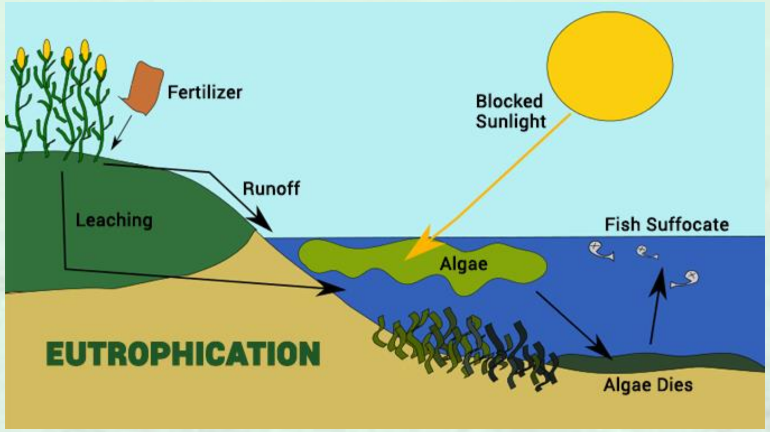
Cyanobacterial Blooms

- **Environmental alterations** may induce cyanobacteria to a massive proliferation in water basins
- Climate change affect frequency, magnitude and duration of cyanobacterial blooms in both freshwater and even marine environments



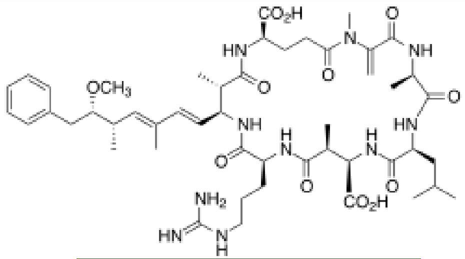
Cyanobacterial Blooms

Major effects on the water quality and functioning of aquatic ecosystems

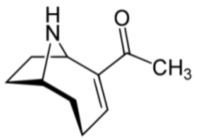


- ❑ **block sunlight to underwater grasses**
- ❑ **consume oxygen in the water leading to fish kill**
- ❑ **produce surface scum and odors**
- ❑ **interfere with the feeding of organisms that filter water to obtain their food**

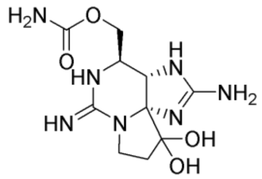
A wide array of cyanotoxins



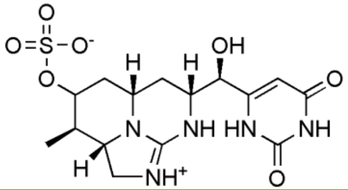
Microcystins



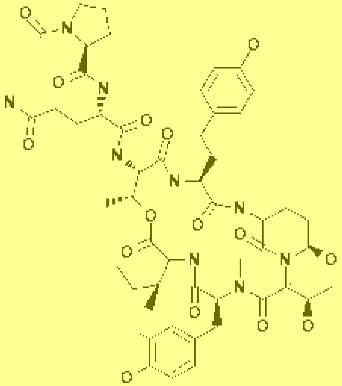
Anatoxin-a



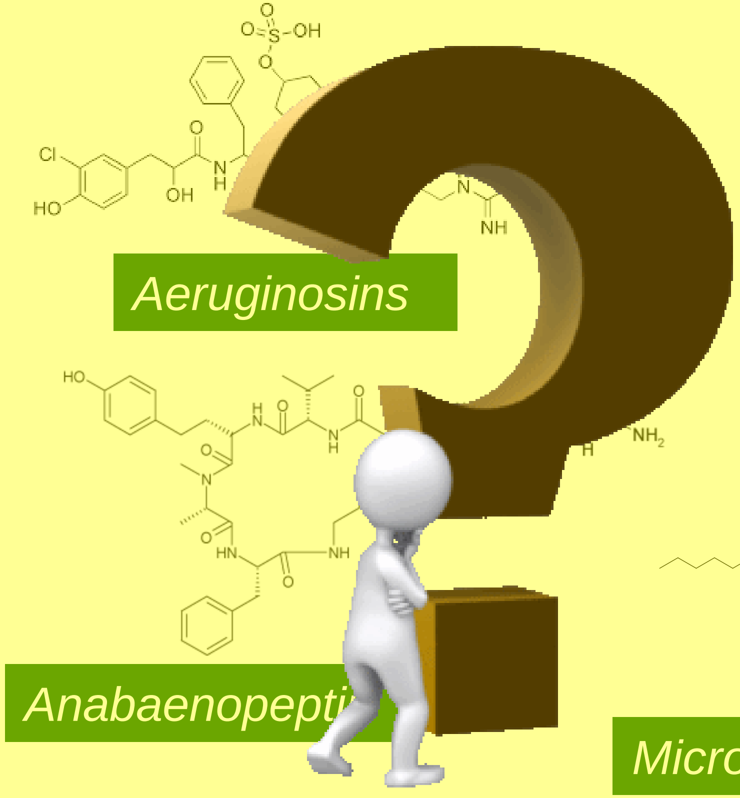
Saxitoxin



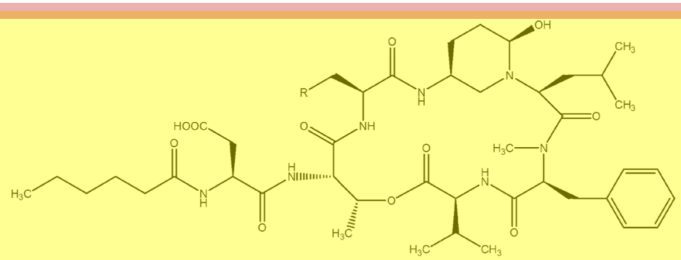
Cylindrospermopsin



Anabaenopeptilides

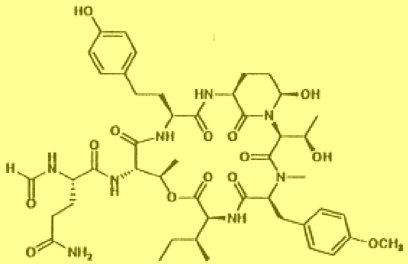


Aeruginosins

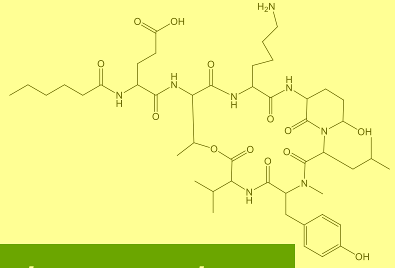


Cyanopeptolins

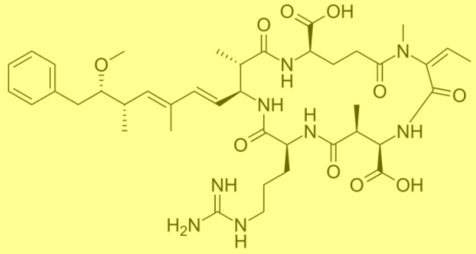
Anabaenopepti



Anabaenopeptilides



Micropeptins



Nodularin

Harmful Cyanobacterial Blooms

CyanoHABs can affect animal and human health

Oral exposure

•injestion of contaminated water during recreational activities
•injestion of drinking water from contaminated reservoires
•consumption of cyanobacteria-based food supplements

Inhalation exposure

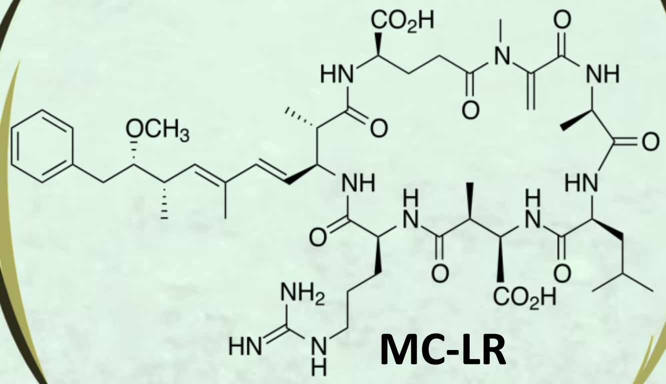
accidental inhalation during recreational activities

Dermal exposure

dermal contact during recreational activities

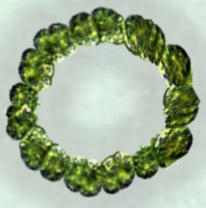
Regulatory settings

World Health Organization



MC-LR

European Commission

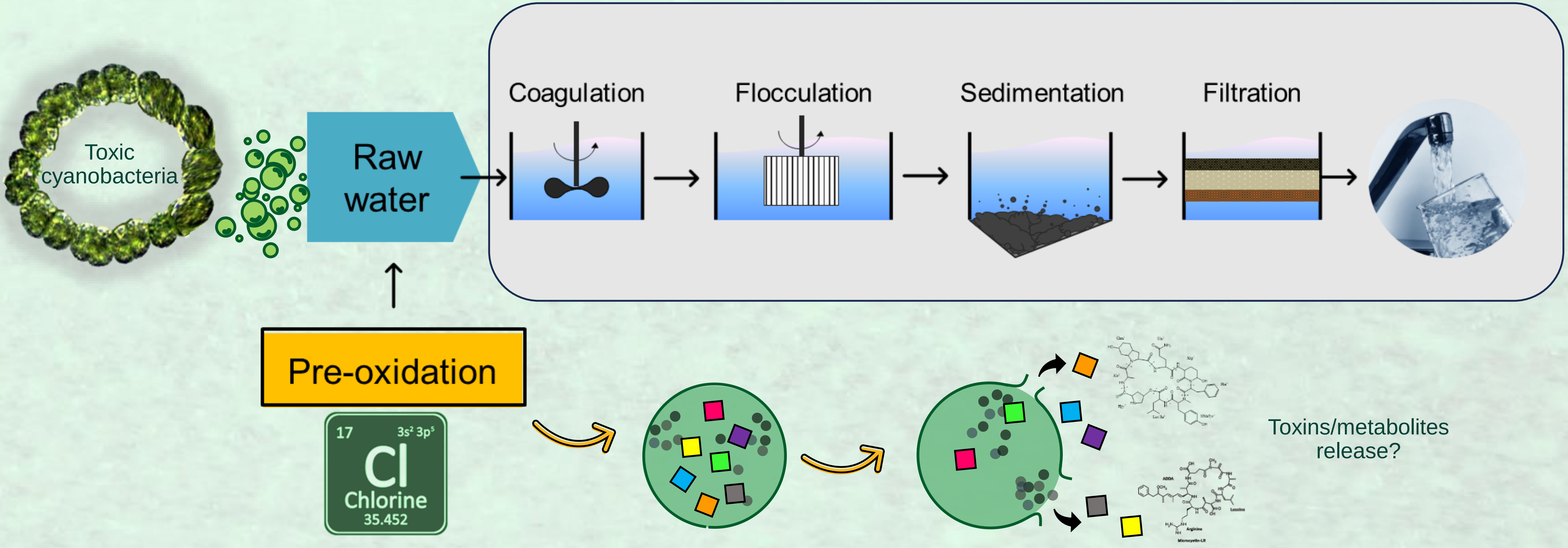


Guidance Value (MCs congeners) = 1 μg/L

Global safe management of drinking water supplies, and cyanobacteria monitoring in water bodies for human consumption represents a priority to protect public health.

Harmful Cyanobacterial Blooms in drinking water reservoirs

- Drinking water treatment plants ➜ **pre-oxidation** conducted using chlorine-based agents to inactivate pathogens, inhibit algal growth, to guarantee the safety of drinking finished water.
- the pre-oxidation of raw surface water is whose efficacy and safety in removing both cyanobacterial cells and cyanotoxins, is still debated.
- Chlorinated oxidants directly added to raw water that **may contain toxic cyanobacteria**
  ➜ Are **toxins**/**other cyanopeptides release or degrade**? **Inactivation of cells**? **Alternatives**?



Low-dosed chlorine-based tratments

NaClO
Sodium hypochlorite

ClO_2
Chlorine dioxide

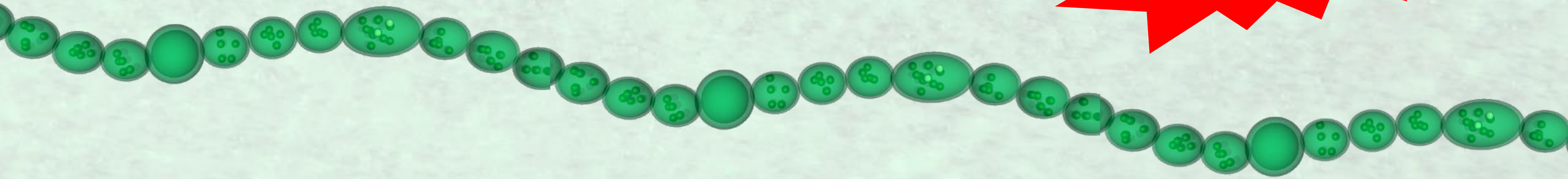
To test two different **chlorine-based tratments**, at different doses and exposure times, on ***Microcystis* sp. culture**, in order to evaluate the efficiency of the water treatment to be used at regulatory level and to face challenges in providing drinking water during a severe cyanobacterial bloom event in source waters.

Romagna Acque Società delle Fonti S.p.A.

Are toxins efficiently removed?

Are disinfection by-products formed?

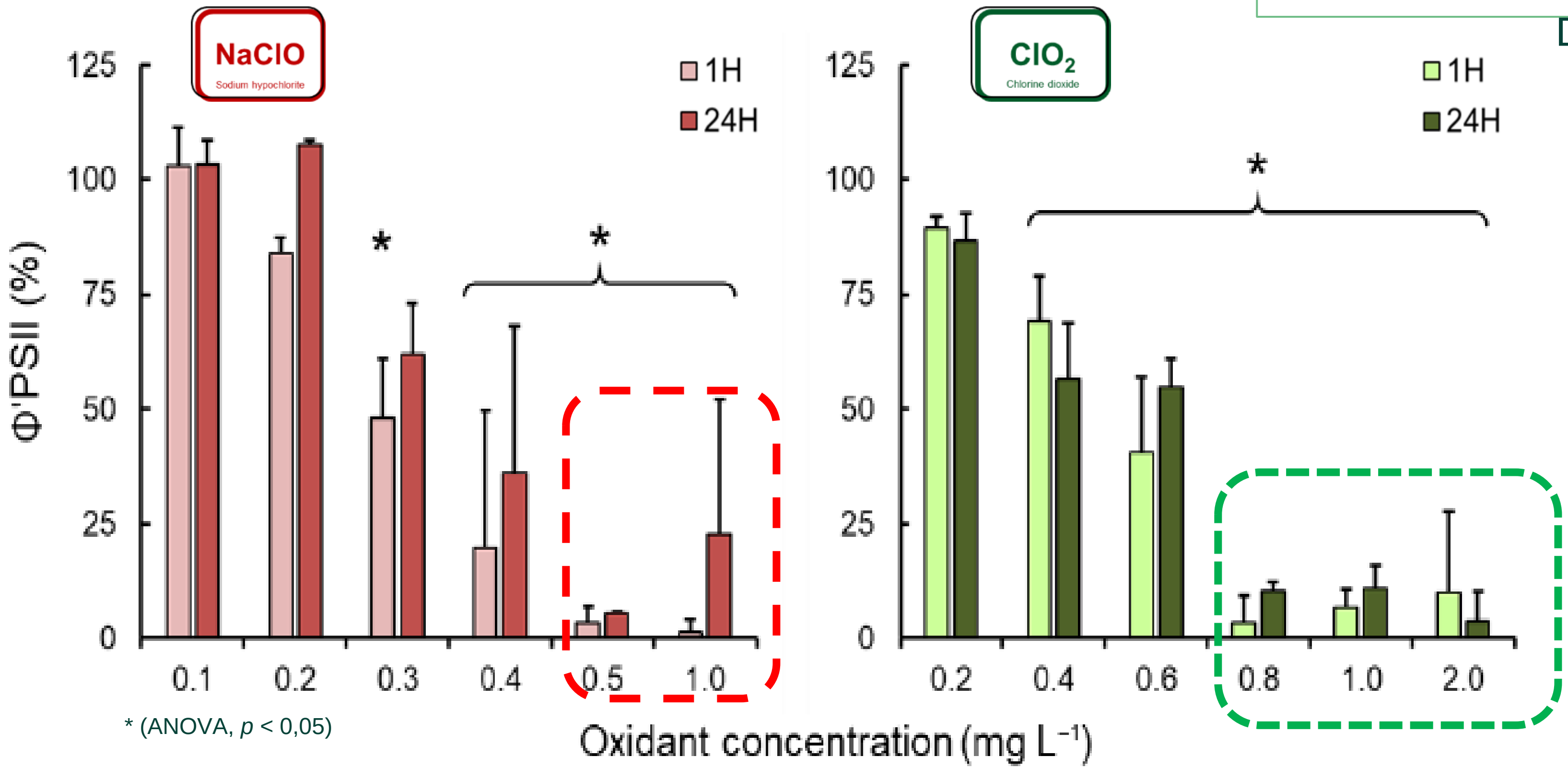
- Cell viability
- Toxin content and fate



Photosynthetic efficiency of *M. aeruginosa*
Preliminary tests

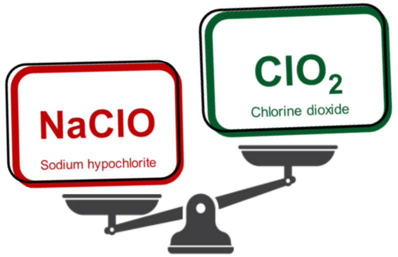
In collaboration with:
Prof. L. Pezzolesi
Prof. R. Pistocchi
Dr. M. Simonazzi
Dr. F. Guerrini

Inhibition of photosynthetic activity (%/ctrl)



* (ANOVA, p < 0,05)

- Inactivation of *M. aeruginosa* cells occurs **at low doses** of both oxidants (0.4-0.8 mg L^{-1}) after 1h
- NaClO appeared affecting cell viability at lower doses compared to ClO_2 (0.5 vs 0.8 mg L^{-1})



Experimental Design

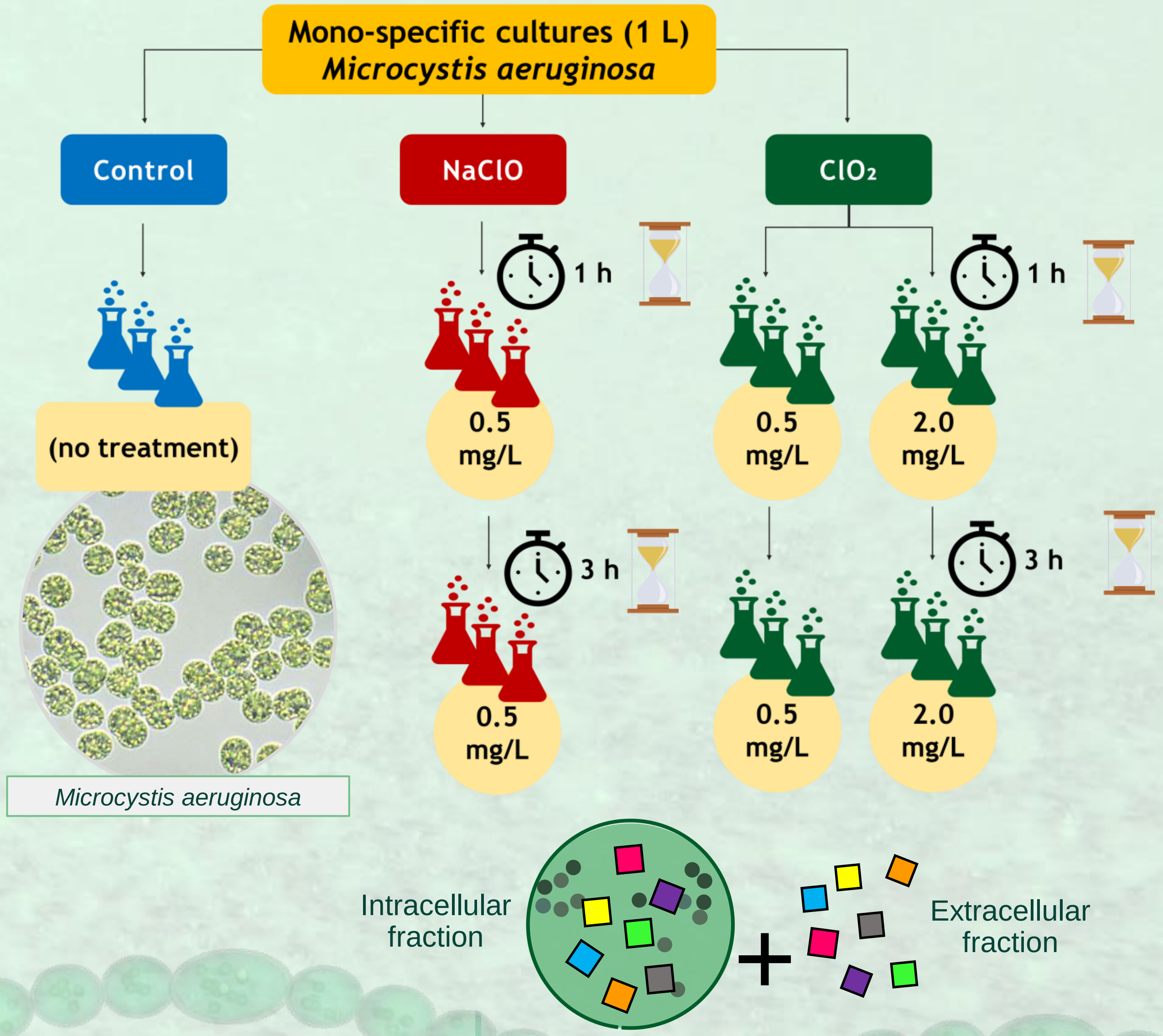
Testing the efficacy of chlorine-based treatments

- Testing common chlorinated oxidants on **mono-specific cultures** of the **toxic cyanobacterium *Microcystis aeruginosa***)

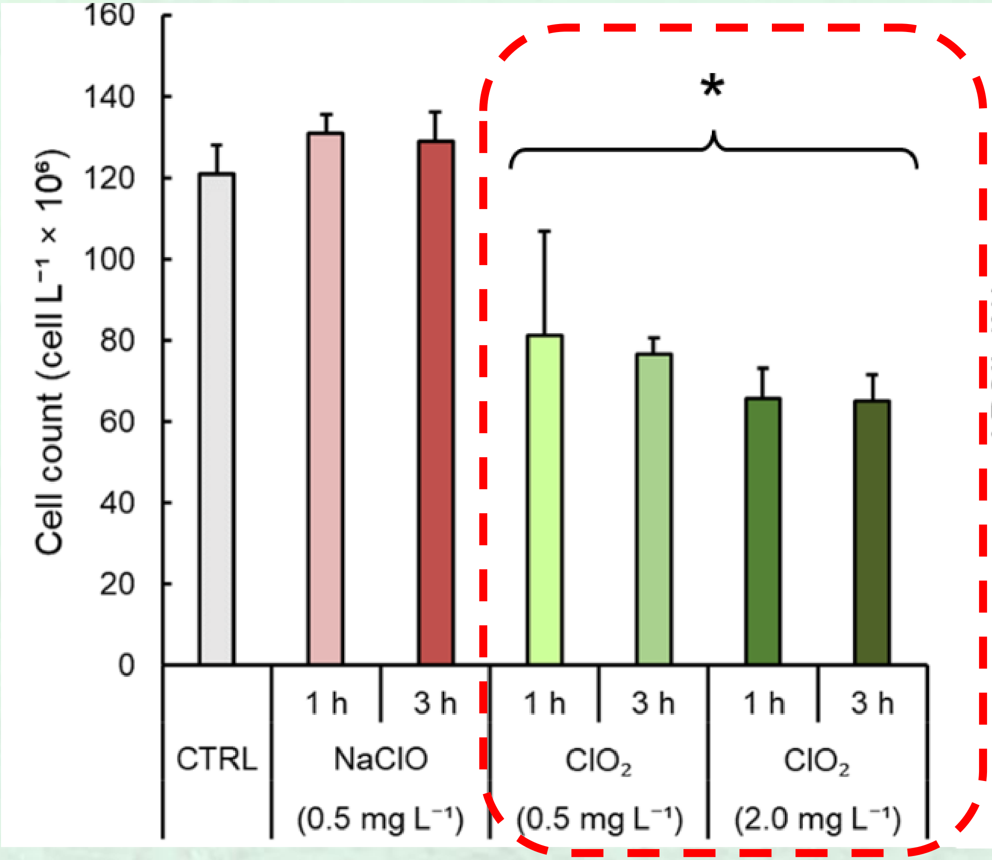
NaClO
Sodium hypochlorite

ClO_2
Chlorine dioxide

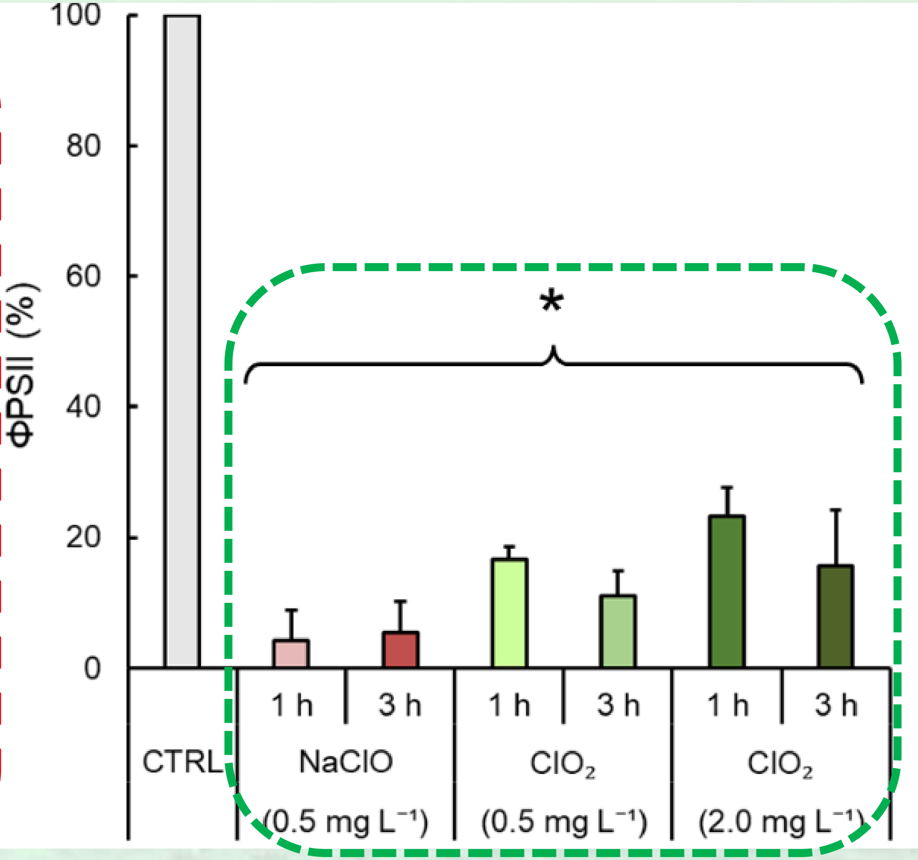
- Understanding the fate of cyanotoxin and other metabolites inside and out cells → centrifugation then **untargeted LC-HRMS**



Cell density and Photosynthetic activity



CELL DENSITY



PHOTOSYNTHETIC EFFICIENCY

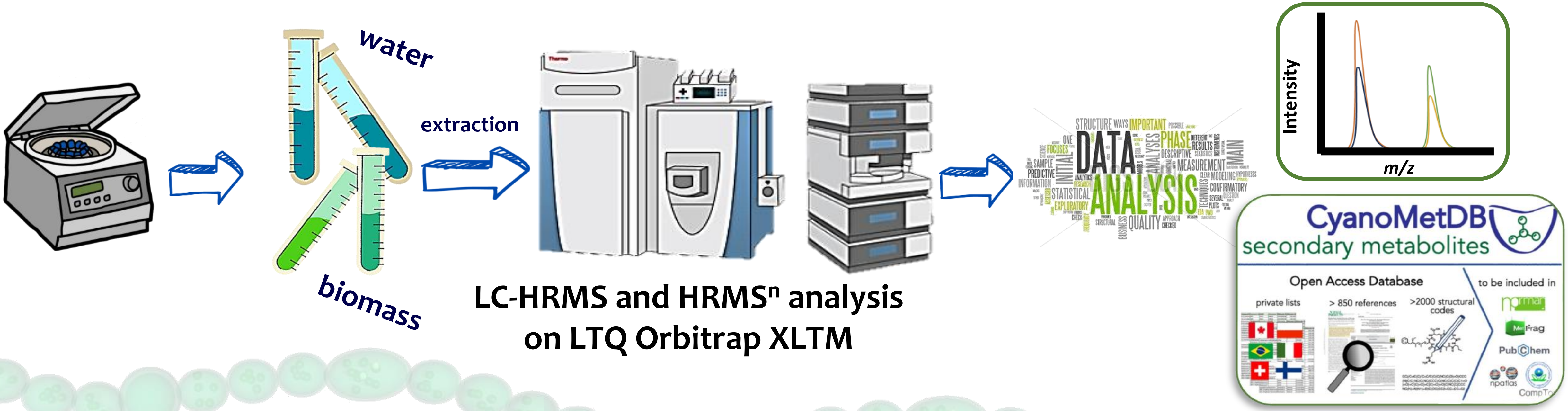
- A significant decrease only for ClO_2 treatments
- No differences among treatment times

- Inhibition (residual activity < 20%) using both oxidants

LC-HRMS investigation

Cyanotoxins quali-quantitative determination

Extra-cellular (water) and **intra-cellular (biomass)** fractions extracted and analyzed by untargeted **LC-HRMS** analyses.



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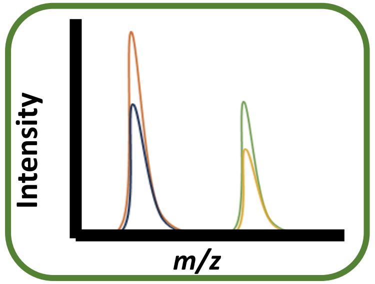
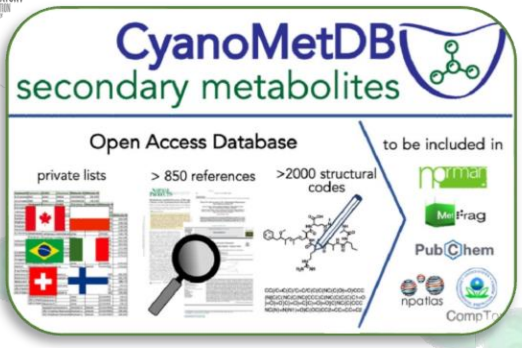
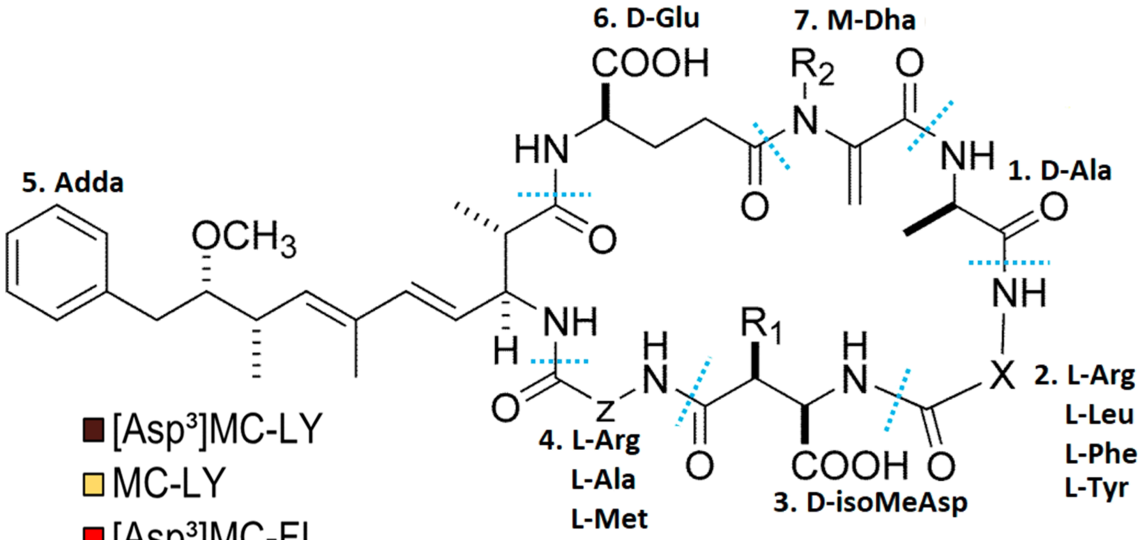


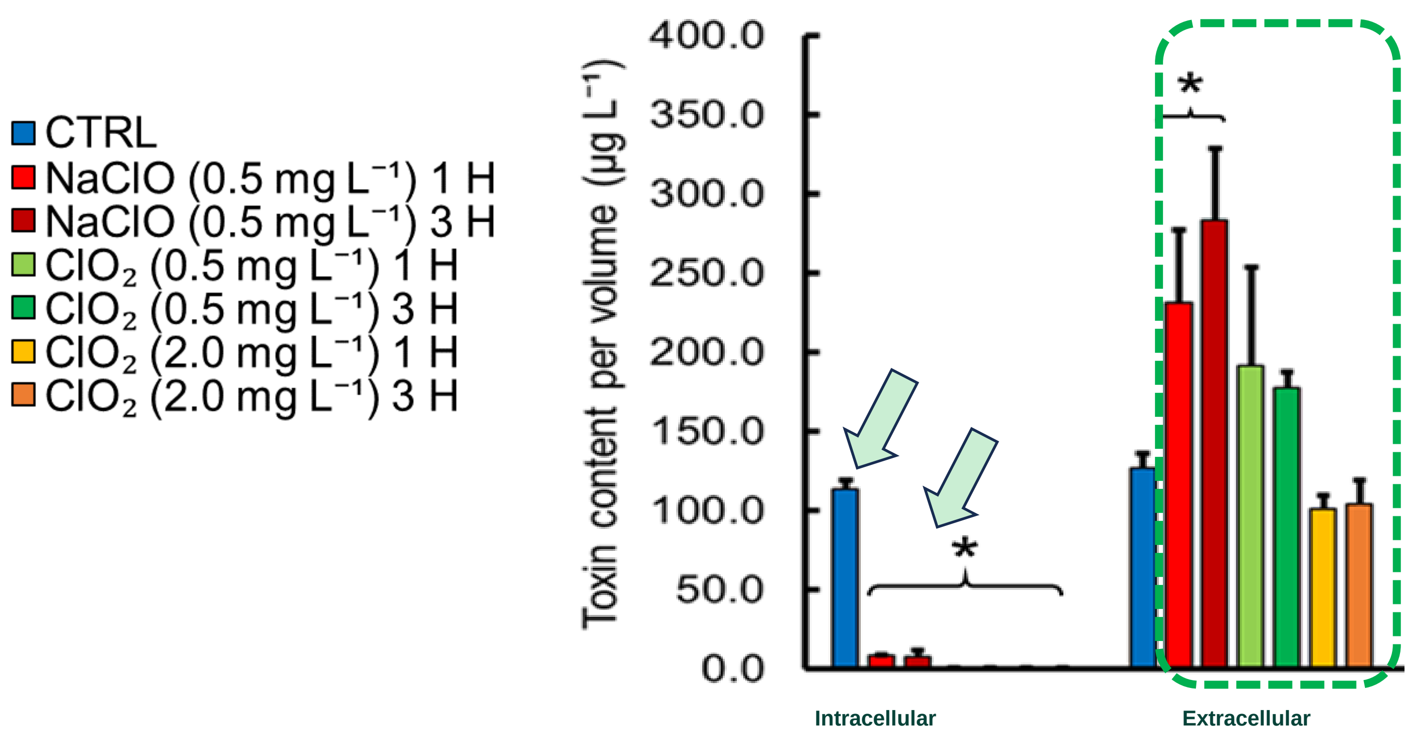


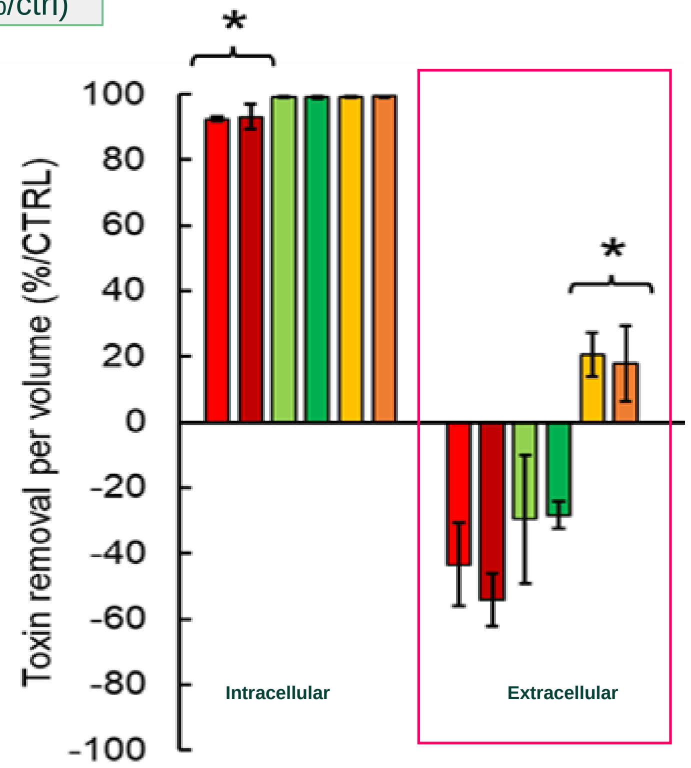


- [Asp³]MC-LY
- MC-LY
- [Asp³]MC-FL
- MC-FL
- [Dha⁷] MC-LR
- MC-LR

Effects of chlorine treatments on toxin content

Toxin content (µg L^{-1}) and percent removal (%/ctrl)





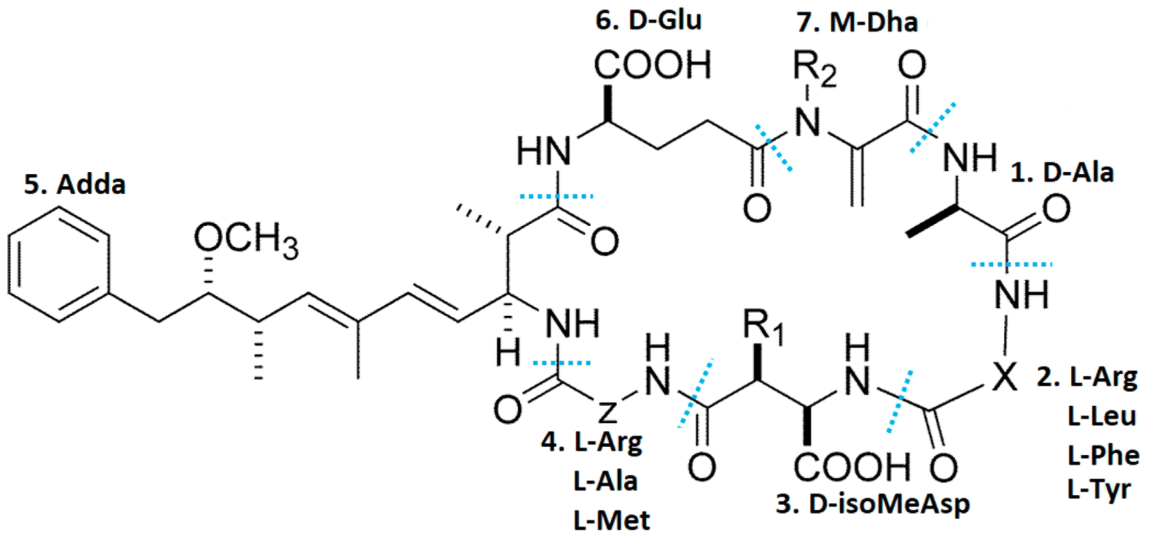
- Intracellular fractions: MC levels lower in treated samples vs controls
- Increase of toxin content in extracellular fractions in treated samples vs controls
- Intracellular toxins removed by chlorinated oxidants, but likely released into water regardless of treatment and time.
- **Only high doses of ClO_2 partially degraded extracellular toxins**

Which disinfection by-products will be formed?

Detoxification treatments:

- **NaClO** (0.5, 2g for 1h e 3h)
- **ClO_2** (0.5, 2g for 1h e 3h)

Microcystins



Biomass: Dihydroxy-MCs

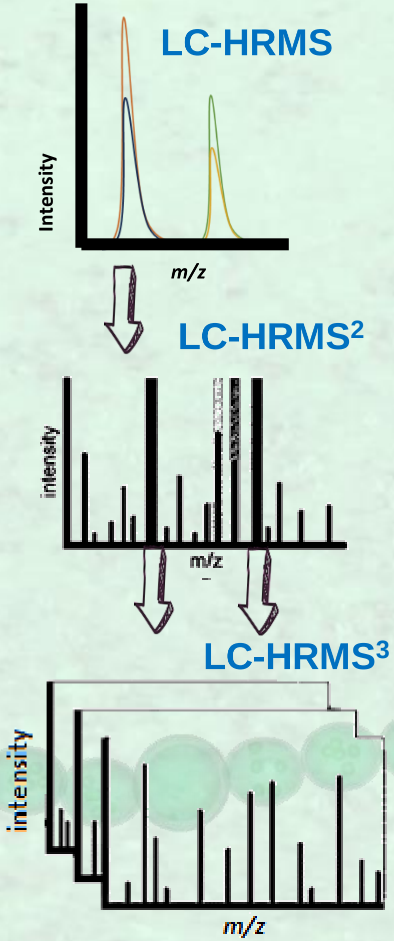
Water: Dihydroxy-MCs + Monochloro-hydroxy-MCs

- Trace levels of MCs oxidized products (dihydroxy and monochloro-hydroxy MCs) have been also detected.
- Chlorine treatments, at the low doses here tested (≤ 2 mg/L), only slightly promote MCs decomposition into the oxidized products, possibly the rate of toxin degradation into nontoxic dihydroxy or monochloro-hydroxy derivatives could be slower than its release due to oxidation-driven cellular damage

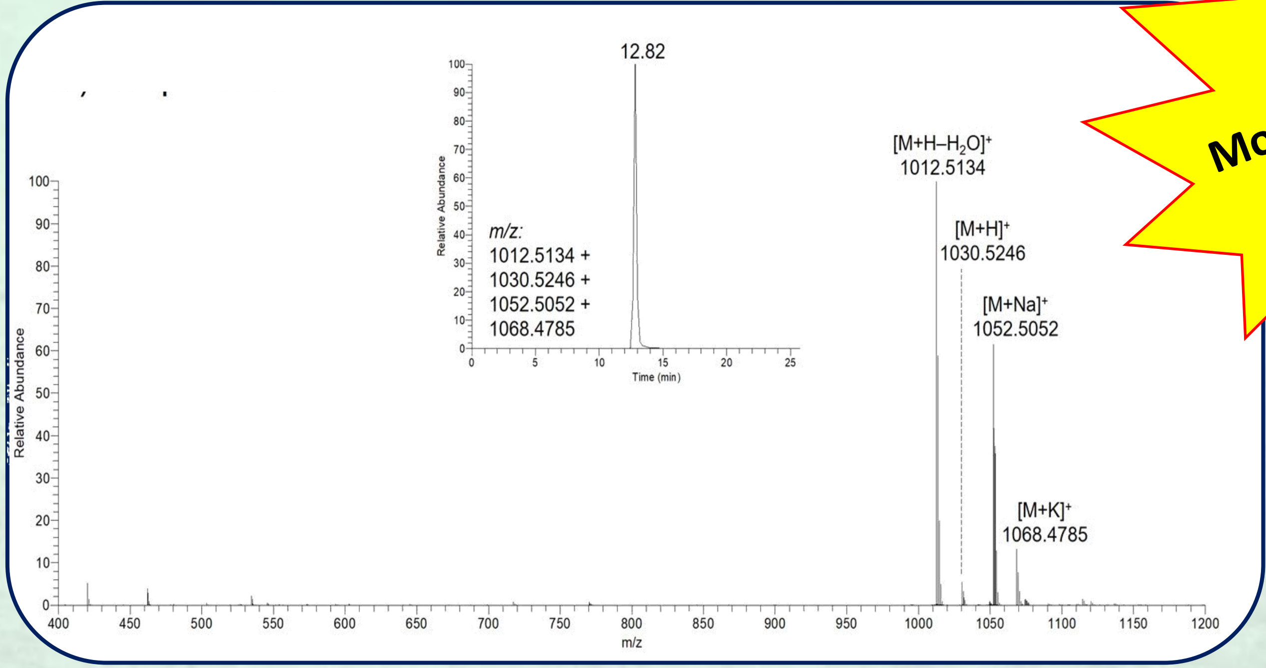
W. Zong et al., Journal of Hazardous Materials, 252– 253 (2013) 293– 299

A new Cyanopeptolin-type peptide

Cyanotoxins quali-quantitative determination



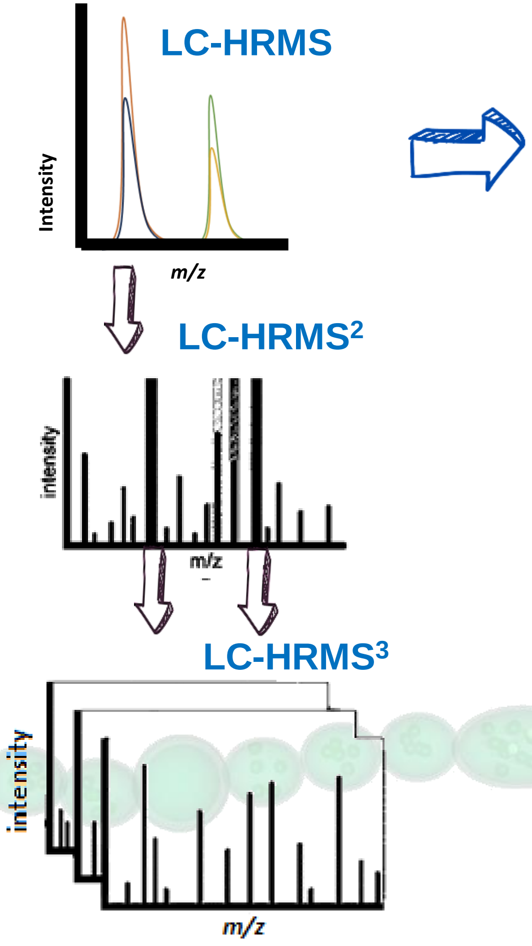
Cyanopeptolin-type peptide-1029

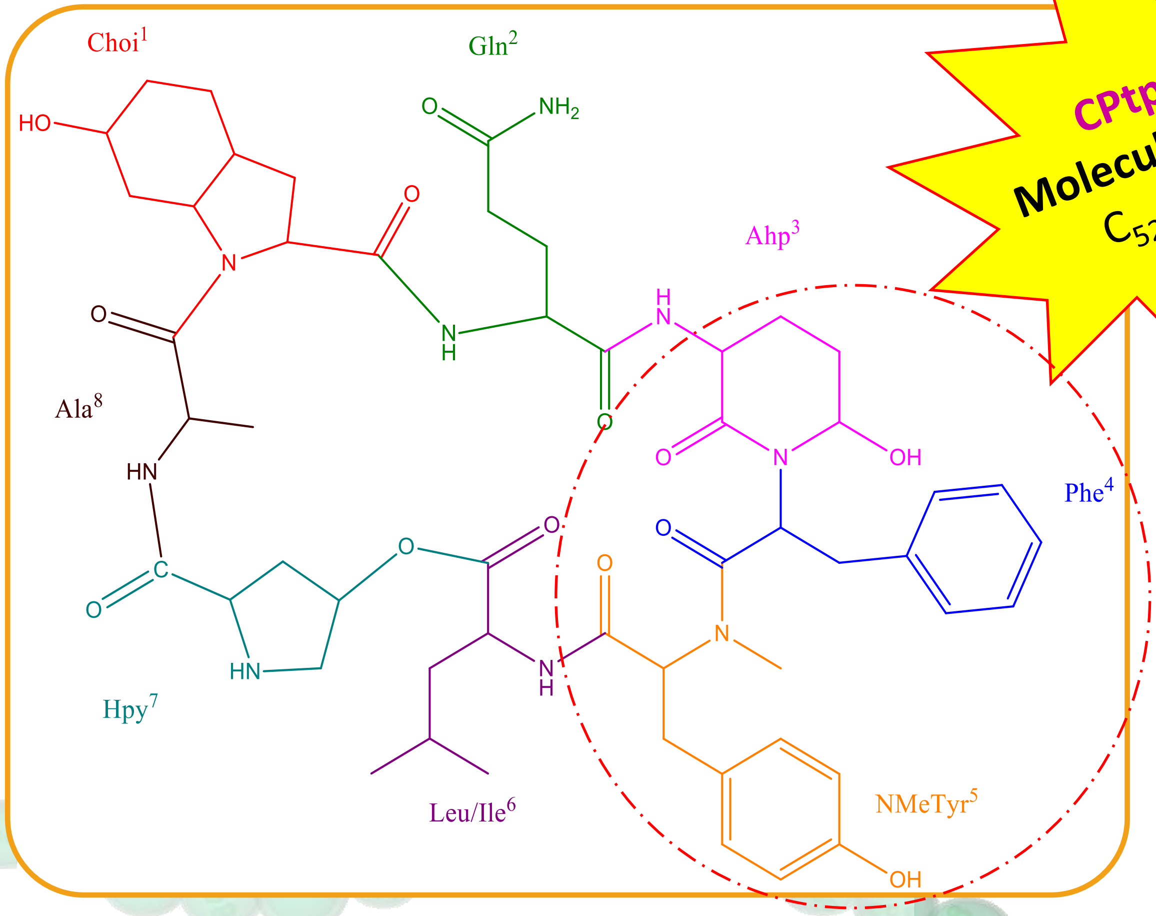


CPtp-1029
Molecular Formula
$C_{52}H_{71}O_{13}N_9$

A new Cyanopeptolin-type peptide

Cyanotoxins quali-quantitative determination





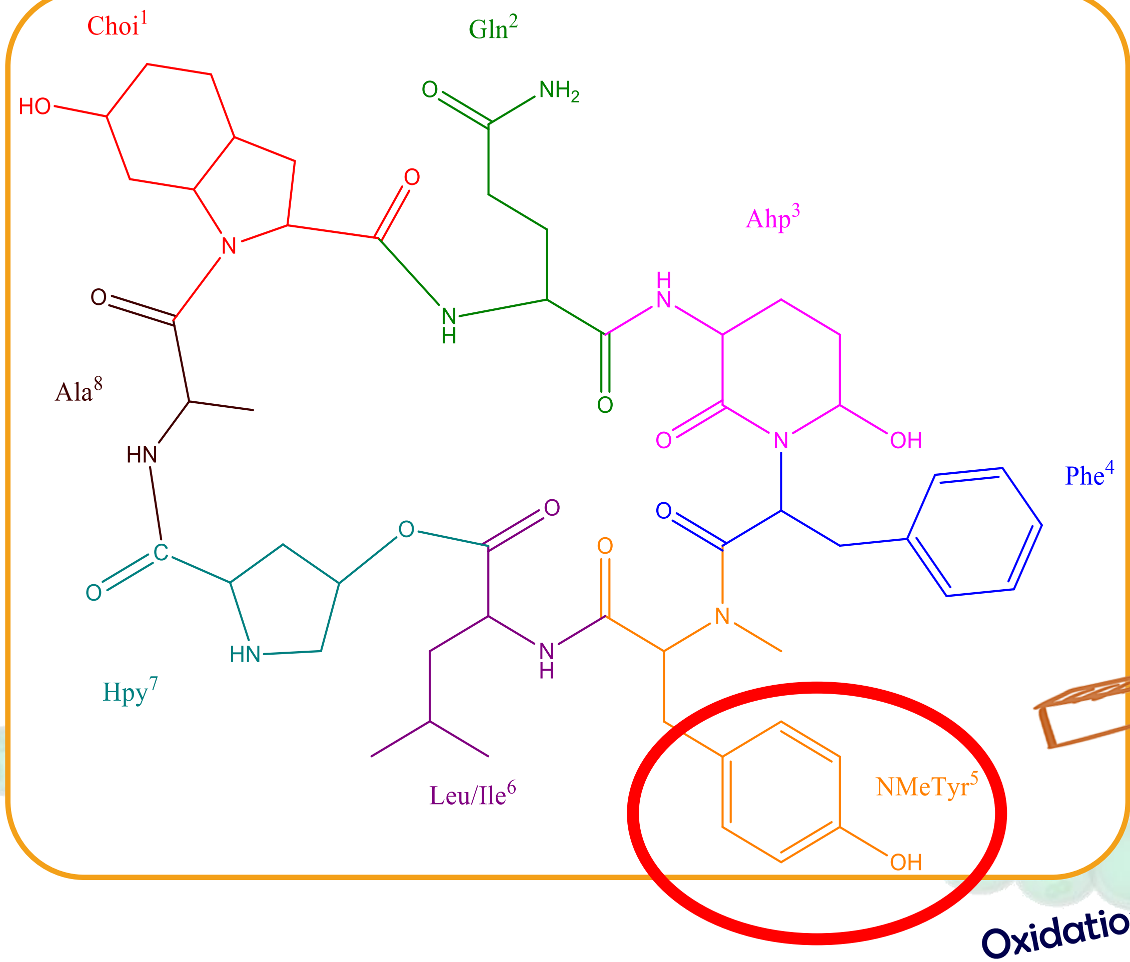
CPtp-1029

Molecular Formula

$C_{52}H_{71}O_{13}N_9$

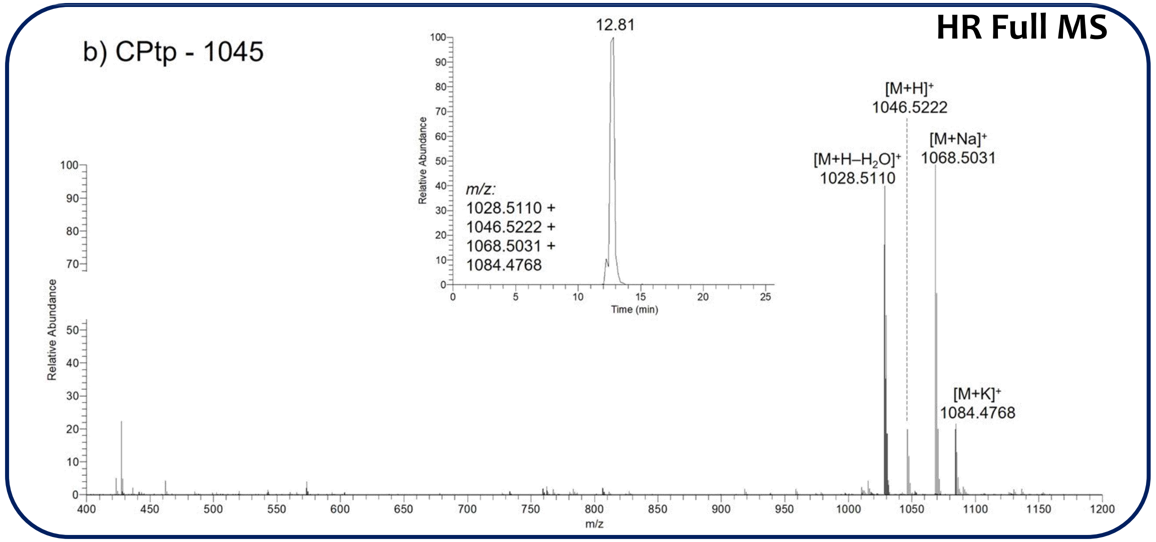
The oxidized Cyanopeptolin-type peptide

CPtp-1045
Molecular Formula
$C_{52}H_{71}O_{14}N_9$



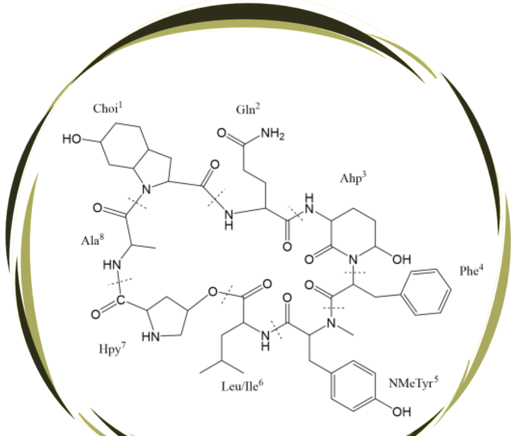
Oxidation site

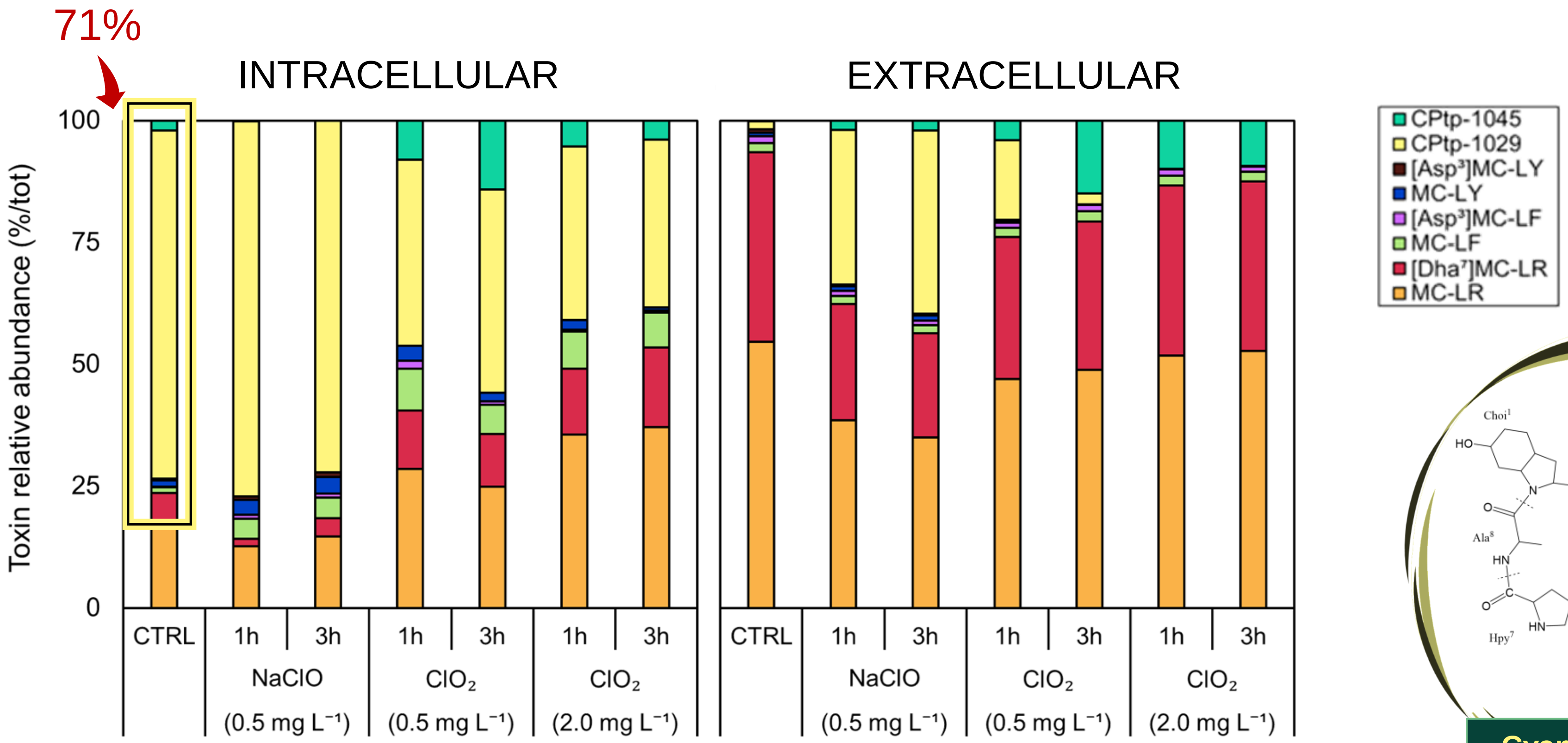
Cyanopeptolin type peptide 1045

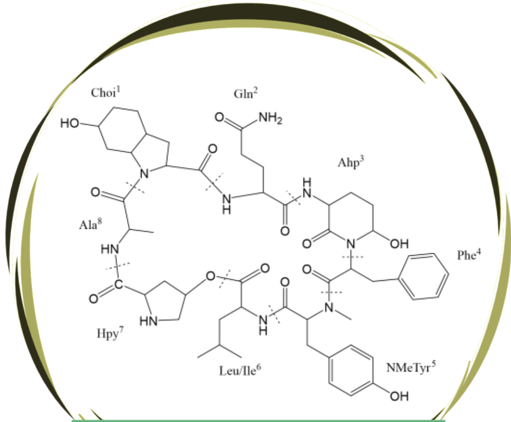


- Minor component of *M. aeruginosa* (2%) biomass
- Only in trace levels in the extracellular fraction

Cyanopeptolins: different oxidants, different fate







Cyanopeptolin-type peptide-1029 (new compound)

Cyanopeptolin-type peptide-1045 (oxidized analogue)

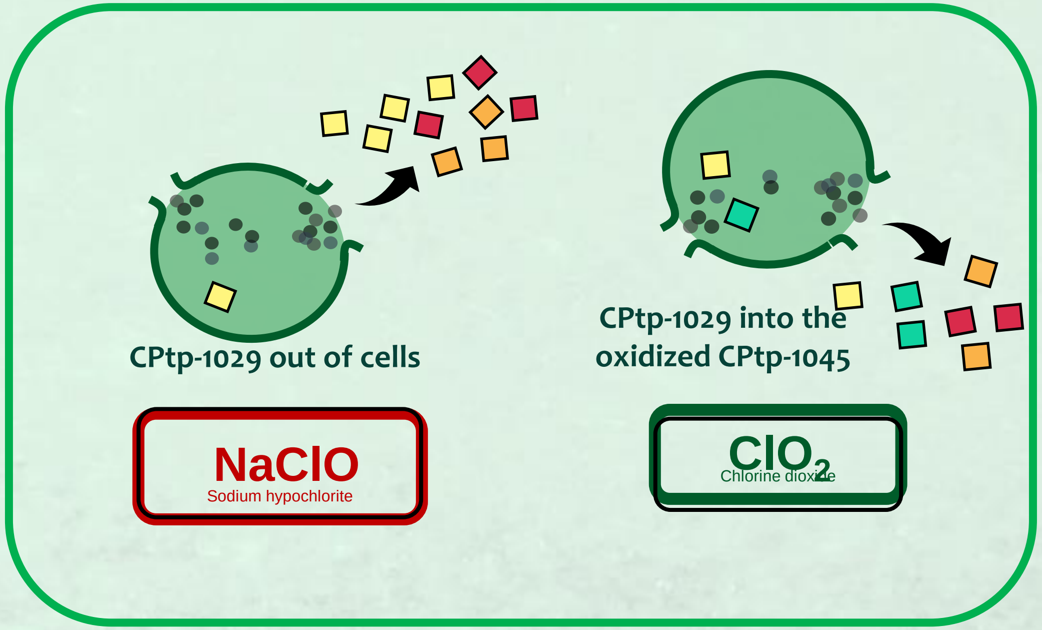
- NaClO-treated cultures: CPtp-1029 was 72-76% of intracellular content/CPtp-1029 extracellular concentrations increased vs controls → only release but not degraded
- ClO_2-treated cultures: CPtp-1029 intracellular content decreased to 33-41%/ CPtp-1029 extracellular concentrations was around 20% (1h, lowest dose) and then drop to around 0%

= **cyanopeptolin-type peptide-1029** → the **most abundant** in intracellular fraction of controls

= **cyanopeptolin-type peptide-1045** → in the extracellular fraction, especially after ClO_2 treatment

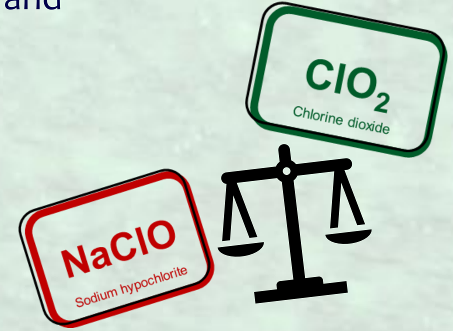
Conclusions

One of the first study assessing the fate of a wide range of cyanobacterial metabolites after drinking water treatment not only MCs but also cyanometabolites not yet regulated. Untargeted LC-HRMS is an important tool to identify highly abundant unknown cyanopeptides.
An unknown **cyanopeptolin-type peptide and its oxidized derivative** - induced by ClO_2 but not NaClO - have been detected and characterized by LC-HRMS^n (n =1-3). Could they be dangerous?



High removal of toxins from biomass and simultaneous increase in water were observed for both oxidants; ClO_2 degraded cyanobacterial cells and total toxins, NaClO possibly stimulated the extracellular toxin release, without an effective removal

The choice of the oxidant to be used during raw water pre-treatments for potabilization purposes, its dose and time, is a key factor to ensure final quality of the water and should be carefully considered to avoid a possible release of toxins and other secondary metabolites in water for drinking purpose.



Thanks for your kind attention!

luciana.tartaglione@unina.it

Luciana Tartaglione| LinkedIn