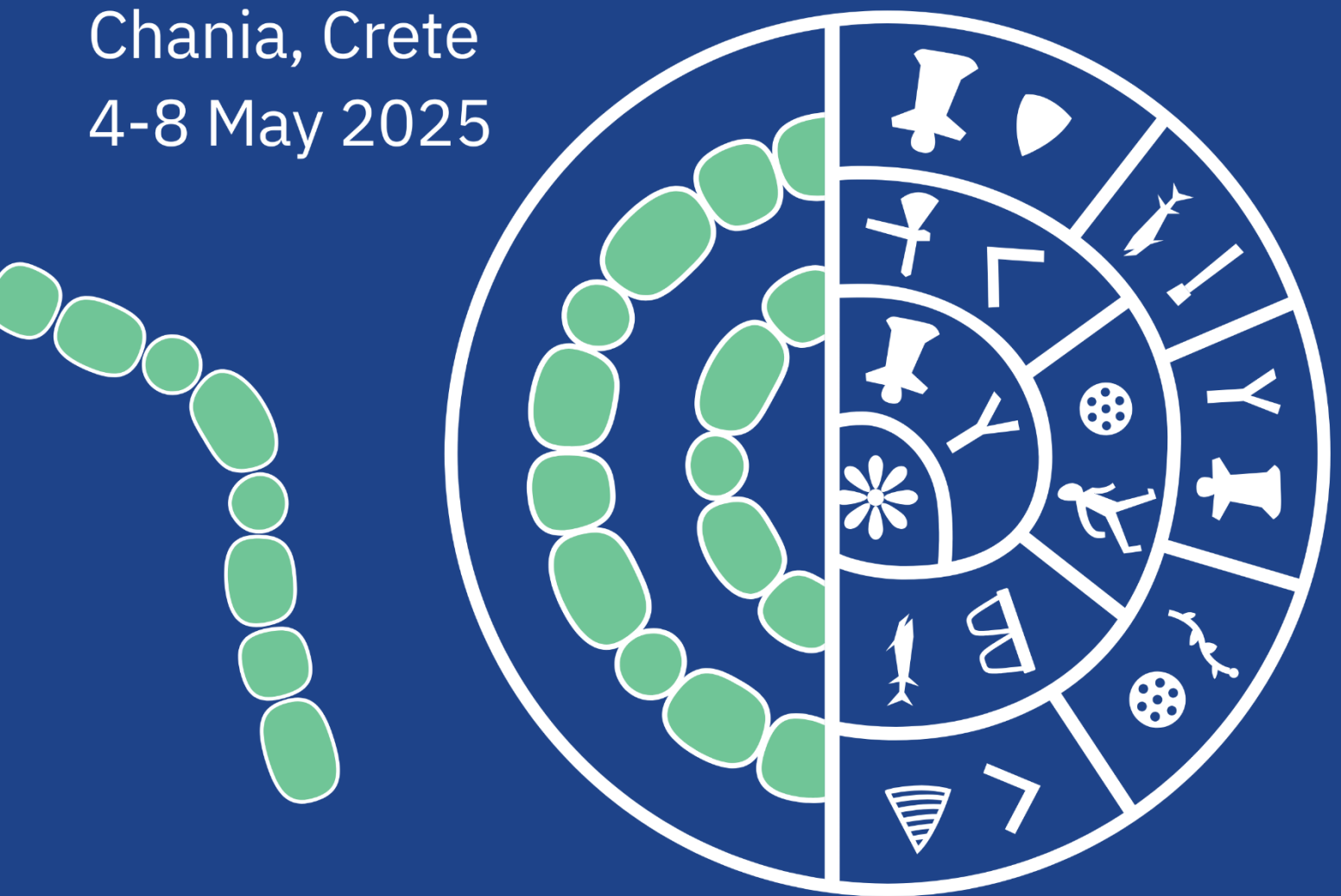


13th International Conference on **t.toxic** cyanobacteria

Chania, Crete
4-8 May 2025



ABSTRACT BOOK

AUSPICES



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We are fully committed to fostering an inclusive and equitable environment at ICTC13, where every attendee is welcomed and valued, regardless of gender, race, ethnicity, nationality, sexual orientation, disability, or any other characteristic. We strive to provide equal opportunities and necessary accommodations for all participants. We also expect all attendees to respect and appreciate the diversity within our community.

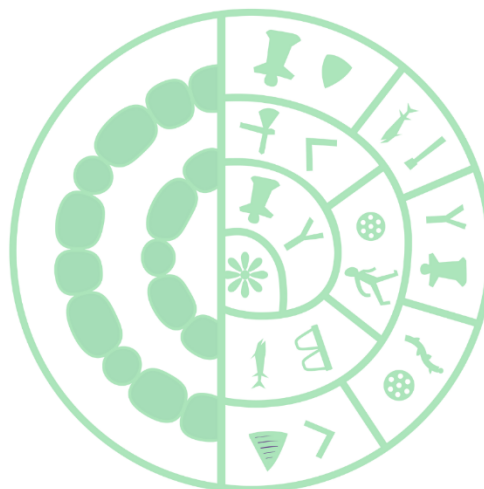


“ Code of conduct

We are dedicated to maintaining a safe and respectful environment at ICTC13. We enforce a zero-tolerance policy for any form of harassment, including but not limited to sexist language, racist behavior, transphobia, ableism, and homophobia. All attendees are expected to treat each other with respect and kindness. Any behavior that makes others feel uncomfortable or unsafe will not be tolerated. If you experience or witness harassment, please report it to our staff or security team immediately.



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WELCOME MESSAGE

Dear colleagues, friends, and fellow cyanobacteria enthusiasts,

It is with great excitement and sincere pleasure that we welcome you to the **13th International Conference on Toxic Cyanobacteria (ICTC13)**, set against the inspiring backdrop of **Chania, Crete, Greece**, a land where science, philosophy, and discovery have deep and enduring roots.

As we gather here to share cutting-edge research, foster collaborations, and address the global challenges posed by toxic cyanobacteria, we are especially proud to celebrate and encourage the contributions of **emerging scientists and early-career researchers**. Your energy, fresh perspectives, and innovative ideas are the lifeblood of this field and the foundation of its future.

Hosting ICTC13 in Greece offers a unique opportunity to connect our modern scientific pursuits with the ancient legacy of thinkers like **Aristotle, Hippocrates, and Theophrastus**, visionaries who laid the groundwork for biology, medicine, and environmental understanding. Their spirit of curiosity and inquiry continues to resonate with us today as we explore the complexities of cyanobacterial dynamics, toxicity, and ecosystem impact.

Chania, located on the westernmost side of Crete, is a city where history and culture intertwine seamlessly. Its Old Town, with narrow, winding streets, showcases a blend of **Venetian, Ottoman, and Greek architecture**, offering a walk back in time. The iconic Venetian harbor, adorned with a historic lighthouse, provides a picturesque waterfront that reflects the city's rich maritime heritage. Beyond its historical allure, Chania boasts stunning beaches like Balos and Elafonissi, and a vibrant culinary scene that captures the essence of **Cretan hospitality**.

We hope that beyond the scientific sessions, you take time to explore the rich culture, history, and natural beauty of Chania, from sun-drenched coastlines to timeless ruins, finding inspiration not only in data and dialogue, but also in the shared human story of seeking knowledge.

Welcome to ICTC13. Let's make this a meeting to remember!

Warm regards,

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KEYNOTE TALKS

The logo for iCTC 13, featuring the text "iCTC 13" in a white, sans-serif font. The letters "i", "C", "T", "C", and "1" each have a small teal dot above them, and the letter "3" has a small teal dot to its left. The logo is set against a dark green background.

Chania, Crete 4-8 May 2025



KEYNOTE TALK

Dissecting the Role of Microcystin on the Carbon Concentrating Mechanism of *Microcystis aeruginosa*

Arthur Guljamow¹, Stefan Timm², Martin Hagemann², Elke Dittmann^{1*}

¹Institute of Biochemistry and Biology, University of Potsdam, Potsdam-Golm, Germany

²Institute of Biological Sciences, University of Rostock

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Studies on the role of microcystin in *Microcystis aeruginosa* have provided a variety of indications of a close link between the toxin and the central carbon metabolism and CO₂ fixation. Not only does the toxin bind to several proteins of the Calvin-Benson cycle, but the loss of microcystin also leads to metabolic reprogramming under high light conditions and to a differential accumulation of products of the key enzyme of the Calvin cycle, RubisCO. These metabolic differences are reflected, among other features, in the reciprocal growth advantages of the wild-type strain PCC 7806 and the microcystin-free mutant at low and high light conditions. ¹

Studies on the role of microcystin for the activity of RubisCO *in vitro* and *in vivo* point to some peculiarities of the carbon-concentrating mechanism (CCM) in *Microcystis* which may contribute to the success of the species in the field. We were able to show a high specificity and affinity for the substrate CO₂ for *Microcystis* RubisCO compared to the non-bloom-forming cyanobacterium *Synechocystis* PCC 6803, independent of the binding of microcystin. However, microcystin promotes the condensation of RubisCO particles below the cytoplasmic membrane of *Microcystis* cells *in vivo*. ² This localization apparently reflects an alternative carbon concentrating mechanism in *Microcystis* that is independent of carboxysomes and strongly impacts the activity of RubisCO. In our view, microcystin contributes significantly to the phenotypic plasticity of *Microcystis* in the adaptation to varying concentrations of inorganic carbon. I will show how we are gradually elucidating the non-canonical CCM of *Microcystis*, because only by understanding the differences in CO₂ fixation in *Microcystis* can we ultimately get the key to understanding the role of microcystin.

Keywords: Microcystis, microcystin, carbon concentrating mechanism

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KEYNOTE TALK

Further insights into *Raphidiopsis* distribution and adaptation from a global pangenome

Anusuya Willis^{1*}, Jason Woodhouse², many collaborators^{3*}

¹ Australian National Algae Culture collection (CSIRO, Hobart, Australia)

² Institute of Inland Fisheries (IGB, Berlin, Germany)

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Raphidiopsis raciborskii is a global cyanobacteria species, including both toxic and non-toxic strains. It occurs, and frequently blooms, in freshwater ecosystems across tropical and temperate environments. To investigate the global genomic diversity of *R. raciborskii* we sequenced the full genomes of ~85 strains from 22 countries, spanning the continents America, Europe, Africa, Asia, and Australia. Previously we reported on geographic isolation and localised adaptation.

Further comparative genomics show a small core genome and a large variable shell genome; however, core functions and physiology are general across species with unique metabolic features occurring only within species clusters. With species clusters occurring in South America, North America – Europe, and Europe – Asia – Australia, indicating taxonomic changes and new species descriptions are needed within *Raphidiopsis*. Speciation is occurring through geographic isolation, with little evidence of rapid invasion.

The *R. raciborskii* global pangenome reveals a generalist species that thrives through flexible physiology rather than localised adaptation.

Keywords: adaptation, cyanobacteria, evolution, population genomics

INVITED TALKS

The logo for iCTC 13 features the text "iCTC 13" in a bold, white, sans-serif font. Each letter has a small teal dot positioned below it. The logo is set against a solid red background.

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INVITED TALK

Taxonomy of cyanobacteria in the age of large genomic datasets

Petr Dvořák

Department of Botany, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic

We recognize thousands of cyanobacterial species. Two hundred years of morphological analyses have suggested that cyanobacteria evolved into coherent and highly diversified lineages. However, phylogenetic reconstructions have shown that most traditional cyanobacterial genera are polyphyletic, and that extensive cryptic diversity complicates taxonomic classification. Here, I advocate for evolutionary species delimitation by identifying species boundaries using population genomics and by investigating the drivers of speciation. As a model, I selected the cosmopolitan cyanobacterium *Microcoleus*. Population structure inference revealed at least twelve species within *Microcoleus*. Species divergence was complicated by intensive gene flow in the form of homologous recombination. The full continuum of divergence was observed, from incipient species to fully diverged species with ongoing gene flow. The reinforcement of species barriers was driven by both geographical and environmental factors (e.g., climate and soil properties). Highly diverged genomic regions were under selection and likely functioned in stress response. The composition of the pangenome was shaped by frequent horizontal gene transfer and selection as well. Morphological analyses revealed a continuum of shapes and dimensions among *Microcoleus* species, ranging from cryptic to distinctly defined species. Moreover, I sequenced the genomes of eight herbarium specimens of *Microcoleus*, including the type specimen, which was almost 200 years old. The herbarium specimen helped connect modern *Microcoleus* species with the original type specimen established by 19th-century researchers, based on genome sequence data.

Keywords: cyanobacteria, evolution, population genomics, speciation, taxonomy

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Acknowledgements and Funding

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INVITED TALK

Cyanotoxins - a pharmacist's perspective

Timo H.J. Niedermeyer¹

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Cyanobacteria are renowned for producing bioactive compounds such as microcystins and saxitoxins, often labelled as “toxins” due to their potential to cause harm. However, as Paracelsus famously observed in the 16th century, “only the dose makes the poison”. Indeed, all pharmaceuticals possess inherent toxicities when misused, yet remain invaluable in medicine. Thus, cyanotoxins with their impressive biological activity, while admittedly being toxins in many contexts, may also hold promise as therapeutic agents or lead structures for drug development.

This talk will explore the dual nature of cyanobacterial toxins. I will first provide an overview of potential applications of cyanobacterial “toxins” in drug discovery, and then focus on microcystins—potent inhibitors of protein phosphatases notorious for causing severe liver damage. Despite their toxicity, microcystins exhibit unique pharmacokinetic and cytotoxic properties that make them intriguing candidates for anti-cancer drug development. Through precursor-directed biosynthesis, we have engineered microcystins amenable to derivatization,¹ enabling their use as payloads in antibody-drug conjugates for targeted cancer therapy.² Initial advances in the structure optimization and bioactivity characterization of microcystins are presented.³

Keywords: cyanotoxins, microcystins, click chemistry, ADCs

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3. Sallandt L.L., et al., *J. Nat. Prod.*, **2025**, 88, 3-14. DOI: 10.1021/acs.jnatprod.4c00688



INVITED TALK

Mitigating Harmful Cyanobacterial Blooms in an increasingly chaotic and extreme global climate

Hans W. Paerl

University of North Carolina at Chapel Hill, Department of Earth, Marine and Environmental Sciences, Institute of Marine Sciences, Morehead City, North Carolina, USA

Globally, we are facing an increasingly chaotic and extreme climate, including record heat waves, droughts and unprecedented storm and precipitation events. On the watershed-scale, these events are altering hydrologic conditions (freshwater discharge, flow, mixing), which in turn are impacting nutrient and organic matter loading to nutrient-sensitive water bodies along the freshwater to marine continuum. These highly dynamic and changing conditions strongly affect cyanobacterial growth rates, nutrient-bloom thresholds, cyanotoxin production and bloom potentials in waterbodies ranging from oligotrophic to eutrophic conditions. In order to establish effective bloom mitigation strategies in the face of increasingly extreme physical and biogeochemical perturbations, we must consider and incorporate changing physical-chemical factors, such as water discharge and residence times, altered light and temperature regimes and nutrient availability in water quality monitoring, experimental and modeling efforts. These efforts should include formulating specific reductions in anthropogenically-generated nutrient (N and P) and sediment loads in the context of a changing climate in order to develop an effective array of mitigation and management strategies, aimed at protecting and ensuring resiliency and safe use of our precious water resources.

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INVITED TALK

Functional Metabolomics Approaches for Understanding Microbial Community Dynamics at the Molecular Level

Daniel Petras^{1, 2}

¹ University of California Riverside Department of Biochemistry, USA

² University of Tuebingen, Interfaculty of Microbiology and Infection Medicine, USA

The chemical composition of the ocean's exo-metabolome represents a fascinating source of chemical entities that are fundamentally important for ecosystem function and planetary processes. Thanks to recent advances in tandem mass spectrometry and computational data analysis tools, we can identify a wide range of metabolites out of this ultra-complex mixture and propose some of their activities through literature knowledge. However, the number of known metabolites and activities represents only a small fraction of all compounds we can detect in these environments. To fully map out the structural and bioactivity space, new methods are needed, as traditional isolation and bioactivity studies do not scale to contemporary non-targeted metabolomics workflows. To address this need, we develop functional metabolomics tools that integrate liquid chromatography tandem mass spectrometry with metagenomics as well as native mass spectrometry. In combination with molecular networking, these workflows allow us to connect the marine meta-metabolome to community composition and protein-metabolite interactions. Taken together, our results provide new insights in the enormous chemical complexity of marine microbial communities and highlight the potential of functional metabolomics workflows for the linking of environmental metabolomics data with microbial communities and biological mechanisms.



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INVITED TALK

Metabolomic Insights into Cyanobacteria: From Toxicity Profiling to Novel Bioactive Compounds

Prof. Dr. Ernani Pinto

University of Sao Paulo, Brazil

Cyanobacteria are widely recognized for their role in harmful algal blooms and their production of cyanotoxins, posing serious threats to aquatic ecosystems and public health. However, recent advances in metabolomics have unveiled a broader chemodiversity among cyanobacteria, including numerous bioactive metabolites and peptides with potential pharmaceutical applications. This lecture will discuss how high-resolution mass spectrometry (LC-MS/MS), combined with bioinformatics tools as well as GNPS molecular networking and CyanoMetDB, has facilitated the annotation of cyanobacterial metabolites and revealed novel compound classes. Studies conducted on biomass and isolates from Brazilian reservoirs identified metabolites such as mycosporine like amino acids, saxitoxins, guanitoxin, and peptides including microcystins, microginins, aeruginosins, and cyanopeptolins, several with strong correlations to bioactivity. These compounds exhibit dual relevance: they can act as environmental toxins, but also as potent inhibitors of specific enzymes such as proteases and phosphatases, or as modulators of molecular targets including sodium channels and cholinesterases. By integrating metabolomic workflows with toxicological screening, this research highlights how cyanobacteria represent both a risk to water safety and a promising resource for drug discovery, emphasizing the importance of monitoring and valorizing microbial secondary metabolites.

Keywords: cyanometabolites, cyanotoxins, metabolomics, bioactive natural products, GNPS and CyanoMetDB



INVITED TALK

Taxonomic and functional metagenomic assessment of cyanobacteria

Nico Salmaso¹

¹ Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

The sequencing and use of single marker genes and the adoption of a polyphasic approach represented a major historical advance in the taxonomy and study of cyanobacteria. Currently, these approaches are still widely used and are helping to fill gaps in the taxonomy, geographic distribution and toxicity of cyanobacterial strains. However, these approaches have important bottlenecks that limit analyses to isolated and cultured specimens, single individuals or colonies, and monospecific environmental samples. In the last decade, the advent of innovative technological methods, particularly the increased use of high-throughput sequencing, has led to important advances in the study of microbial communities. The increasing use of amplicon sequencing approaches (metabarcoding) has paved the way for the study of microbial communities in environmental samples and has rapidly become a mature and de facto standard in many laboratories. However, metabarcoding for the identification of bacteria and cyanobacteria has several drawbacks, mainly due to the short length and resulting low taxonomic resolution of the 16S rRNA gene after bioinformatic processing (around 400 bp), which mostly limits classification to the genus level. Recent improvements in this approach (selection of non-ribosomal marker genes and sequencing of 16S rRNA long reads) have not broadened the scope of this approach, which remains limited to the taxonomic classification of microbial communities. A breakthrough has been the implementation of genomic and metagenomic methods, either based on short read assembly (whole genome shotgun sequencing) or long read assembly (Pacific Biosciences-PacBio and Oxford Nanopore Technologies-ONT), which allow the reconstruction of draft or circular cyanobacterial genomes. These techniques are being applied to a wide range of samples (from isolates to complex microbial communities), providing information at the strain level and on species metabolic functions and community functioning following genome annotation. At the taxonomic level, the calculation of average nucleotide identity (ANI) and/or digital DNA-DNA hybridisation (dDDH) values, complemented by phylogenomic analyses based on the selection of single-copy marker genes or (from pangenomic analyses) single-copy core genes, represent an increasingly adopted approach for genome-based species delineation and evolutionary relationships among cyanobacteria. Overall, the study of cyanobacterial taxonomy and ecology is undergoing rapid and extensive development. These changes will be critically discussed by reporting on specific case studies from ongoing research and literature, highlighting the drawbacks and limitations as well as the prospects for development of the different approaches used in the modern study of cyanobacteria.

Keywords: metabarcoding, full shotgun sequencing, long-read sequencing, taxonomic classification, genome annotation

Acknowledgements and Funding

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INVITED TALK

Effective In-Lake Measures to Control Cyanobacterial Blooms

Petra M. Visser

*Dep. Freshwater and Marine Ecology-IBED, University of Amsterdam, **The Netherlands***

Cyanobacterial blooms pose a growing threat to aquatic ecosystems, public health, and water security worldwide. In-lake measures play a vital role in mitigating these blooms, especially when decreasing external nutrient loading is insufficient or takes too long to become effective. This lecture will provide a comprehensive overview of the most effective biological, physical and chemical control in-lake strategies currently available. Special attention will be given to two increasingly applied techniques: deep artificial mixing and hydrogen peroxide treatment. Deep artificial mixing disrupts water column stratification, reducing the competitive advantage of buoyant cyanobacteria, and suppressing bloom formation. Case studies will illustrate how this method affects the phytoplankton in the lake. Hydrogen peroxide offers a selective chemical control option, targeting cyanobacteria while minimizing harm to non-target organisms. Recent field trials and experimental insights will be shared, highlighting both the potential and the limitations of this approach. Furthermore, also innovative and emerging approaches will be highlighted that show promise for future in-lake cyanobacterial bloom control. This lecture will combine scientific insights with practical case studies to support evidence-based decision-making for the sustainable management of cyanobacterial blooms.

ORAL PRESENTATIONS

The logo for iCTC 13, featuring the text "iCTC 13" in white on a red background. The letters are stylized with green dots above the 'i', 'C', 'T', and 'C', and below the '1' and '3'.

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O1 - 263

Relationship between Clickable Cyanotoxin Content and Labeling by Click Reaction in Bloom-Forming Cyanobacteria *Microcystis aeruginosa* and *Planktothrix agardhii*

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It has been shown that promiscuous adenylation (A) domains in non-ribosomal peptide synthesis (NRPS) allow for the incorporation of clickable non-natural amino acids (non-AAAs) into their peptide products, i.e. microcystins (MCs) or anabaenopeptins (APs). In a second step, click chemistry is used for visualization of chemically modified cyanopeptides [1]. In this study time lapse experiments have been performed using pulsed feeding of non-natural amino acids (nonAAAs) in order to observe the build-up or decline of azide- or alkyne-modified peptides, i.e. clickable MCs or APs as recorded via standard peptide extraction and LC-MS analysis [2]. Two cyanobacteria *Microcystis aeruginosa* and *Planktothrix agardhii* were grown under maximum-growth rate conditions (0.1-0.4 and 0.2-0.4 per day, respectively) in the presence of nonAAAs for 0-168 h and subsequently labeled using Alexa Fluor488 (A488) for visualizing MC/AP. Vice versa the decline of the azide- or alkyne-modified MC or AP was observed by growing in the presence of nonAAAs for 48 h and subsequent transfer of cells to new medium and labeling using A488 at 0-96 h (*M. aeruginosa*) or at 0-192 h (*P. agardhii*). In general, the increase of clickable MC/AP in peptide extracts was observed fast, reaching its maximum after 24-48 h. A decrease of clickable MC/AP in peptide extracts was observed linearly with time. The proportion of clickable MC/AP in peptide extracts (or in microgram of MC/AP per mg of dry weight) related linearly with Alexa Fluor488 labeling intensity as recorded via laser-scanning microscopy for individual cells. At the population level the flow cytometry-based quantification of labeled subpopulations also correlated with clickable MC/APs proportion in corresponding peptide extracts. It is concluded that chemical modification of MC/AP can be used to track intra- and extracellular dynamics in synthesis using both analytical chemistry and high-resolution imaging.

Keywords: microcystin synthesis; anabaenopeptin synthesis; time-lapse experiments; pulse-feeding; unnatural amino acids; click-chemistry; fluorescent labeling; laser-scanning microscopy; flow cytometry

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O2 – 247

The Role of Interacting Anthropogenic and Climatic Drivers on Late 20th Century Development of Harmful Cyanobacterial Blooms in Utikuma Lake, Alberta

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Eutrophication has degraded water quality, disrupted ecosystems, and increased health risks through associated development of harmful cyanobacterial blooms (cyanoHABs) in many Canadian lakes, while also impacting recreational and commercial fishing and tourism¹. A 140-year sediment core record from Utikuma Lake, Alberta, dated using ²¹⁰Pb, revealed drivers of its transition to a hypereutrophic state, with cyanoHABs becoming prominent since the 1990s. End-member modeling analysis (EMMA) of sediment grain size data linked catchment runoff to specific sedimentological processes. Elemental X-ray fluorescence (XRF) analysis was used to assess changes in productivity, precipitation, weathering, and runoff. Microcystin congeners (LA, LR, [Dha⁷] LR, and RR), well-preserved in sedimentary records, were quantified using tandem mass spectrometry.

Five end members (EMs) were identified, and linked to sedimentary processes such as precipitation events, spring freshets, and anthropogenic catchment disturbances. CONISS analysis of EMMA and XRF data defined five limnological phases since the late 19th century, each marked by unique patterns of productivity, weathering, and runoff driven by natural and anthropogenic factors. A major shift occurred in 1996, characterized by increased productivity and runoff, leading to hypereutrophic conditions and appearance of persistent cyanoHABs. This limnological shift was triggered by the deliberate release of effluent from decommissioned sewage lagoons, causing the collapse of Alberta's largest commercial whitefish fishery.

Spectral and wavelet analyses of XRF and EMMA data revealed the role of climate oscillations—Arctic Oscillation, El Niño-Southern Oscillation, North Atlantic Oscillation, and Pacific Decadal Oscillation—in influencing productivity and runoff. There has also been significant climatic warming in the region, beginning with recovery from the Little Ice Age in the late 19th century. Since 1950, Alberta's winter temperatures have risen by 0.5–1°C per decade, reducing lake ice cover duration and directly affecting lake productivity. These findings reveal the complex interplay of climatic and anthropogenic factors influencing cyanoHAB occurrence in Utikuma Lake.

Keywords: Lake C, Eutrophication, Catchment Runoff, Climate Oscillations and Warming, Paleolimnology

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O3 - 164

Methods and Reference Materials for Measurement of Complex Anatoxin Profiles in Benthic Cyanobacteria

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Anatoxins are increasingly linked to animal fatalities worldwide and present a risk to human health due to their occurrence in drinking water reservoirs. In Atlantic Canada, where cyanobacteria and their toxins are an emerging issue, anatoxin-producing *Microcoleus*-dominated benthic cyanobacterial mats have been responsible for at least seven dog deaths since 2018.^{1,2} Analysis of anatoxins is important for enabling research and monitoring, but can be challenging due to their high polarity, chemical instability and chemical interferences. The very high spatial and temporal variability in benthic mat occurrence and ATX concentration makes research in the field difficult, often requiring analysis of a large number of samples. To enable the study of toxic benthic cyanobacteria in Atlantic Canada, we have developed a toolbox of broadly applicable mass spectrometry-based methods for the analysis of anatoxins. These include targeted multi-class LC-MS/MS methods, LC-high-resolution MS methods for non-target analysis, and direct ionization methods for high-throughput quantitative screening.

Direct analysis in real time-high resolution tandem mass spectrometry (DART-HRMS/MS)³ has now been used to measure anatoxins in hundreds of benthic cyanobacterial mat samples from the Wolastoq (Saint John River) near Fredericton, Canada. This revealed spatial trends and intra- and inter-annual variability in mat toxicity in this highly impacted system. Several new anatoxin analogues were identified by non-target LC-HRMS/MS.⁴ The formation of conjugates helps explain the poor stability of anatoxins and accounted for up to 14% of ATXs in field samples. 10-hydroxy-anatoxins represent new biosynthetic products that accounted for up to 38% of ATXs in *Microcoleus*-dominated mats. New calibration solutions and matrix reference materials are being developed to enable the broader profiling of a wider range of ATXs. This includes a new certified reference material for homoanatoxin-a and feasibility studies on the production of calibration solutions for isomers of dihydroanatoxin-a and 10-hydroxy-dihydroanatoxin-a.

Keywords: Anatoxin-a, Mass Spectrometry, *Microcoleus*, Reference Materials, Benthic Cyanobacteria

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O4 – 23

Identification of 7-Deoxy-desulfo-argino-cylindrospermopsin, the Missing Piece in Cylindrospermopsin Biosynthesis

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Cylindrospermopsin, a major cyanotoxin, is produced by freshwater cyanobacteria. Its biosynthesis starts from arginine and glycine and involves five polyketide synthases and several tailoring enzymes.

The first enzyme of this pathway, CyrA, an amidino-transferase has been studied in vitro by the Neilan group¹ and we have characterized CyrI, the hydroxylase², and CyrJ, the sulfotransferase³, catalysing respectively the last and penultimate steps of this biosynthetic pathway.

We report here the identification of a new biosynthetic intermediate⁴, 7-deoxy-desulfo-argino-cylindrospermopsin in several cylindrospermopsin-producing cyanobacteria using mass spectrometry experiments. We have purified this new metabolite and established its structure by 1D and 2D NMR spectroscopy. Using labeled arginines in isotopic incorporation experiments we have shown that arginine is fully incorporated into 7-deoxy-desulfo-argino-cylindrospermopsin and that the uracil ring of cylindrospermopsin originates from the guanidino moiety of arginine, thus solving a long-standing puzzling questions.

We have purified to homogeneity the two putatively involved enzymes, CyrG and CyrH, from the cylindrospermopsin producing strain, *Oscillatoria* sp. PCC 6506, and showed that CyrG is a zinc-dependent hydrolase, homologous to adenosine deaminases, that transforms 7-deoxy-desulfo-argino-cylindrospermopsin into 7-deoxy-desulfo-cylindrospermopsin and ornithine. CyrH contained almost no metal and showed no such activity even in the presence of excess metal.

Using structure-based alignments and secondary structure predictions we propose that the fifth and last polyketide synthase CyrF, in cylindrospermopsin biosynthesis, contains an unprecedented C-terminal domain homologous to N-acetyltransferases. We suggest that this domain catalyzes the condensation of the CyrF product with arginine to give 7-deoxy-desulfo-argino-cylindrospermopsin. This would be an unprecedented termination step for a polyketide synthase.

Keywords: cylindrospermopsin, specialized metabolite biosynthesis, Cyr enzymes

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Searching for the cylindrospermopsin producer in a Dutch estuarine shellfish production area

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In the Netherlands, official shellfish production areas are monitored for regulated marine toxins. However, in Dutch estuaries, cylindrospermopsins are found in shellfish from summer up to winter. Cylindrospermopsins are not regulated in shellfish, but their presence might pose a food safety risk. For management purposes, it is helpful if the cylindrospermopsin producer can be identified. Routine phytoplankton monitoring did not reveal the presence of known cylindrospermopsin producers. The aim of this study therefore was to identify the cylindrospermopsin producer in our production areas. We selected a hydrodynamically isolated production area and performed a survey on cylindrospermopsins in mussel, water, seston and epibiota. Additionally, species were identified by light microscopy and molecular techniques in seston, epibiota and mussel (molecular techniques only). During the study period (January up to harvesting in August), cylindrospermopsins were detected in nearly all mussel samples, with the highest concentrations found at the moment of harvesting (140 µg/kg ww cylindrospermopsin and 74 µg/kg ww 7-deoxy-cylindrospermopsin). In the epibiota of the rope culture, toxins followed the same pattern, but the concentrations were orders of magnitude higher: 15 mg/kg dw cylindrospermopsin and 16 mg/kg ww 7-deoxy-cylindrospermopsin). No toxins were detected in water and seston. Although cyanobacteria were identified by light microscopy, there were no known cylindrospermopsin producers present that could be related to the toxin dynamics in shellfish and epibiota. 16S rRNA analysis also did not show a clear trend, and no CYN biosynthesis genes were identified by qPCR. Amplicon sequencing identified actinobacteria in epibiota as a group that followed toxin dynamics. As cylindrospermopsin biosynthesis genes have been identified in some actinobacteria, further study is needed to determine whether these organisms are indeed responsible for the observed cylindrospermopsin accumulation. In conclusion, cylindrospermopsins seem to be produced in the epibiota, but the actual producer has not been identified yet.

Keywords: cylindrospermopsin, shellfish, estuary

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OMICs characterization of Kamptonema strains from BACA and PCC reveals taxonomic and cyanometabolite diversity

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Kamptonema is a recent genus derived from Phormidium based on genetic and morphological data, with currently eleven recognized species. Some Kamptonema species have been characterized as toxic, with cylindrospermopsin, anatoxin and homoanatoxin reports. A collaboration between the Pasteur Cultures of Cyanobacteria (PCC) collection and the Bank of Algae and Cyanobacteria of the Azores (BACA) allowed the analysis of 17 Kamptonema strains from different habitats and countries, covering a wide latitudinal and longitudinal gradient. This work includes the assembly of draft genomes of eight Kamptonema strains from BACA and six from PCC to perform genomic analysis of all strains. This work provided evidence for a new species through phylogenomics and ANI/AAI analyses, along with 16S rRNA gene phylogeny and p-distance, 16S-23S rRNA ITS secondary structure, and morphology analyses. An extensive analysis of biosynthetic gene clusters (BGCs) coding for natural products was also conducted using genome mining. The potential production of cyanotoxins, such as cylindrospermopsin and homoanatoxin-a, inferred from genomics was confirmed by ELISA and ESI-HR-LC-MS/MS, resulting in the identification of new toxic strains. BGCs analysis also revealed other cyanometabolites, such as novel cyanobactins and proteusins, as well as geosmin, whose distribution among strains seemed to be mainly taxa related. This work collects 14 new draft genomes of Kamptonema, gathering information on taxonomy, toxicity and metabolism, with evidence for a new toxic species.

Keywords: cylindrospermopsin, homoanatoxin, RiPPs, new taxa, genomics

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Metabolic profile of *Raphidiopsis raciborskii* extracts

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Cyanobacteria are known to synthesize a diverse array of metabolites with potential applications in various fields. Although a comprehensive database of known cyanobacterial metabolites has been recently published, knowledge of the metabolite profiles of *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera & al. 2018 remains limited. Therefore, our research aimed to explore the lesser-studied metabolites of this cyanobacterial species. To achieve this, *R. raciborskii* strains from diverse geographical origins and with varying toxicity profiles were analyzed. Most of the examined strains were non-cylindrospermopsin-producing (isolated from Poland and Israel), while two cylindrospermopsin-producing strains (CS-505 and CS-506) were isolated from Australia.

The toxic potential of these strains was initially assessed by comparing the presence and concentration of cylindrospermopsin using solid-phase extraction (SPE) coupled with high-performance liquid chromatography (HPLC), where CS-505 showed a higher amount of this metabolite compared to CS-506. Untargeted metabolomics was conducted using Ultra High Pressure Liquid Chromatography (UHPLC)-Orbitrap Exploris 120 Mass Spectrometer. Data analysis, performed with Compound Discoverer 3.3 Software and annotation through ChemSpider and CyanoMetDB, identified 881 unique compounds based on their mass-to-charge ratio (m/z) and enabled the annotation of 124 compounds. Further, the Variable Importance in Projection (VIP) score was utilized to determine each metabolite's contribution to group differentiation, facilitating the identification of the most significantly altered metabolites through the OPLS-DA model (Orthogonal Partial Least Squares Discriminant Analysis). Notably, the toxic strains (CS-505 and CS-506) and two non-toxic Polish strains (UAM/DH-KmRr and UAM/DH-PnRr) displayed greater differentiation compared to other strains, which remained clustered. Horizontal clustering of metabolite abundance revealed that certain strains, particularly the toxic ones (CS-505 and CS-506), exhibited a higher number of unique compounds compared to the others. These findings suggest distinct metabolite profiles among strains, prompting further analysis of their extracts to better understand their biological significance.

Keywords: *Raphidiopsis raciborskii*, metabolome, cylindrospermopsin

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Peptides from Baltic cyanobacteria as potent anti SARS-CoV-2 agents

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Antiviral activity of cyanobacterial metabolites, such as lectins and polysaccharides, has been well documented. The compounds mainly inhibit or prevent the infection caused by human immunodeficiency viruses (HIV). In our preliminary experiments, extracts from Baltic cyanobacteria, representing different genera, were screened for activity against SARS-CoV-2. Of the sixty tested samples, the most potent activity was recorded for filamentous cyanobacteria of the orders *Pseudanabaena*, *Anabaena*, *Nostoc*, *Nodularia* and *Spirulina*. In the presented study, two classes of cyanopeptides, known as serine proteases inhibitors, were identified as the most potent anti-SARS-CoV-2 compounds: cyanopeptolins (CPs) isolated from *Nostoc edaphicum* CCNP1411 and spumigins (SPUs) isolated from *Nodularia spumigena* CCNP1401. Here, the peptides inhibited the viral infection in human A549^{ACE2/TMPRSS2} cells and in the primary human airway epithelium, without cytotoxic effects in the cells. CPs with Arg bound directly to the coronaviral S protein and thus, prevented virus entry to host cells. The peptides were active against all SARS-CoV-2 variants tested (Wuhan, Omicron, Alpha and Delta), except for Wuhan. Spumigins showed different mechanism of action. They inhibited the activity of host cell serine protease TMPRSS2, which in turn hampered virus entry and replication. Unlike CPs, SPUs caused a significant decrease in virus infection for all SARS-CoV-2 variants tested. The results of our study confirmed the significance of cyanopeptides as natural products with manifold biological activity and high pharmaceutical potential.

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A metabolomics approach to investigate the responses of *Microcystis aeruginosa* to UV-B stress

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Cyanobacterium *Microcystis aeruginosa* is a prominent species associated with harmful algal blooms, which pose significant ecological and public health risks. This study investigates the effects of UV-B radiation on *M. aeruginosa* PCC7806, focusing on metabolite alterations. Advanced analytical techniques were employed to assess these changes. Using untargeted high-resolution mass spectrometry (HRMS) in combination with computational workflows, including MZmine [1] for feature detection and alignment, GNPS2 [2,3] for feature-based molecular networking and spectral annotation, and SIRIUS [4] for enhanced structural elucidation, we conducted a comprehensive analysis of the metabolic responses to UV-B stress. The experiments involved exposure of *M. aeruginosa* cultures to various doses of UV-B radiation and analysis of intracellular metabolites after specified incubation times. Our findings indicate that UV-B exposure induced alterations in the metabolomic profile of *M. aeruginosa*. Statistical analysis by the FBMN STATS [5] workflow, revealed significant changes in several compounds, including cyclic peptides, mycosporine-like amino acids, pterins and glutathione pathway metabolites. The results contribute to exploring markers of UV-B stress responses in cyanobacteria, providing valuable insights into their potential role in survival - adaptation mechanisms. Furthermore, the study enhances our understanding of how cyanobacteria respond to various stressors, to inform future strategies for managing harmful algal blooms in aquatic ecosystems.

Keywords: cyanobacterial stress, UV-B radiation, untargeted analysis, computational metabolomics, GNPS2

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Updates of CyanoMetDB - a comprehensive public database of secondary metabolites from cyanobacteria

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CyanoMetDB is a comprehensive database of secondary metabolites from cyanobacteria made openly-accessible as a dataset, which is highly curated with an international team of experts since 2021. CyanoMetDB is presented as a flat-file database comprising the following core fields: compound identifier code, compound name, compound class, molecular formula, molecular weight, monoisotopic mass, reference(s) related to the structure elucidation, SMILES string, InChI string, InChIKey, IUPAC name, whether nuclear magnetic resonance spectroscopy (NMR) was used for the structure elucidation, and the cyanobacterium or field sample type used to extract the compound from for structure elucidation. Since September 2024, Version03 of CyanoMetDB is available presenting the structural information of 3085 metabolites from 1156 primary references published between 1967 and early 2024. In December 2024, we have surpassed 5000 downloads of the database. We collaborate with MetFrag, PubChem, NORMAN Suspect List Exchange Classification, The Natural Products Atlas and since 2024 also with MIBiG to ensure that we share the most up-to-date metabolite information across these platforms. We expanded our efforts from the structural database to provide highly curated reference mass spectra (MS/MS) through MassBank. We have systematically measured spectra for 346 metabolites on an ESI Orbitrap MS platform to record spectra in positive and negative mode both with 9 collision energies (i.e., 18 spectral types) and 5-10 replicates per spectra type. The obtained raw data were processed using an open-source R package RMassBank, which is responsible for automatic MS and MS/MS recalibration, clean-up and intensity correction, as well as gathering spectral meta data to generating exports that can be deposited as new entries in MassBank. In total the stringent data processing approved spectra of 165 metabolites for upload. New projects within CyanoMetDB are ongoing, a new Version 04 is planned for 2025, and we welcome new teams to join us and contribute.

Keywords: database, open-access, metabolites, SMILES, dereplication

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O11 - 240

(Ahp)-Cyclodepsipeptide and its possible detoxification pathway

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Cyanobacterial harmful algal blooms (CyanoHABs) are becoming increasingly frequent and severe due to environmental drivers such as eutrophication and climate change.¹⁻⁴ These blooms pose significant ecological, economic, and health risks, largely attributed to the production of diverse secondary metabolites, including cyanotoxins.^{5,6} Among these metabolites, the 3-amino-6-hydroxy piperidone (Ahp)-cyclodepsipeptides—a large family of over 200 compounds—have garnered attention for their notable cytotoxic and protease-inhibitory properties.⁷ This study focuses on a recent CyanoHAB dominated by *Phormidium* species, aiming to evaluate its ecological impact through comprehensive chemical and biological analyses.

Biomass samples were collected from the bloom, and crude extracts demonstrated potent cytotoxic activity against *Thamnocephalus platyurus*. The metabolites in the mixture were initially analyzed using a targeted list of characteristic MS/MS fragments to identify cyanotoxin classes. The results of the analysis showed the presence of (Ahp)-cyclodepsipeptides. A chemoselective approach was employed to probe the functional characteristics of these natural products. The compounds were subsequently isolated, and their structures elucidated through HR-ESIMS and NMR analysis. Biological assays revealed that the newly identified metabolites exhibited strong cytotoxic activity against *Thamnocephalus platyurus*.

Interestingly, truncated derivatives of these compounds were also identified, isolated, and characterized within the crude extract. Unlike their parent compounds, these truncated derivatives displayed no cytotoxicity, suggesting the existence of a detoxification pathway for these cyanotoxins. This finding offers new insights into the ecological dynamics of CyanoHABs and the potential mechanisms to mitigate the toxicity of cyanobacteria.

Keywords: Cyanobacteria, bloom, cyanotoxin, (Ahp)-cyclodepsipeptides

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O12 - 178

Selected peptides (microcystins, anabaenopeptins and microginins) of past and present cyanobacterial presence in a large shallow lake (Lake Balaton)

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Cyanobacteria are ancient microorganisms that played an important role in shaping the Earth's atmosphere. They are increasingly causing aquatic blooms due to global warming and anthropogenic impacts. Many eutrophic water bodies are currently monitored for cyanobacterial toxins, but little is known about the historical occurrence of the toxins. Currently used paleolimnological methods are perfectly adequate for monitoring past cyanobacterial mass occurrences, but do not provide information on the presence and dynamics of the cyanotoxins [1]. Lake Balaton is one of the largest shallow lakes in Central Europe and it is important from an economic, ecological, touristic and recreational point of view. In our study, we aimed to investigate the history of bioactive cyanobacterial metabolites in Lake Balaton. For the study, sediment core samples (35-40 cm depth) were taken from Keszthely basin of Lake Balaton, sliced and ²¹⁰Pb dating were performed. Viable photosynthetic microorganisms were cultured from the samples and after extraction with methanol, their toxin content was measured using the HPLC-MS/MS method [2]. The upper 20 cm layer of the sediment covers an interval of about 100 years. We cultured the species *Microcystis*, *Aphanizomenon*, *Dolichospermum* (*Anabaena*) and *Raphidiopsis* (*Cylindrospermopsis*) from the sediment. Their occurrence is well correlated with historical records of aquatic blooms in the lake [3]. We found various cyanobacterial bioactive peptides and toxins in the cultured sediment core samples. The studies revealed that the species producing bioactive peptides, such as microginins and anabaenopeptins, could have been present in the water body much earlier. The layer from a depth of 5-10 cm was the richest in peptide metabolites. The examination of the most recent sediments shows that the frequency of occurrence of microcystins has decreased recently, but the occurrence of microginins and especially anabaenopeptins seems to be increasing in the future.

Keywords: cyanobacteria, paleolimnology, peptides, cyanotoxins

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O13 - 143

In vitro study of the intestinal and renal detoxification of Microcystin-LR catalyzed by Glutathione-S-Transferase (GST): organ and species differences

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Microcystins are detoxified through conjugation with glutathione (GSH), a reaction that occurs both spontaneously and catalyzed by GSTs.

This study, using in vitro systems such as single recombinant enzymes and human and rat renal and intestinal cytosols, investigated the extrahepatic detoxification process of Microcystin-LR (MCLR) in the kidney and intestine of human and rat, to determine the possible differences of organ and species and increase the toxicokinetic information useful for the animal-human extrapolation.

The five human recombinant GSTs tested (also expressed in the intestine and kidney) showed this Cl_i ranking: GST O1 > A2 > T2 > M4 > A4, with the O1 isoform four times more efficient than A4.

Three of the four cytosols (rat and human kidney and human intestine) showed an atypical saturated kinetic curve indicating positive cooperativity. This can be explained by the different expression of the various GST isoforms in the various organs, for example the GSTP1 isoform, mainly expressed in the kidney and intestine but not in the liver, showed positive cooperativity in the reaction with MCLR¹.

Comparing the data obtained on the intestinal and renal cytosols with those obtained previously in the liver, both for humans and rats², it was highlighted that:

- in the rat the liver conjugation of MCLR with GSH is approximately 2 and 7 times more efficient than in the intestine and kidney.
- In human, the enzymatic efficiency is comparable in the three organs.
- The interspecies comparison highlights that the hepatic Cl_i in the rat is 2 times higher than human, while it is comparable in the intestine and 3 times lower in the kidney.

In conclusion, the intestine and kidney are also relevant in the MCLR detoxification reaction; in the intestine, there are no significant differences between human and rat, while human shows more efficient renal detoxification.

Keywords: Cyanotoxin, Microcystin-LR, extrahepatic metabolism, human and rat

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O15 - 36

Distinct ecotoxicological impacts induced by different *Microcystis* genotypes to Medaka fish

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One of the most prevalent and notorious bloom-forming freshwater cyanobacterial genus is *Microcystis*, whose toxicological impacts yet remain incompletely investigated. Based on our previous studies, we hypothesize that some emerging *Microcystis* metabolites, in addition to microcystins (MCs), are of (eco)toxicological concerns and should be further investigated. To this end, we explore the ecotoxicological potential of different *Microcystis* genotypes producing various bio-active metabolite cocktails, particularly cyanopeptides of different structural families including MCs, cyanopeptolins, microginins, anabaenopetins, aeruginosins or microcyclamides, on embryo/larvae and adult Medaka fish model.

Embryo and larvae exposures to the extracts of the four distinct *Microcystis* genotypes -comprising two MC-producing (PMC 728.11 and 807.12) and two non-MC-producing (PMC 810.12 and 826.12) strains – showed that PMC 810.12 and 826.12, respectively, producing microginins, aeruginosins and microcyclamides exhibit early toxicity and teratogenicity, when PMC 728.11 presented rather larvae toxicity on hatched larvae, in agreement with its high MC content. In addition, we handled a 4-days microcosm experiment with adult female Medaka exposed to environmental concentrations of these four *Microcystis* strain cultures to observe the microbiome and metabolome responses in various organs. While fishes exposed to PMC 728.11 exhibited microbiota dysbiosis signature, the PMC 826.12 strain remarkably perturbs the fish digestion process inducing even more pronounced microbial and metabolic alterations than the PMC 728.11 strain, when the two others provoke more moderated perturbations. These findings then highlight toxic effects on fish exposed to non-MC producing cyanobacteria and suggest the complexity of effects potentially occurring on fish naturally exposed to cyanobacterial bio-active and toxic compounds during blooms of *Microcystis*.

Keywords: Ecotoxicology, Cyanobacteria, Cyanotoxins, Microbiome, Metabolomics, Multi-omics

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O16 - 277

Ecosystem risk and mammalian susceptibility of the stable, lipid soluble toxin aetokthonotoxin (AETX) from the epiphytic cyanobacterium *Aetokthonos hydrillicola*

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Consumption of aetokthonotoxin—eagle killer toxin (AETX) induces vacuolar myelinopathy (VM) in fish and wildlife. AETX is a lipid soluble, pentabrominated indole alkaloid produced by the epiphytic cyanobacterium, *Aetokthonos hydrillicola*. *A. hydrillicola* grows on aquatic plants and produces AETX when bromide is bioavailable. Birds, reptiles, and mammals that consume aquatic vegetation with AETX-producing cyanobacteria can develop the neurological disease Vacuolar Myelinopathy (VM) and transfer the toxin up the food web. We have detected AETX in plants, invertebrates, birds, amphibians, reptiles, and mammals from disease locations. Birds of prey and mammalian predators develop VM from preying on these neurologically impaired taxa. Man-made reservoirs and wetland impoundments provide critical overwintering and migratory habitat in North American watersheds. Due to the prevalence of aggressive co-invasions of submerged aquatic vegetation and toxic epiphytic cyanobacteria, these altered wetland habitats present a risky stopover for birds. We have documented *A. hydrillicola* growing in critical migratory wetlands in Louisiana, and in new watersheds from Texas to New York. AETX was also detected in tissues in Florida panthers diagnosed with Feline Leukomyelinopathy (FLM), which presents with clinical and histological features similar to VM. The severity of VM induced in mice correlated with the AETX dose (5ppm, 10ppm, 20ppm) with which they were gavaged. The recent development of a LAMP protocol for detection of the *A. hydrillicola* ITS region and AETX biosynthesis genes will allow us to track the cyanobacterium and the potential for AETX production. *A. hydrillicola* produces two toxins, the neurotoxin AETX and additional statin derivatives including aetokthonostatin (AEST). These can be detected using HPLC/MS/MS and more recently high resolution MALDI⁴. Understanding the synergistic relationship between these novel biotoxins is critical to making informed decisions for improving the health of our watersheds for wildlife and people.

Keywords: eagle killer, aetokthonotoxin, Hydrilla, bioaccumulation

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In collaboration with Dr. Phillips in the Proteomic and Mass Spectrometry Laboratory, we have developed a rapid and precise method for measuring the concentration of AETX in plants, fish, wildlife, and mammal tissues. The Bruker Solarix 12T FTICR purchased using an NIH grant (NIH S10 OD025118) provides ultra-high resolution and mass accuracy peaks using MALDI (Matrix-assisted laser desorption/ionization).

Monitoring and modelling expanding risk of hydrilla, neurotoxic *Aetokthonos hydrillicola*, and vacuolar myelinopathy (FP00024538). US Department of Interior, AWD003243G1.



O17 - 221

Tracking harmful cyanobacteria and their effects on the Eastern oyster, *Crassostrea virginica*, in Louisiana estuaries using integrative methods

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The emergence of toxigenic cyanobacteria in estuarine systems raises concerns regarding the impacts of fitness and public health risks of exposure to valuable shellfish species like the eastern oyster, *Crassostrea virginica*. The freshwater cyanobacterium, *Microcystis aeruginosa*, is of particular concern due to its salt tolerance¹, production of microcystins, ability to outcompete other phytoplankton species with inputs of nutrient-rich freshwater², and documented presence in oligo- and mesohaline Louisiana estuaries^{3,4}. This spatiotemporal overlap of eastern oysters and *M. aeruginosa* makes monitoring efforts and research resolving eastern oyster feeding on this, and similar, species of pressing importance. However, individual cells of *M. aeruginosa* are small (2-4 µm) and can also form large colonies (100 µm) comprised of hundreds to thousands of cells, both of which are difficult to enumerate accurately in mixed communities. Furthermore, morphological identification of *M. aeruginosa*, or other cyanobacteria species that produce toxins non-constitutively, does not indicate if harmful cyanotoxins are present.

Through a combination of methods ranging from imaging microscopy, toxin analyses, and molecular techniques (real-time quantitative PCR; RT-qPCR) we are detecting low concentrations (5-250 cells mL⁻¹) of *M. aeruginosa* in local oyster habitats, determining the portion of those cells with the microcystin gene (0-65%), and quantifying microcystins in discrete water and time-integrative resin and oyster tissue samples. We also used RT-qPCR to quantify possible rejection of a non-toxic strain of *M. aeruginosa* in lab-based oyster feeding experiments. These results showed that rejection of *M. aeruginosa* was consistent even with alternative prey available, although total pseudofeces production increased with a diet rich in the cyanobacterium. Methods detecting genes, cells, and toxins are allowing us to better understand toxigenic cyanobacteria as components of natural estuarine phytoplankton communities, relating species abundance to toxin accumulation in oyster tissues, and understanding oyster feeding behavior as these cells become more abundant in prey communities.

Keywords: *Microcystis aeruginosa*; oysters; shellfish; selective feeding

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Small cysts going up, lake hairs disappearing: Is it all Greek to you? Unravelling the Greek language contribution in Cyanobacteria

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Etymology is a key component in nomenclature, often carrying key information for an organism. Greek and Latin languages have been used for centuries in synthesizing the names of living organisms. However, there are surprisingly few studies about each language's contribution, which is sometimes crucial for understanding the structure, function, and ecology of species and/or avoiding misunderstandings around names' meaning and origin. Further, the literature surrounding the etymology of these scientific terms is limited and not accessible to the international scientific community, because of their Latin and Greek origin. This study examined the etymology of the sum of the taxa of Cyanobacteria, namely 520 genera and 4797 species, as well as the terms used to describe them. The nomenclature databases cyanodb and algaebase were used for finding and recording the genera, whereas the terms were recorded in glossaries of books specialized on the study of Cyanobacteria [1,2,3]. They were studied for their linguistic origin and to identify Greek and Latin components in the terms. Analysis of the etymology of Greek words was based on specialized dictionaries [4,5]. The results showed that almost 75% of the generic names and 68% of the terminology, respectively, consisted of Greek components. This research intends to make the literature referring to the etymology taxa and terms accessible and lead to a better understanding of the words used to describe Cyanobacteria.

Keywords: terminology, etymology, Latin and Greek terms, morphology, ecology

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O19 - 249

Gas vesicle genotype diversity in *Planktothrix rubescens*: Exploring drivers of seasonal and interannual variability

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Active buoyancy control through an interplay between gas vesicles and carbohydrate storage is an important factor in the ecology of the harmful cyanobacterium *Planktothrix rubescens*. The buoyancy provided by gas vesicles allows *P. rubescens* to float up in lakes after deep mixis, to depths where light conditions support phototrophic growth. *P. rubescens* populations consist of subpopulations that vary in the size of their gas vesicles (GV-genotypes). Vesicle size determines their resistance to hydrostatic pressure during deep mixis and the resource investment required for vesicle synthesis. We investigated how GV-genotype-dependent differences affect filament survival and competitive performance during deep mixis and stratification. Over three years, we followed seasonal changes in GV-genotype frequencies in the deep mixing Lake Zurich (Switzerland) using flow cytometric sorting of filaments and single filament PCR. These results were set in context with physico-chemical monitoring data to better understand environmental drivers shaping the gas vesicle gene pool diversity in *P. rubescens*. Strains with less robust gas vesicles had higher mortality during deep mixis but a growth advantage during summer stratification and autumn surface mixis. This pattern fits the expectation of a trade-off between robustness and resource investment, explaining the persistence of both, weak and strong GV-genotypes. However, long-term data indicate a general decline in the abundance of weak GV-genotypes. Additionally, their advantage became negligible when overall winter survivability was high. This suggests that the selection for GV-genotypes depends strongly, but not exclusively, on deep mixing survivability and resource economy. Strain-dependent variability in responses to nutrient and light limitations could contribute to interannual variations in GV-genotype dynamics, promoting genetic variability in the *P. rubescens* population of Lake Zurich. This intraspecific diversity likely strengthens the species' adaptability to shifting environmental conditions in freshwater ecosystems.

Keywords: Seasonal successions, Intraspecific trait variation, Trade-off, Deep convective mixing, Gas vesicles

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O20 - 84

Unveiling Cyanobacterial Diversity at LEGE-CC: Taxonomic Legacy and Future Prospects

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The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), housed at the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) in Matosinhos, Portugal, is one of the world's most significant repositories of cyanobacterial and microalgal strains. Considering cyanobacteria, the collection houses 1,200 strains isolated from five continents, classified into fourteen orders. Among these, thirteen new genera and two new species have been formally described using a polyphasic approach. These include *Geminobacterium*, *Lusitaniella*, *Calenema*¹, *Leptolyngbyopsis*, *Pseudoleptolyngbya*², *Eurychoronema*³, *Ciimarium*⁴, *Pseudolimnoccocus*, *Eucapsopsis*, *Acaryochloridopsis*, *Vasconcelosia*⁵, *Zarconia*, *Romeriopsis*⁶, *Hyella patelloides*, and *Jaaginema littorale*¹. Additionally, a new microalgae genus, *Endolithella*⁷, has also been described. Taxonomic studies on LEGE-CC strains are ongoing and are conducted using a comprehensive approach, including 16S rRNA phylogenetic analysis, 16S-23S ITS secondary structures, as well as morphological and ecological characteristics. Our studies have revealed that numerous strains remain unidentified and should be described as new genera. Among them, the filamentous marine strains LEGE 07170 and LEGE 191244 (Oculatellales), isolated from Portugal and Morocco, are distinguishable from other Oculatellaceae genera by the presence of vacuole-like structures adjacent to the cell cross-walls. Similarly, the extremophile strain LEGE 231229 (Oscillatoriales), collected from an alkaline-brackish lake in the Bolivian Altiplano, exhibits a *Lyngbya*-like morphology but differs from its closest phylogenetic relatives by the presence of aerotopes. Additionally, strains LEGE 6306, LEGE 6307, LEGE 10375, and LEGE 11436 (Prochlorococcaceae), isolated from planktonic samples in Portugal, represent four distinct new genera that are morphologically similar to *Cyanobium* and *Ciimarium*. Additionally, the strains LEGE 181157, LEGE 181224, LEGE 181227 are a new species of *Gibliniella* (Nodosilineales) differing from the type species *G. alaskensis* by the presence of abundant diffuent mucilaginous envelopes and constrictions at the cell cross-walls. These discoveries, along with the numerous unidentified strains within the LEGE-CC collection, underscore its immense potential for uncovering and describing new cyanobacterial taxa.

Keywords: Cyanobacteria, Biodiversity, LEGE-CC

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Potential of Cyanobacterial Blooms for Sustainable Biodiesel Production

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Cyanobacterial blooms have a negative impact on aquatic ecosystems, reaching high population densities under favorable environmental conditions and forming visible scum on the water surface (Akcaalan et al., 2014). However, this substantial biomass offers great potential for the production of economically viable products such as biodiesel and bioactive natural compounds (Madusanka and Manage; 2018; Duman et al., 2019). Thus, the exploitation of cyanobacterial blooms could provide both environmental and economic benefits. To explore this potential, water samples were collected from two artificial rainwater harvesting ponds in Gümüşyaka and Çakıl (Istanbul, Türkiye) between June and October 2024. Phytoplankton counts were performed to identify the dominant species, and biodiesel yield and quality were analyzed, focusing on the fatty acid methyl ester (FAME) content. The results showed that cyanobacteria represented more than 80% of the total algal abundance in both ponds. In Çakıl, *Microcystis* sp. was identified as the dominant species, while *Aphanizomenon* sp. and *Planktothrix agardhii* were dominant in Gümüşyaka. FAME content analysis showed that biodiesel yields ranged between 45% and 60% in Çakıl and between 40% and 50 % by weight in Gümüşyaka. In addition, the fatty acid composition met biodiesel quality standards (EN 14214 and ASTM D6751). These results showed higher FAME content compared to laboratory-scale cultures, suggesting that the natural stress conditions in these environments increased FAME production. The study highlights the significant potential of biomass derived from cyanobacterial blooms for sustainable and economically viable biodiesel production. This approach not only supports the bioenergy sector, but also helps mitigate the environmental impacts of harmful algal blooms.

Keywords: Cyanobacteria Bloom, Biodiesel, FAME, Rainwater Harvesting Ponds

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Critical salinity thresholds and iron limitation: Decoding the role of salinity on growth and toxicogenicity of cyanobacterial blooms

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Climate change is pushing lakes toward critical thresholds that favor toxigenic cyanobacterial blooms. In this study, we investigated how nutrient limitation (P-limitation = $TP \leq 0.05$ mg/L, N-limitation = $TN \leq 0.8$ mg/L, P and N co-limitation = $TP \leq 0.05$ mg/L and $TN \leq 0.8$ mg/L, and neither P nor N limitation = $TP > 0.05$ mg/L and $TN > 0.8$ mg/L), salinity, and Fe availability interact to regulate cyanobacterial growth and toxin production in lakes within the LWW. Analysis of 100+ lakes revealed a range of specific conductivity (a proxy for salinity) from 100 to $> 100,000$ $\mu S/cm$, spanning critical thresholds: the freshwater-to-saline transition ($\sim 2,000$ $\mu S/cm$) and saline-to-seawater threshold ($\sim 50,000$ $\mu S/cm$). Cyanobacterial species composition varied with salinity: *Planktothrix* and *Dolichospermum* dominated in the lowest salinity waters, *Microcystis* thrived near the freshwater-saline interface, and *Nodularia* dominated in saline environments. Nutrient limitation patterns also varied with salinity. In fresh lakes ($< 2,000$ $\mu S/cm$), nutrient conditions included all combinations of P and/or N limitation, with biologically available Fe³⁺ (presented as the $pFe = 22-26$) often acting as a co-limiting factor. In lakes with $> 2,000$ $\mu S/cm$, lakes were neither P nor N limited, but Fe-limitation became severe, and concentrations of siderophores that scavenge Fe increased. Cyanotoxin concentrations rose as lakes approached the freshwater-saline transition ($\sim 2,000$ $\mu S/cm$), peaked around 8,000 $\mu S/cm$, but declined beyond 20,000 $\mu S/cm$ as cyanobacteria biomass declined, with a shift from microcystin to nodularin-R as salinity increased. These findings highlight how rising salinity and Fe limitation interact to intensify cyanobacterial bloom toxicity, providing critical insights into the complex responses of inland waters ecosystems under climate change.

Keywords: Canadian prairies, salinity, nutrient-limitation, cyanobacteria species, cyanotoxin induction

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Ironing out the answer: What limits cyanobacteria in freshwater lakes in the Canadian Prairies?

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The Canadian prairies is a notable hotspot for cyanobacteria-dominated lakes. Our study found minor variations in cyanobacterial genera across these lakes yet observed significant differences in standing biomass, as the lakes ranged from oligotrophic to hypereutrophic. A correlational analysis of nutrients, specifically total phosphorus (TP) and total nitrogen (TN) revealed that the limiting nutrients varied considerably across the region. Of the lakes studied, cyanobacterial biomass was P-limited in 21 lakes, N-limited in 3 lakes, and co-limited by both P and N in 23 lakes. Surprisingly, the biomass was limited by neither P nor N in 32 lakes. In these lakes, iron (Fe) emerged as the most likely limiting nutrient, given a relatively narrow range of free ferric ions (pFe) between 18 and 26. Cyanobacteria can create biomass during Fe stress by producing Fe-scavenging siderophores that target pFe. However, in neither P- nor N-limited lakes, there was a lack of correlation between siderophore concentrations and cyanobacterial biomass ($r = 0.05$), indicating that the siderophores were unable to scavenge pFe and thereby utilize the available P and N to produce further cyanobacterial biomass. Our findings suggest that these Fe-starved eutrophic lakes exhibited a paradox of slow-growing yet high cyanobacterial biomass, challenging the notion that only oligotrophic lakes embody slow-growing metabolisms. Overall, our study emphasizes the importance of moving beyond the classic macronutrient paradigm to better understand cyanobacterial dynamics. By integrating both macro-nutrients (P and N) and micro-nutrients (specifically Fe) considerations into existing nutrient management strategies, cyanobacterial dominance can be more effectively mitigated.

Keywords: Canadian prairies, nutrients, iron, siderophores, cyanobacteria speciation

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O24 - 237

Nutrition strategy and non-algal turbidity sustained the dominance and alternative shifts of bloom-forming filamentous cyanobacteria: a case study in large shallow Lake Honghu, China

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Cyanobacterial blooms (CyanoHABs) have markedly increased over the past few decades and are projected to continue rising due to the combined impacts of eutrophication, elevated CO₂ levels, and global warming. The proliferation and persistence of bloom-forming filamentous cyanobacteria (FC) are increasing across various geographical regions. Numerous studies have elucidated the ecological dominance of FC; however, there has been less focus on their nutrient utilization strategies. Recent research indicates that certain filamentous cyanobacteria may utilize phosphonates (phosphonic acids) in aquatic ecosystems to support their growth. In N-deficient seasons, *Cylindrospermum raciborskii* can maintain a relatively high biomass by utilizing nitrogen released from cyanophycin nitrogen rather than relying solely on fixed nitrogen. A case study of Honghu lake, a shallow lake with a surface area of 350 Km², reveals a significant decline in submerged plants following a sustained high water level resulting from major flooding in 2016. Since then, the deterioration of water quality has coincided with the dominance of bloom-forming filamentous cyanobacteria, characterized by high spatiotemporal variability in their succession. Nutrient strategy and non-algal turbidity have contributed to the sustained dominance of FC over the past years. Additionally, water temperature has shaped the structure of, with low-temperature-tolerance species thriving in spring and winter, while high-temperature-tolerant species dominate in summer and autumn. The heterogeneity of non-algal turbidity may alter competitive outcomes among filamentous cyanobacteria or promote their coexistence. In conclusion, it is imperative and urgent to address the increasing challenges related to the mechanisms and control of filamentous cyanobacteria.

Keywords: Honghu Lake, Filamentous cyanobacteria, Nutrition strategy, Non-algal turbidity, Algal succession



O25 - 32

Multilevel Analysis of Thermal Stress Impact on Energy Budget and Photosynthetic Performance in *Microcystis aeruginosa*

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Thermal tolerance in cyanobacteria has gained renewed attention due to the increasing temperature fluctuations associated with climate change, which intensify the frequency of harmful cyanobacterial blooms. These blooms, driven by temperature, light, and nutrient availability, pose significant ecological and economic threats to freshwater systems globally. Among these, *Microcystis aeruginosa*, a toxic cyanobacterium known for producing microcystins, threatens drinking water reservoirs and aquatic ecosystems worldwide. Understanding the physiological responses of *M. aeruginosa* to temperature is crucial for developing strategies to mitigate its impact. In our study, we employed an integrated approach combining Membrane Inlet Mass Spectrometry (MIMS) and a Joliot-Type Spectrophotometer (JTS) to investigate photosynthetic performance under temperature fluctuations. This advanced configuration enables simultaneous measurements of gas exchange and redox reactions within the photosynthetic apparatus, providing direct correlations between oxygen dynamics, electron transport, and overall photosynthetic activity. These insights pave the way for a deeper understanding of the mechanisms that regulate thermal tolerance in cyanobacteria.

In this study, we applied the combined system to evaluate the response of two *M. aeruginosa* strains, the frequently studied PCC7806 and the local Lake Kinneret strain C-1004, to heat shock conditions. The combined MIMS and JTS methodology offered detailed insights into photosynthetic and respiratory regulation under thermal stress. This approach complements standard biochemical and molecular analyses, providing a robust toolkit for exploring the physiological and metabolic adjustments in cyanobacteria. Our findings highlight the importance of integrated techniques in climate change research, enabling a deeper understanding of how abrupt environmental shifts impact aquatic photosynthesis. Future research will focus on using this platform to complement omics data of thermal sensitivity in the local *M. aeruginosa* strain and to predict long-term acclimation potential in cyanobacteria, contributing to strategies for addressing the effects of climate change on aquatic ecosystems.

Keywords: Cyanobacteria, *Microcystis aeruginosa*, Thermal tolerance, Photosynthetic performance, Heat shock response

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O26 - 256

Combined efectos of abiotic and biótico stressors on cyanobacterial metabolism

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Water pollution from chemical substances, such as the herbicide metolachlor (MET), and solid waste, such as cigarette butts (CBs), poses significant risks to aquatic ecosystems. For instance, MET can disrupt the growth and general metabolism of non-target microorganisms. Similarly, CBs, one of the most common forms of global litter, contain many chemicals that can be released into water and can negatively affect microorganisms such as cyanobacteria and fungi. Despite their prevalence, little is known about the combined effects of these pollutants and biotic stressors on phytoplankton. This study investigated the metabolic response of the toxigenic cyanobacterium *Planktothrix agardhii* when exposed to both biotic (the chytrid parasite *Rhizophydium megarrhizum*) and abiotic stressors (CB leachate equivalent to 1 CB L⁻¹ and MET at 100 µg L⁻¹). We measured key metabolic biomarkers, including microcystin levels, protein and lipid content, lipid peroxidation, and the enzymatic activity of superoxide dismutase (SOD) and glutathione-S-transferase (GST). MET exposure did not significantly alter protein or lipid content or enzyme activities in either infected or uninfected cultures. However, cyanobacteria simultaneously exposed to MET and chytrids exhibited reduced microcystin levels and lipoperoxidation, indicating an oxidative stress response countered by non-enzymatic mechanisms. In contrast, CB leachate reduced protein and lipid content and microcystin concentrations in the cyanobacteria only in the absence of the parasite, suggesting oxidative stress and adverse effects on general metabolism. Interestingly, simultaneous exposure to CB leachate and chytrids appeared to mitigate the negative effects on cyanobacteria. Thus, CB leachate could potentially enable cyanobacterial growth by inhibiting chytrid infections in an epidemic scenario. This study highlights the complex responses of cyanobacteria under simultaneous abiotic and biotic stressors, revealing how anthropogenic pollutants shape aquatic microbial interactions and broader ecological processes.

Keywords: chytrids, oxidative stress, pollutants, herbicide, cigarette butts

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Are toxic cyanobacteria competitively superior at elevated CO₂ and temperature?

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Competition is among the fundamental organismal interactions and main agents of natural selection shaping the diversity of ecological communities. Competitively fitter species displace the less-fit ones. In phytoplankton communities, competitive superiority is often attributed to cyanobacteria, which dominate in the phytoplankton structure of numerous eutrophic lakes worldwide and are capable of forming toxic blooms across the vegetative season. Climate projections assume substantial changes in key climatic variables by the end of this century and predict that cyanobacteria dominance can be perpetuated further with the increasing share of toxic genotypes in the population. The aim of this study was to determine whether toxic genotypes of most common bloom-forming cyanobacteria gain competitive superiority in simulated warmer and richer in carbon dioxide climate conditions. Experiments involved co-culturing of two strains of the same lake origin but different capability to produce toxic metabolites (anabaenopeptins, anatoxin-a, cylindrospermopsin, microcystin, saxitoxin or none) and different taxonomic affiliation (*Aphanizomenon gracile*, *Aphanizomenon klebahnii*, *Chrysochloris bergii*, *Cuspidothrix issatchenkoi*, *Dolichospermum planctonicum*, *Planktothrix agardhii* or *Raphidiopsis raciborskii*) for 50 days under each of the two climate regimes (ambient: 20°C/430 ppm of CO₂, elevated: 26°C/1200 ppm of CO₂). Procedural controls were constituted of monocultures of competitors respective to the co-culture. The strains capable to produce toxic metabolites often achieved higher biovolume than their competitors in both climate conditions, and this effect was in most cases exacerbated when co-cultures were exposed to 26°C and 1200 ppm of CO₂. Analysis of chlorophyll-*a* concentration showed that total biomass of two co-cultured strains was usually lower than the summarized biomass of both respective monocultures. However, there were few exceptions where higher biomass was noted in co-cultures than in monocultures together. To conclude, our results demonstrate that toxic cyanobacteria genotypes have the potential to be competitively superior under climate change.

Keywords: bloom-forming cyanobacteria, climate change, competition, cyanotoxins, experimental ecology

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Complex interaction between cyanophage CL131 and freshwater cyanobacterium *Aphanizomenon flos-aquae* during the viral infection

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Cyanophages are important ecological and biogeochemical drivers and are considered as key player in governing critical cellular processes and metabolic pathways. Viral infections influence physiology, dynamics and mortality of the attacked microbial populations which in turn impacts whole aquatic environments. Thus, understanding the extent of ongoing metabolic alterations during the infection process is critical for integrating viruses into microbial community dynamic, food web models as well as cyanobacterial bloom formation. Among numerous bloom-forming taxa, nitrogen fixing species, such as *Aphanizomenon spp.*, often dominate quantitatively and in terms of biomass in many temperate lakes worldwide. These cyanobacteria coexist with their specific viruses which are believed to significantly influence their physiology and dynamics however, mechanisms of such interaction have not been understood. Here we used multi-omics approach to investigate host physiological and metabolic alterations during infection of freshwater bloom-forming diazotrophic cyanobacterium *Aphanizomenon flos-aquae* by CL131 cyanophage. The main objectives were i) to determine whether and to what degree changes in host metabolism are triggered by viral-mediated disruption of host transcription and translation machinery, and ii) to evaluate the level of host stress response and activation of antiphage defense mechanisms to prevent progeny production. Our findings indicate that infected *A. flos-aquae* cells display substantial variability in their gene expression level, protein contents and metabolite profiles, suggesting that phage infection affects not only photosynthesis and central metabolism of the host but also other metabolic cycles/pathways crucial for viral demands. Furthermore, while the proteomic and metabolomic data are generally consistent, we observed a disrupted relationship between transcription and translation machinery, presumably to favour viral progeny production. In summary, the patterns of host stress response and host metabolism takeover indicate novel strategies of infected cells and cyanophages, distinct from marine individuals and unicellular *M. aeruginosa* species.

Keywords: *Aphanizomenon flos-aquae*, cyanophage infection, host-phage interaction

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O29 - 96

Genomic insights into *Raphidiopsis* (*Cylindrospermopsis*) *brookii* isolates from northwest Ohio

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Common around the globe in small and large freshwater systems, cyanobacterial harmful algal blooms (cHABs) cause degradation of ecosystems and can be dangerous to humans and wildlife. In Ohio, both microcystins and saxitoxins are common. *Cylindrospermopsis* are also found, but are less frequently detected. These cyanotoxins range in effects in humans and wildlife but frequently co-occur and have compounding effects. Moving into the next several decades, climate change will continue to favorably impact cHABs and cause longer and more intense bloom seasons. *Raphidiopsis* is regarded as a planktonic tropical cyanobacterial genus hypothesized to have originated in Africa. Now common in Africa, South America, and Australia, over the past several decades there have been increased incidences in Europe and North America. *Raphidiopsis* has also previously been found in the Midwest region of the United States, in multiple Ohio waterbodies (Sandusky Bay, Harsha Lake) and both reservoirs and natural lakes in Indiana. We isolated and sequenced the genome of three strains of *Raphidiopsis* from a local lake in northwest Ohio that is plagued by mixed cyanobacterial blooms. These strains were unique from other high quality *Raphidiopsis* genomes, forming a distinct branch on a phylogenomic tree. We also discovered a deletion of many key genes for performing nitrogen fixation in one Ohio strain and another strain from Brazil. By investigating the genomic features within the three Ohio strains and others, we can find what features have made *Raphidiopsis* successful in this lake.

Keywords: Metagenomics, cyanobacteria, climate change, harmful algal bloom

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O31 - 33

Defining the Nostocales ADA clade (*Anabaena-Dolichospermum-Aphanizomenon-Cuspidothrix*) through genome sequencing

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The ADA clade of bloom-forming Nostocales cyanobacteria has recently been proposed as a genus-level group based on phylogenomic analyses (1). Studies from the Pacific NW region of the US and from Finland in particular have established members of the ADA clade as significant contributors to CyanoHABs in temperate regions, with the capacity to produce a range of cyanotoxins and the taste-and-odor compound geosmin (2-4). The 45 genomes available in 2021 were spread across 8 species-level subclades (5), with some branches represented by only 1 or 2 genomes. In order to more deeply assess the diversity of genotypes and the range of species-level subgroups represented by the ADA clade, we collected >100 cultured strains from colleagues around the world. The strains were selected on the basis of provisional phylogenetic placement from 16S rRNA alignments, aiming to achieve good representation of all subclades and different toxigenicities, and to represent different parts of the world as well as possible. A report will be given on the progress of genome sequencing, which is prioritizing Oxford Nanopore sequencing in order to obtain as complete genomes as possible. The finalized collection of new genomes will be merged with existing genomes and any metagenome-assembled genomes that become available, and colleagues will be invited to assist in analyzing the dataset. The goals of the study include (1) to determine the subclade structure of the ADA clade, to identify the species within the ADA genus and to allow the proposal of a rational taxonomy for this group; (2) to determine the scope of genetic diversity within the clade, which by including the genes for the four major cyanotoxins (microcystin, anatoxin-a, cylindrospermopsin, and saxitoxin) is already known to be extraordinary; and (3) to obtain clues to understand the evolution and physiological properties of members of this clade that underlie their importance in harmful blooms.

Keywords: Nostocales, ADA clade, genome sequences

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O32 - 40

Understanding the scale and drivers of benthic *Microcoleus* blooms: Insights from 20 years of monitoring in Aotearoa-New Zealand

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Since the early 1990s there have been reports of anatoxin-induced neurotoxicosis in animals from benthic cyanobacterial mats attributed to species from Oscillatoriales group I. In the 30 years since, reports of benthic, anatoxin-producing cyanobacterial mats have been increasing rapidly¹, with over 16 countries across Africa, Europe, North America, Oceania, and South-East Asia impacted. In New Zealand, proliferations in 2004/05 led to significant research effort and the development of consistent guidelines for monitoring the extent of potentially toxic proliferations of *Microcoleus* (previously *Phormidium*)^{2,3}. This has resulted in a spatially (> 1000 sites) and temporally (> 20 years) comprehensive dataset. We compiled data from 16 local authorities alongside environmental covariates when available. We aimed to test whether proliferations are increasing in frequency and severity, understand bloom phenology and determine whether there is apparent time-series clustering among sites. Of the 178 sites with sufficient data between 2015 and 2024, trend analysis revealed increasing cover at 47 sites and decreasing cover at 38 sites. Proliferations typically occurred over the summer months, with the peak (maximum cover) occurring at most sites in mid-summer since 2012. The initiation of proliferations was approximately normally distributed with the peak centred around mid-summer, although there were a substantial number of sites where the first proliferation (>20% cover) occurred between mid-winter and mid-summer. Strong regional variation in the timing of initial and peak proliferation were clear. These results highlight the significant challenges for predictive modelling and generalisation associated with variation occurring across scales from individual rocks to entire nations and from sub-daily to decadal timescales. Together, these results underscore the need for in-depth investigations and the need for caution when generalising results from datasets with limited spatial or temporal scale to *Microcoleus* proliferations more broadly.

Keywords: benthic cyanobacteria, monitoring, anatoxins, *Microcoleus*, phenology

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AI Meets Microscopy: Revolutionizing Algal Bloom Monitoring with Machine Learning and Image Segmentation

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The rising frequency and severity of algal blooms threaten water quality, ecosystems, and public health, necessitating accurate species-level identification for effective management. Traditional microscopy, the standard for algal and cyanobacterial identification, is time-consuming, labour-intensive, and prone to human error. Machine learning driven image recognition technologies have shown to be promising tools to enhance this process¹. This study reviews and evaluates the transformative potential of artificial intelligence (AI), specifically machine learning models, in automating algal bloom identification from microscopic images.

A systematic review was conducted on the application of AI-based image analysis workflows, including image acquisition, pre-processing, and model architectures. Key insights distinguish classification and detection tasks, providing a structured framework for advancing research in this field. Building on the review findings, extensive image datasets were utilized to evaluate the Fast Segment Anything Model (FastSAM), a Vision Transformer (ViT)-based image segmentation algorithm, for its effectiveness in segmenting algal and cyanobacterial cells from microscopic images.

FastSAM was used for cell segmentation, clustering and identification on images taken from varying water quality backgrounds. FastSAM demonstrated promising accuracy, achieving 60-97% similarity with manual segmentation, including 97% accuracy for single-cell identification. Clustering evaluation metrics, such as Spectral Angle Mapper (84-94%), highlighted the method's potential for precise cell shape segmentation and community characterization. These findings underscore the ability of machine learning to enhance efficiency and accuracy in algal bloom monitoring.

This study highlights the biological diversity and ecological importance of cyanobacterial and algal communities while addressing challenges in integrating machine learning into environmental monitoring. By automating species identification and quantification, these advancements support ecological research, water quality monitoring, and public health protection. The integration of AI-driven tools like FastSAM represents a significant leap forward in microbial ecology, enabling better understanding and mitigation of the complex roles algae and cyanobacteria play in aquatic ecosystems.

Keywords: Artificial Intelligence (AI), Machine Learning, Segmentation, Clustering and Identification

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O34 - 196

Development of novel monitoring techniques to address cyanobacterial blooms in Ecuador

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Cyanobacterial blooms and its toxin production represent a growing environmental and public health concern in Ecuador, particularly in the Daule River basin and seven coastal water reservoirs. Conventional toxin detection methods such as HPLC and Elisa are expensive and logistically challenging in this region. This study evaluates environmental DNA (eDNA) and environmental RNA (eRNA) as alternative approach for monitoring potential toxic bloom events. We conducted a longitudinal and temporal analysis of water samples between 2022-2024, targeting five strategic locations along the Daule's river basin; and seven water reservoirs during 2024. The research focused on detecting three microcystin synthesis genes (mcyA, mcyB, and mcyE) and two cylindrospermopsin synthesis genes (cyrA and cyrC). Additionally, strain-specific primers were employed to differentiate between *Microcystis aeruginosa* and *Planktothrix agardhii*, the predominantly bloom-forming cyanobacteria in Ecuador. Molecular analysis revealed distinct spatiotemporal distribution patterns of cyanotoxin genes. Microcystin-related genes showed intermittent detection in river samples between 2022-2024, with peak occurrence in 2022 and a subsequent decline. Conversely, cylindrospermopsin-related genes maintained consistent presence across all seven reservoir locations during the second semester of 2024, with higher prevalence in lentic water bodies compared to the Daule River. These results indicate a habitat preference: microcystin-producing species predominantly occur in riverine systems, while cylindrospermopsin-producing species dominate in reservoir environments. The observed distribution patterns suggest that cyanobacterial species composition is significantly influenced by water body type and associated environmental factors. This study validates eDNA/eRNA techniques as sensitive method for early detection and monitoring of harmful algal blooms, presenting a viable alternative to traditional analytical approaches in resource-constrained environments.

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O35 - 251

Metabolomics for the detection of cyanopeptides from cultures, blooms and sediment cores

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Cyanobacterial blooms have emerged as a major threat to freshwater ecosystems around the world. Cyanopeptide studies have largely focused on microcystins, which are regulated in many jurisdictions. Despite this, the identity and concentration of most cyanopeptides released into waterways by blooms and the risks they pose to ecosystem health are unknown. Common bloom forming species produce diverse mixtures of other cyanopeptides (i.e. cyanopeptolins, anabaenopeptins, microginins), that have received less research attention. A major challenge for studying cyanobacterial metabolites is their immense structural diversity. To better understand cyanobacterial natural products chemistry, we use LC-MS based metabolomic approaches to decipher their cyanopeptide profiles. From cultures grown in the lab, we examined the strain-specific cyanopeptide profiles for several *Microcystis* and *Planktothrix* strains. Each strain generated unique cyanopeptide profiles. Most identified metabolites belong to non-microcystin cyanopeptide groups. Similar metabolomic approaches were applied to blooms and sediment cores to determine the toxigenic potential of cyanobacterial populations in Eastern Ontario, Canada. Select microcystins were the most commonly detected cyanopeptides, however, cyanopeptolins and anabaenopeptins were the most diverse groups. Quantifying common cyanopeptides in sediment cores in combination with other paleolimnological data (i.e. geochemistry, pigments, diatoms, age-depth models) enabled land-use changes around specific watercourses promoting cyanobacterial blooms to be identified. The major objective of these studies is to address the paucity of information on the environmental concentrations and biological activities of poorly studied cyanopeptides from common bloom forming cyanobacteria.

Keywords: Cyanopeptides, metabolomics, cultures, bloom, sediment

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O36 - 24

Sudden harmful algal blooms emphasize the detrimental effects of re-eutrophication on the long-term stability of lake restoration

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In temperate lakes, phosphorus (P) precipitation is among the most effective treatments to mitigate lake eutrophication. However, after a period of high effectiveness, studies have shown possible re-eutrophication and the return of harmful algal blooms. While such abrupt ecological changes were attributed to the internal P loading, the role of lake warming and its potential synergistic effects with internal loading, thus far, has been understudied. Here, in an eutrophic lake (BarleberSee) in central Germany, we quantified the driving mechanisms of the abrupt re-eutrophication and sudden harmful algal blooms (both diatom and cyanobacteria) 30 years after the P precipitation. We combined a process-based modelling approach with long-term in situ high-frequency monitoring dataset covering contrasting trophic states. We found that: 1) Internal P release accounted for 68% of the cyanobacterial biomass proliferation, while lake warming contributed to 32%, including direct effects via promoting growth (18%) and synergistic effects via intensifying internal P loading (14%). The synergy was attributed to prolonged lake hypolimnion warming and oxygen depletion. 2) Diatom blooms in winter was dually controlled by light and temperature, rather than by light alone. Water temperature played a further indirect role in initiating the bloom through ice-thaw, which increased light exposure. These mechanisms differ from those typically responsible for spring diatom blooms and contributed to the high peak biomass. Our study unravels the substantial role of lake warming in mediating seasonal succession of lake phytoplankton communities, promoting both winter diatom and summer cyanobacterial blooms, in re-eutrophicated lakes even after decades of successful restoration. We emphasize the need of more attention on the loss of long-term stability in lake restoration under a changing environment.

Keywords: harmful algal bloom; high frequency monitoring; phosphorus precipitation; internal loading; climate change

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Mitigating Realistic Cyanotoxin Health Risks and Enhancing *Vicia faba* Productivity through Innovative Water Treatment

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The contamination of surface water used for crop irrigation by toxic cyanotoxins poses significant public health risks. Cyanotoxins adversely affect plant growth and can accumulate in edible tissues, endangering human health. This study evaluated the effects of irrigating faba bean (*Vicia faba*) with raw water from the cyanotoxin-contaminated Lalla Takerkoust Dam (Morocco), well water free from contamination, and dam water treated using a Multi-Soil Layering (MSL) system. The experiment, conducted from October 2019 to March 2020 during the peak of cyanobacterial bloom period, involved quantifying microcystins (MC) in water and faba bean seeds using liquid chromatography–mass spectrometry (LC-MS). Raw dam water contained MC levels exceeding 30 µg/L during the growth period, surpassing the WHO guideline for safe water (<1 µg/L MC-LR). In contrast, treated dam water and well water maintained MC levels below this threshold. Growth and yield parameters were assessed, and the Kruskal-Wallis rank sum test revealed significant differences ($p < 0.001$) across irrigation treatments for root, shoot, and fruit fresh weights, as well as the number of pods and grains per pod. Faba beans irrigated with treated water achieved the highest yield (45 quintals/hectare), followed by well water (38 quintals/hectare) and raw dam water (32 quintals/hectare). However, seeds irrigated with contaminated water accumulated up to 224 µg MC/kg dry weight (DW), presenting significant health risks with hazard quotients of 1.5 for adults and 3.6 for children. This study underscores that irrigating crops with cyanotoxin-contaminated water reduces yields and endangers consumer health. Conversely, MSL-treated water enhances crop productivity while minimizing health risks, demonstrating the potential of innovative water treatment systems in agriculture.

Keywords: Microcystins, Morocco, Takerkoust Dam, Faba bean, Crop irrigation, Multi-Soil Layering

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O38 - 149

Diversity and distribution of cyanotoxins along geographical and climatic gradients in Poland and Lithuania

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Cyanobacterial blooms are a persistent, recurring problem, particularly in freshwaters. Paleological and contemporary data indicate that they are becoming more severe due to the ongoing eutrophication of waters and climate change. Cyanobacteria can produce various that negatively affect aquatic and water-related organisms and humans. With global warming tropical and subtropical potentially toxic species are known to invade new areas as observed for *Raphidiopsis raciborskii* in temperate Europe.

The present research aimed to investigate the occurrence and diversity of toxins regarding the composition and structure of cyanobacteria, with special attention given to invasive cyanobacterial taxa in about 90 lakes in Poland and Lithuania. The study was carried out in geographical and climatic gradients from western Poland, characterized by a milder climate, through the cooler eastern part of Poland to even cooler regions in Lithuania. The toxins were analyzed in untargeted analysis using HPLC and LC-MS/MS using an Agilent 1200 chromatograph coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer

The study confirmed the presence of cyanotoxins in 67% of sampled lakes. It revealed the gradient in the diversity of cyanotoxins, with higher in south-western Poland and lower in north-eastern Poland and Lithuania. We detected 41 congeners of microcystins, two of anapenopeptins and micropeptins, and one congener of cylindrospermopsin. The presence of anatoxins was not confirmed. The study also demonstrated that cylindrospermopsin was detected in seven western and two eastern Poland lakes. The toxin was present only in lakes where *R. raciborskii* was identified but not in all lakes where this species occurred. This is an interesting result since *R. raciborskii* strains in Europe have not been proven to produce cylindrospermopsins. Multiparameter analyses and SVM modeling are used to elucidate environmental conditions and cyanobacterial assemblages that support the biosynthesis of cylindrospermopsin as well as other identified toxins.

Keywords: diversity of cyanotoxins, environmental gradients, invasive cyanobacteria, machine learning, *Raphidiopsis raciborskii*

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Diversity and Toxicity of Marine Benthic Cyanobacterial Proliferations from Florida (USA)

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Benthic cyanobacterial mats (BCMs), or benthic cyanoHABs, in marine environments are on the rise, partly driven by nutrient loading and climate change. Reports of BCMs occurring across the Florida coast have increased from mangroves to seagrass beds and corals, demonstrating a need to understand the diversity and physiology of this threat. To monitor this environmental health hazard, benthic proliferations were sampled between 2022 and 2023 to investigate their diversity and toxicity. Cyanobacterial mats were collected, eDNA was extracted in triplicate, and the 16S rRNA of bacteria including cyanobacteria, were amplified as well as the 18S rRNA of associated eukaryotes. In addition to amplicon sequencing, isolation, taxonomic characterization, and genome sequencing of select strains as well as toxin analysis of environmental samples and isolates through ELISA and LC-MS/MS were also applied. Nutrient and metal analyses were also investigated to potentially reveal drivers of the proliferations. Lastly, metagenomes of select field samples were also investigated. Results indicated that benthic mats are diverse primarily composed of the large filamentous cyanobacteria *Dapis*, *Okeania*, and *Sirenicapillaria* while acting as a reservoir for several bloom-forming dinoflagellates including *Amphidinium* and *Alexandrium*. Heterotrophic bacteria revealed a core bacterial consort among samples. Toxin analyses revealed the presence of anatoxin-a, dihydroanatoxin-a, cylindrospermopsins, and microcystins. A novel microcystin congener was uncovered that dominates these BCMs in relatively higher concentrations than other toxins. Environmental variables revealed significant differences across samples and bays, and large differences between benthic and rafting proliferations. Results from this study indicate a need to further assess the diversity and toxicity of these blooms by not only investigating their cyanobacterial and eukaryotic community structure but also the production of novel toxins.

Keywords: genomes, 16S amplicon, benthic cyanobacteria mats BCMs, anatoxin, microcystin

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O40 - 139

The uncertain future of the Burgundy Blood Alga in Peri-alpine Lakes

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The purple colored *Planktothrix rubescens* ("Burgundy blood alga") is the dominant bloom forming, toxigenic cyanobacterium in deep peri-alpine lakes. The long-term population dynamics are driven by eutrophication / reoligotrophication and climate change. Re-oligotrophication allows more light to penetrate down to the metalimnion, where the Deep Chlorophyll Maxima (DCM) of *Planktothrix* are formed. Climate warming strengthens lakes' stratification and enhances the stability - buoyancy frequency - of the metalimnion. Interactions between re-oligotrophication and climate change therefore may expand *Planktothrix* habitat in deep lakes, but only up to a point. We present results from a study showing how ultimately *Planktothrix*' tendency to maintain neutral buoyancy forces this cyanobacterium to leave its position in the metalimnion and sink into the hypolimnion, approaching a zone of deep turbulence. Additionally, we will show how maximal physical stability at the depth of the DCM is relevant for co-existence of different *Planktothrix* morphotypes, which differ in key traits like gas-vacuole content and Chla:phycoerythrin ratio. Intraspecific diversity, critical for population resilience, is maximal in summer, during the peak of stratification, but is lost with lake overturn in fall. Finally, we present data from a third study where we combined response curve experiments with dynamical modeling, showing how *Planktothrix* preference for relatively deep, cold and dark layers allows the cyanobacterium to escape the worst of infection by parasitic chytrid fungi. All these studies contribute to a deeper understanding of future *Planktothrix* development in changing peri-alpine lakes, and help lake management to better deal with surprises, such as a shift of the blooming period from summer to winter.

Keywords: Deep lakes, Stratification, Climate change, Chytrid parasites

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Extra-Tropical Storms as an Emergent Contributor to Harmful Algal Bloom Development in the Canadian Maritimes

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Intense storms can pose a serious threat to lake ecosystem functioning and services, triggering significant changes in nutrient cycling^[1]. In shallow lakes, storm-driven waves can resuspend nutrient-rich lakebed sediments, whilst heavy rainfall can accelerate nutrient runoff from catchments. The resulting biogeochemical changes can alter ecological conditions to favour cyanobacteria over other phytoplankton, and the development of harmful cyanobacterial blooms (CyanoHABs). Whilst a mechanistic link between storm forcing and lake community shifts has been observed via in-situ monitoring, it remains undemonstrated in palaeolimnological records.

This study analysed dated (²¹⁰Pb; ¹³⁷Cs) sediment cores from three lakes in southwestern New Brunswick, Canada, a region which has recently experienced a rise in lake CyanoHABs. Multi-proxy sediment analyses (end-member modelling analysis of grain-size distributions (EMMA), ITRAX-XRF; stable isotopes: δ C; δ N), were used to explore links between the drivers of limnological change (e.g., climate variability, historic storms, land use change), and past episodes of CyanoHAB development. Cyanobacterial responses were investigated using taxonomically-specific sedimentary pigments, including aphanizophyll (N-fixing), echinenone (total cyanobacteria), canthaxanthin and myxoxanthophyll. Microcystin congeners LA, LR, [Dha⁷] LR and RR were quantified using high-resolution tandem mass spectrometry (HRMS/MS).

Although decoupling the compounding drivers of lake cyanobacteria occurrence from sedimentary records is challenging, initial EMMA, and ITRAX-XRF derived ratios (Br+Cl/Al), which are linked to precipitation and air mass patterns, showed co-variance with increases in productivity. δ N, which represents fossil nitrogen inputs, exhibited the strongest correlation with productivity proxies. This suggests that catchment-mediated processes such as agriculture and urbanization, strongly influenced the development of CyanoHAB. A late 20th century increase in cyanobacteria concentrations is attributed to heightened nutrient inputs. This set the stage for significant CyanoHAB occurrences in 2015, likely attributable to lake fertilisation triggered by the passage of extra-tropical storm Arthur in 2014.

Keywords: lake cyanoHABs, palaeolimnology, storms, land use change, climate change

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O42 - 06

Enhancing Harmful Algal Bloom Predictions by Coupling Machine Learning Models with a Data Assimilation Technique

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Abstract-Harmful Algal Blooms (HABs) are detrimental to humans, pets, livestock, wildlife and the ecosystem due to their ability to produce undesirable toxins, making effective monitoring necessary. While traditionally numerical models such as the Water Quality Simulation Program (WASP), have been used to model and monitor HABs using chlorophyll-a as an indicator, the emergence of sensor-based high-frequency water quality data and machine learning (ML) technologies such as deep learning (DL) models, have led to the development of data-driven ML models and decision support tools. DL models are more dynamic in the sense that they capture patterns and structures in the water quality data which are difficult to describe in traditional approaches; however, these data-centric models are limited by the model structure and uncertainty in generating results. In the current study, to improve chlorophyll-a predictions and reduce uncertainty in model results, the DL models were coupled with a data assimilation (DA) technique. DA is a technique that combines models with new sources of observations to estimate the evolving state of a system over time. The current study was conducted one day ahead prediction of chlorophyll-a as an indicator of HABs using Long Short-Term Memory (LSTM) and Gated Recurrent Unit (GRU) models. The Ensemble Kalman Filter (EnKF) approach was computed to assimilate chlorophyll-a values to improve the model prediction at every time step. New observations were assimilated daily, 2, 5, 7, 15 days, monthly and randomly. The results with DA showed improved chlorophyll-a predicted values compared to models without DA. Increasing DA frequency led to improved predictions, with daily assimilation achieving an RMSE of 0.02 µg/l for LSTM and 0.03 µg/l for GRU and, while monthly assimilation resulted in an RMSE of 3.59 µg/l for LSTM and 3.63 µg/l for GRU, highlighting the importance of DA for improving ML model accuracy.

Keywords: HABs, Sensor-based, Machine Learning, Deep Learning, Data Assimilation

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O43 - 87

Persistence of potentially toxin-generating cyanobacteria in drinking water treatment plants across Eastern Australia

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The accumulation of algae and cyanobacteria within drinking water treatment plants (DWTPs) poses significant challenges, particularly due to the persistence of potentially toxin-generating cyanobacteria taxa. The risk to water quality is heightened as this is downstream of one or more treatment barriers and may hinder supernatant recycling. This study analysed historical data spanning 10-30 years from 16 Eastern Australia DWTPs to understand the accumulation of algae and potentially toxin-generating cyanobacterial communities through DWTP processes and determining the best mitigation approach.

Common genera found in the raw inlet to DWTPs included cyanobacteria (*Cyanogranis*, *Aphanocapsa*), green algae (*Scenedesmus*, *Monoraphidium*, *Crucigenia*), and diatoms (*Urosolenia*, *Centrics*, *Aulacoseira*). Notably, cyanobacteria taxa, including some with toxicogenic potential, such as *Synechococcales*, *Cyanonephron*, *Chroococcales*, *Microcystis*, *Raphidiopsis*, and *Aphanizomenonaceae* were reported at high average cell concentrations (10^5 cells/mL), and the corresponding cyanotoxins such as Deoxycylindrospermopsins ($<11\mu\text{g/L}$), Cylindrospermopsins ($<6.5\mu\text{g/L}$), and Microcystins ($<2\mu\text{g/L}$) were recorded. While downstream analysis of DWTP processes was less comprehensive, existing data showed the presence of potentially toxic *Pseudanabaena* and *Planktolyngbya* cells after filtration, with no detection of cyanotoxins. Similar cyanobacterial communities such as *Synechococcales*, *Planktolyngbya*, *Chroococcales*, and *Aphanizomenonaceae*; *Pseudanabaena*, *Aphanothece*, and *Aphanocapsa*; and *Cyanocatenula*, *Aphanocapsa*, unidentified *Chroococcales*, and *Planktolyngbya* were detected in backwash lagoon supernatants, sludge thickeners, and supernatant return, respectively. Again, all cyanotoxins were below $1\mu\text{g/L}$ in these units across all monitored years, with only one detection of $0.8\text{--}1.1\mu\text{g/L}$ Deoxycylindrospermopsin in the sludge thickener supernatants in two adjacent DWTPs. No cyanobacteria data was available. This research revealed the existence of potentially toxicogenic cyanobacteria in later DWTP phases, posing a risk of reintroducing them to the water treatment process. Further work will focus on multivariate analysis of cell concentrations, biovolumes, hydraulic and operational data, and water quality to better define potential risks to product safety and to understand the build-up of algae and potentially toxin-producing cyanobacteria.

Keywords: Algal bloom, sludge lagoon, supernatant return, water treatment process

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O44 - 101

Phylogenomics of anatoxin-a-producing *Microcoleus* species at a local and global geographic scale

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Microcoleus anatoxicus and closely related *Microcoleus* species [*Matx*] are ubiquitous benthic cyanobacteria capable of producing anatoxin-a derivatives. Their proliferation is commonly associated with the death of vertebrates, including domestic animals, at the shores of rivers and lakes. Despite recent sequencing efforts, we lack an overview of the genomic diversity and biogeography of this problematic *Microcoleus* clade. Here, we analyze a set of a dozen new complete *Matx* genomes and metagenome-assembled genomes (MAGs) collected, over the span of four years, from a small Swiss river, *L'Areuse*. This river flows through a complex landscape involving dramatic changes in topography, geology, and levels of human impact. We compare the *Matx* sequences from *L'Areuse* with genomes from other regions of Switzerland and around the world. At the scale of the river, we find that proliferations happening in a given area over the years are caused by the same lineage (Average Nucleotide Identity [ANI] >99.9%), while different lineages (ANI <99.5%) proliferate in different areas along the river. Nevertheless, the genomes collected in *L'Areuse* form a coherent group clearly distinct, in terms of ANI, genome structure, and gene content, from sequences collected elsewhere in Switzerland or on other continents. In addition, the anatoxin-a biosynthesis operon found in local *Matx* populations is unique, confirming that this genomic region is prone to recombination and diversification. Overall, our results suggest that *Matx* populations present a quite strong geographic structure, in particular compared to planktonic cyanobacteria; this structure could be linked to dispersal barriers or, alternatively, to local adaptations which could be key to understand the ecological success of *M. anatoxicus*, both globally and locally.

Keywords: *Microcoleus*, anatoxin-a, phylogeny, genomics, biogeography

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O45 - 88

Significance of cyanotoxins in lakes for irrigation and drinking water for livestock

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The Norwegian Lake Akersvannet is used for recreation, irrigation of agricultural areas and by livestock. However, there are no guidelines from food and health authorities on how the situation in such waters and their use should be monitored. In a pilot phase in 2022, Akersvannet was investigated for its suitability as irrigation water for vegetable production. Water samples from the lake were examined for potentially toxigenic cyanobacteria and for the cyanotoxin microcystin. In addition, selected vegetables (lettuce, celeriac, red onion and parsnip) and soil samples from areas that were irrigated with lake water were tested for microcystins. From August onwards, the cyanobacterial biomass increased and dominated the phytoplankton until October. In the samples microcystin concentrations up to 11.9 µg L⁻¹ were measured and are thus well above the WHO guideline value for drinking water of 1 µg L⁻¹. In cyanobacterial films or clumps, microcystin concentrations up to 35000 µg L⁻¹ were measured. These microcystin levels can be hazardous to the health of large animals such as cattle or even lethal to smaller animals such as dogs - an incident that happened in 2021. The cyanobacterium *Microcystis* is the main producer of microcystins in Akersvannet. Only in two lettuce samples were low microcystin concentrations between 2 and 5 ng g⁻¹ detected. All other vegetable samples and soil samples showed values in the range of the background values of the controls and are therefore assumed to be negative. Based on these results, an adult human weighing 60 kilos can eat 480 g of lettuce daily without exceeding the tolerable daily intake for microcystin. In 2024, the follow-up project 'BEDREVANN', funded by the Regional Research Fund Vestfold Telemark, was launched to develop an early warning system and further increase knowledge about the effects of cyanotoxin-containing irrigation water on fruit, vegetables and soil.

Keywords: Lake Akersvannet, irrigation water, vegetables, cyanobacteria, cyanotoxins

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O46 -127

Taxonomic, metabolic, and toxigenic diversity of cyanobacteria in the Sacramento-San Joaquin Delta: Insights from genome-resolved metagenomics and environmental drivers

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Cyanobacterial blooms are a recurring challenge in freshwater ecosystems, including the Sacramento-San Joaquin Delta—a critical drinking water source for California—where they pose significant ecological and public health concerns. Recent increases in the diversity and abundance of potentially toxigenic cyanobacteria in the Delta have raised concerns about novel cyanotoxin producers. To investigate these threats, we conducted a genome-resolved study in 2023, integrating metagenomics, toxin analyses, and water quality assessments across ten Delta sites over eight months.

We recovered over 200 high-quality metagenome-assembled genomes (MAGs) of cyanobacteria, with the highest numbers belonging to toxigenic genera such as *Microcystis*, *Aphanizomenon*/*Dolichospermum*/*Anabaena* (ADA clade), and *Planktothrix*. Additionally, a substantial number of bins were recovered for non-toxigenic genera like *Pseudanabaena* and the benthic genus *Koinonema*. Microcystin biosynthesis genes were detected exclusively in *Microcystis* genomes, reinforcing its role as the primary microcystin producer in the Delta. *Planktothrix* genomes contained biosynthetic gene clusters (BGCs) for unknown bioactive compounds, while *Aphanizomenon* and *Dolichospermum* harbored BGCs for geosmin, a taste-and-odor compound impacting water quality.

Environmental analyses revealed that water temperature explained 15.2% of community variation ($p < 0.0001$), with other factors like dissolved oxygen, nitrogen species, and total alkalinity also significantly influencing cyanobacterial composition. Seasonal and spatial dynamics, particularly in the north and south Delta during summer and fall, highlighted shifts in cyanobacterial dominance.

This study underscores the importance of genome-resolved approaches in understanding cyanoHAB diversity and dynamics. By integrating environmental drivers and toxin profiling, these findings provide critical insights for predicting cyanoHAB responses to future hydrologic and water management scenarios.

Keywords: Cyanobacterial Harmful Algal Blooms, Sacramento-San Joaquin Delta, Genome-Resolved Metagenomics, Cyanotoxins, Environmental Drivers

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O47 - 182

Comparison of the impact of differentiated cyanobacterial morphotypes and bacterial communities in relation to complex water quality threats - an intercontinental study

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Cyanobacterial harmful blooms (cHBs) dominated by coccoidal colonies of *Microcystis* are well described globally. However, cHBs dominated by filamentous cyanobacteria, e.g. *Aphanizomenon* and *Planktothrix*, also occur globally and may produce different compositions of cyanotoxins. Bacteria that co-occur with cyanobacteria, called the cyanosphere, play an important role by altering the ambient chemical composition, physiology, and development/collapse of cHBs. The cyanosphere also provides a suitable environment for pathogenic bacteria and antibiotic resistance genes (ARGs). These hazards associated with cHBs highlight the need to extend knowledge about blooms dominated by filamentous cyanobacteria, especially since cHBs are expected to occur more frequently in the context of climate change and growing anthropopressure. This study focused on i) toxigenicity, ii) diversity of associated bacteria, iii) presence of pathogenic bacteria, iv) ARGs profiles, and v) diversity and abundance of bacterial nutrient-cycling genes. Samples were collected from freshwaters containing bloom-forming cyanobacteria (*Microcystis*, *Aphanizomenon* or *Planktothrix*), in Poland, USA, Republic of Korea, Singapore and Guatemala. Differences in cyanobacterial taxa were reflected in toxigenicity, e.g. a wider variety of cyanotoxin genes detected for a water body containing a mixed bloom of *Microcystis*, *Snowella* and *Aphanizomenon*. Numerous correlations were observed between relative abundances of *Microcystis* and bacteria (24 total interactions), while lower correlations were observed for *Planktothrix* and *Aphanizomenon* (14 and 4 total interactions, respectively). *Microcystis* blooms showed high abundance of potentially pathogenic bacteria belonging to *Roseomonas*, while *Planktothrix* blooms showed strong co-dominance with the potential pathogen *Flavobacterium*. Shotgun metagenomics suggest that anthropogenic pressure and land use are important factors influencing ARGs diversity in cHBs. Furthermore, non-diazotrophic cyanobacteria (*Microcystis* or *Planktothrix*) appeared to have higher dependencies on their attached consortia compared to diazotrophic cyanobacteria (*Aphanizomenon*). This was inferred from the higher detection of nitrogen-degrading and assimilatory genes for the non-diazotrophic cyanobacteria, allowing them to thrive in nitrogen-limited environments.

Keywords: cyanosphere, 16S rRNA amplicon sequencing, shotgun metagenomics, anthropopressure, ARGs

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O48 - 202

Toxic cyanobacteria's journey from aquatic to terrestrial environments: Soil colonization and Impacts on microbiome, crops, and cyanotoxin risks

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Proper management of the enormous amount of drinking water treatment residuals (WTRs) generated daily is of significant importance. Land application of WTR is one of the popular approaches. However, when the source water is affected by harmful cyanobacterial blooms (HCBs), concerns need to be addressed regarding the impact of HCB-affected WTR on soil and crops. This study provides a deeper insight into the fate of freshwater cyanobacteria and cyanotoxin after being introduced into agricultural soil.

Two WTRs, from source water of different HAB intensities in Ohio, USA, were applied to greenhouse soil at a 5% rate (dry weight basis) for soybean cultivation, Ohio's most common and essential crop. Freeze and thaw (F&T) treatment was conducted for half of the WTR to inactivate viable cyanobacteria. The microbiome, cyanotoxins, and physicochemical attributes of WTRs were first characterized. Microcystin (MC), saxitoxin, and β -Methylamino-L-alanine (BMAA) were detected and quantified in both WTRs. Core toxic cyanobacterial genera of the two WTRs are different, both containing MC-producing *Planktothrix* and *Microcystis*. To investigate the dynamic of total and viable MC-producing cyanobacteria in WTR-amended soil, MC synthesis genes were quantified in the extracted soil DNA and RNA, respectively.

Results showed that both WTRs contained MC, saxitoxin, and β -Methylamino-L-alanine (BMAA). Nanopore sequencing revealed different core cyanobacterial genera, but both showed MC-producing *Planktothrix* and *Microcystis*. MC-producing *Planktothrix* can better survive and accommodate in agricultural soil than MC-producing *Microcystis*. Colonized MC producers were persistently releasing the toxin into the soil, resulting in an elevated MC concentration in the WTR-amended group (no F&T treatment) at the harvest time. Although no food safety risk was associated with human uptake of MC from soybean consumption, WTR amendment (high MC concentration group, no F&T treatment) lead to impaired soybean productivity. Therefore, for the HAB-affected WTR, the application rate needs to be systematically justified to minimize the long-term negative impacts of toxic cyanobacteria and other WTR biochemical attributes on food security and environmental health.

Keywords: *Microcystis*, *Planktothrix*, water treatment residuals, cyanotoxin, soybean

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O49 - 08

From water to fork: accumulation of microcystins in fruits and vegetables

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Toxic cyanobacterial blooms are commonly observed in freshwater reservoirs, ponds, lakes and canals. These waterbodies are also well-known irrigation water sources for crop production. This overlap has resulted in the accumulation of cyanotoxins, particularly microcystin congeners (MCs), in fruits, vegetables, and culinary herbs. Yet, data on the influence of accumulation based on crop type, irrigation methods, and the prevalence of the MCs in products on the consumer market is incomplete.

Initiating our studies, a UHPLC-MS/MS method was validated in strawberries, carrots, lettuce, and basil to quantify eight MCs and Nodularin. Thereafter, multiple lab and mid-scale greenhouse experiments were performed to assess the MCs accumulation. Specifically, the accumulation and depuration of MCs was investigated at lab-scale for basil. Additionally, strawberries were exposed to MC-LR or naturally obtained toxic cyanobacteria biomass using the same setup. Drip and/or spray irrigation were evaluated for strawberries, carrots, and lettuce exposed to MC-LR fortified irrigation under relevant greenhouse conditions. Furthermore, fruit, vegetable, and basil samples were collected from the consumer market.

Accumulation of MCs in crops was established for most irrigation approaches. MCs were quantified in basil roots and leaves after 7 days. Strawberries grown in the lab or undergoing drip irrigation in a greenhouse showed accumulation in the roots and the foliage. These results show that MCs can be transported through the roots, to the subsequent plant parts. Moreover, MC-LR was quantified on strawberries after spray irrigation. Similarly, a combination of spray and drip irrigation resulted in MC-LR accumulation in lettuce. The consumer market sampling demonstrated the presence of MC-LR in one basil food item, indicating that the prevalence of MCs contamination in crops might still be low. However, MCs accumulation should be monitored as the contamination frequency might change in different regions due to climate change, resulting in additional human exposure to MCs.

Keywords: Horticulture, Microcystins, Public Health, irrigation

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Unmonitored Risks: Cyanotoxins in Marine Seafood and Their Impacts on Food Security

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Freshwater cyanotoxins produced by harmful algal blooms (FHABs) are a global threat to coastal ecosystems, with documented impacts on marine shellfish, mammals, and seabirds. Despite these risks, routine monitoring for cyanotoxins in marine environments remains limited, raising significant food safety concerns, particularly for subsistence harvesters. The Salish Sea region, encompassing Washington, USA, and British Columbia, Canada, is rich in culturally, economically, and ecologically significant seafood. For the Coast Salish Peoples and the Lummi Nation, who have harvested seafood for millennia, safe and sustainable access to these resources is a critical food security priority. While marine toxin surveillance is robust, freshwater toxins in shellfish remain unmonitored, despite their routine exposure to these contaminants.

From 2017 to 2024, we collected an extensive dataset documenting over 200 occurrences of microcystins across the marine food web, including sediment, shellfish, fish, ducks, seabirds, crabs, snails, and feces. Guided by the One Health framework, we aim to better understand the freshwater transport of these understudied toxins into marine ecosystems. Using solid-phase adsorption toxin transfer (SPATT) (2023–2024), we are tracking pathways that link freshwater toxin sources to marine exposures. By integrating freshwater and marine monitoring, our study provides critical data to help inform tribal policy development, safeguarding marine species and the communities that depend on them. This research underscores the value of a One Health approach in addressing the interconnected health of ecosystems, wildlife, and humans, and fostering resilient, sustainable food systems.

Keywords: microcystins, shellfish, One Health Approach, subsistence harvest, food safety

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O51 - 82

Macrophyte-associated toxic cyanobacteria in a mesotrophic urban lake - abundance of toxins and risk assessment

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Benthic cyanobacteria are increasingly recognized in many freshwaters, mostly rivers, worldwide. They can colonize different substrates and produce potentially lethal toxins. Here we analyse long-term data of a German lake in which benthic toxic cyanobacteria were detected after its recovery from eutrophication.

Dimictic Lake Tegel in Berlin was hypertrophic due to strong anthropogenic pressure causing epilimnion total phosphorus (TP) concentrations of up to 800 µg/L until the 1980's when restoration measures were implemented to improve water quality for recreation and to ensure drinking water quality (which is produced via bank filtration). In the course of the restoration, epilimnion TP concentrations dropped to 20-29 µg/L in summer planktonic cyanobacteria disappeared transparency increased from 0.5 to > 4m, submerged macrophytes re-appeared and the lake is now mesotrophic. However, in 2017 dog deaths led to the detection of anatoxin producing cyanobacteria (*Microcoleus*) associated to submerged macrophytes, mainly the rootless moss *Fontinalis antipyretica*, and since then these macrophyte-associated cyanobacteria appear regularly in Lake Tegel. The presentation shows toxin data from a 5-year-period of sampling macrophytes washed ashore at beaches and from a one-year intensive screening at various transects, and it presents an assessment of human health risks.

In a current collaborative project (DIVATOX), further questions about the putative interaction between macrophyte - associated cyanobacteria, macrophytes and their microbiome, (a)biotic influences on growth and toxin production of *Microcoleus* as well as (eco)toxicological evaluation of anatoxins, will be addressed.

Keywords: *Microcoleus*, anatoxin, macrophytes, risk assessment

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O52 - 188

CYANOACTION – COST Action CA23160, Effective Lake management: reducing cyanobacteria by actions in the catchment

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The increasing occurrence of toxic cyanobacterial blooms as a consequence of anthropogenic eutrophication in freshwater ecosystems threatens water security and quality worldwide for humans and animals and severely disrupts ecosystem balance. Besides, significant economic losses to water companies, fisheries, recreational activities, and other businesses caused by cyanobacterial blooms require greater attention from policymakers. Therefore, the implementation of thorough monitoring strategies for cyanobacteria, along with evaluation of the risks of bloom incidents and a plan for prevention and control measures, are now required in water safety plans by water suppliers. A suite of in-lake control methods exists, but little guidance exists on their efficiency. Decreasing nutrient input from the catchment is crucial for long-term prevention/ reduction of blooms and would be possible by the transition towards more sustainable agricultural practices, but it requires implementation strategies.

The COST action, CYANOACTION, started in the autumn of 2024, aims to increase and broaden capabilities throughout Europe for effective and sustainable management of water resources using a catchment-wide approach. To achieve this, CYANOACTION seeks to establish a unique network of experts, end users, and stakeholders. Gathering and distributing information on early warning detection methods and experience in the effectiveness of both long and short-term control methods will underpin Decision Support Tools for water managers and authorities. These tools will ensure the selection of appropriate methods tailored to specific water bodies. The overarching goal is to increase the mutual exchange between scientific knowledge and management practices in order to decrease cyanobacterial blooms while considering the diverse characteristics of water bodies, their catchment, and the regulatory frameworks across Europe.

Keywords: COST, cyanobacterial blooms, control methods, eutrophication

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A sustainable and innovative management system for toxic cyanobacteria blooming of surface waters with combined energy production, sustainable agriculture and food safety (CYANOTECH)

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The blooming of toxic cyanobacteria has become more persistent and prevalent globally as a direct result of increased inputs of nutrient-rich waste into aquatic systems, increasing temperatures, and CO₂ emissions [1]. Besides the aesthetic and environmental effects of cyanobacteria harmful blooms (Cyano-HABs), a number of economic sectors including tourism, fishery, food, health, and water industries, experience annual losses in the range of millions USD [2]. With so many diverse sectors of modern living being affected by the same problem, it is crucial to develop and apply innovative and sustainable management systems for toxic cyanobacteria that can be easily adapted into current infrastructures. The CYanoTech project aimed to address water availability, quality, and scarcity due to anthropogenic activities (such as intense agriculture and livestock activities) by applying an innovative approach for safeguarding surface water quality from further deterioration, while utilizing harmful waste as raw materials for the production of fertilizers, food, and energy [3]. Specifically, the CYanoTech approach comprises of the removal of the excess aquatic biomass (cyanobacteria cells and algae) from water with a low-energy non-mechanical separation technology, the treatment of the aquatic biomass for the production of energy and biofertilizers (struvite), and the application of treated and untreated surface water in hydroponic cultures that produces safe for consumptions crops (cyanotoxins-free crops). The energy produced can be utilized to run all the aforementioned processes making it self-sustainable. Finally, a Life-Cycle-Analysis confirmed that the CyanoTech approach significantly reduces the environmental impact of cyano-HABs.

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Playing to combat cyanobacteria: A multi-stakeholder consensus-building approach to address eutrophication in waterbodies

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The primary approach to sustainably combating cyanobacterial blooms in water bodies focuses on restoring water quality by addressing eutrophication. However, implementing this approach is often complex, as it requires the involvement of numerous stakeholders within a given territory to develop effective action strategies. These stakeholders may have divergent interests and face use conflicts. As a result, consensus-building processes are essential, and serious games are increasingly playing a key role in these efforts. These games allow stakeholders to enhance knowledge and awareness of environmental issues while fostering collective decision-making.

The NoCyano project (New Approach for the Management of Cyanobacterial Blooms in Moselle Ponds) was developed within this broader context. It brings together research teams in biological/environmental sciences and social sciences, along with consulting firms and local authorities. The serious game created as part of NoCyano, is based on a simplified representation of the targeted territory and its ponds and on a modeling approach of water and nutrient flows. Players' actions, reflecting their management of various activities and practices, influence nutrients concentrations in the ponds and, consequently, the likelihood of cyanobacterial blooms. The game involves seven stakeholder roles, such as farmers, fishers, local authorities with participants assuming roles different from their real-life ones during the session. After testing the game with various groups of stakeholders, it appears that the strategies developed by these groups of players can range from highly individualistic to highly collective, with corresponding impacts on nutrients level and cyanobacterial bloom occurrence in the ponds. Following the gaming sessions, workshops are held to collaboratively define a new governance framework for the ponds and discuss the levers of action for mitigating eutrophication. The serious game has also been used as an educational tool with Master's level students in environmental sciences and humanities.

Keywords: Cyanobacteria, eutrophication, serious game, concerted management

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20years of experiences with cyanobacterial blooms management: Big thinks depends on small details

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There is an abundant literature describing the principles of the lake restoration projects around the globe, resulting from the experiences of several past decades. Structure of classical projects are dominantly based on the phosphorus paradigm in catchments and on the in-lake methods working mostly with nutrients management combined with the autecology of dominant cyanobacterial species.

Practical experiences proved, that at least the last decade, thanks to the climate changes, classical approaches are not sufficient in this changing world and more parameters we must take in to account to reach the aim- the prevention and mitigation of cyanobacterial blooms.

The shift of dominants from colonial buoyant *Microcystis* to dispersed filamentous cyanobacteria like *Raphidiopsis* sp., *Planktothrix* sp., or even to picocyanobacteria require novel methods, even the combination of methods which can be effective in the changing world.

This contribution will demonstrate the experiences from the lake restoration projects using methods from the near-nature methods for nutrients management in catchment, classical Al, Fe salts, humic substances, or probiotic bacteria up to novel in-situ AOP production of peroxides. All projects can look perfectly up to the day, when we would like to realise all scientifically proved methods in another lake. Every lake is an original, catchment is the important part of the game and detailed scientific preparation of the cyanobacterial management can be finally dependent on the biotic interaction of the submersed macrophytes, or the composition and abundance of the fish assemblages. All parameters which can affect the cyanobacterial biomass and blooms composition are important and success depend on the precise scientific preparation taking into account all chemical, biological and climatic details in the changing world.

Keywords: cyanobacterial blooms management, lake restoration projects



O56 - 201

Effects of hydroxyl radicals (H₂O₂/UVC) on natural organic matter from a cyanobacterial bloom

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Freshwater cyanobacteria blooms are considered a major threat to ecosystems and human health, mainly due to the production of a wide range of secondary metabolites, including potent toxins. More than 3000 cyano-metabolites are documented in the literature, with the largest group featuring at least one peptide bond. Reactions of cyano-peptides with reactive species such as the hydroxyl radical have attracted attention, to gain insights on transformation pathways in the natural environment and during advanced oxidation water treatment. Nevertheless, there is a knowledge gap regarding the effects of reactive species on complex mixtures of cyano-metabolites. In this study, we investigated the effects of hydroxyl radicals produced by H₂O₂/UVC on natural organic matter extracted from a cyanobacterial bloom in Lake Kastoria, Greece. Untargeted LC-HRMS analysis was applied to determine the composition of the aqueous samples pre- and post-treatment. Data analysis included pre-processing in MZmine, library search, molecular networking (FBMN) in GNPS2, compound and compound-class level annotations in SIRIUS and the CMMC workflow (GNPS2) to provide insights into co-existing microbial communities. ChemProp2 was further used to show the directionality of potential transformations within networks during H₂O₂/UVC treatment. Various transformation reactions such as hydroxylation, oxidative cleavage and isomerization were observed, depending on the experimental conditions and the molecular structures of the compounds. We discuss the applicability of HRMS combined with computational tools to investigate transformation pathways in complex mixtures of cyano-metabolites during advanced water treatment and natural degradation processes.

Keywords: Cyanobacteria metabolites, hydroxyl radical, advanced oxidation processes, transformation products, GNPS2

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Controlling cyanobacterial blooms with hydrogen peroxide: implications for non-target species and the role of green algae

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Addition of hydrogen peroxide (H₂O₂) is an effective method to acutely suppress toxic cyanobacterial blooms in lakes. In this presentation, two aspects are discussed that may have implications for the mitigation of cyanobacteria with H₂O₂. First, a reliable H₂O₂ risk assessment to identify potential effects on non-target species is currently hampered by a lack of appropriate ecotoxicity data. Therefore, we investigated the 24-h toxicity of H₂O₂ to three cyanobacteria (*Planktothrix agardhii*, *Microcystis aeruginosa*, *Anabaena* sp.) and 23 non-target species (six green algae, eight zooplankton and nine macroinvertebrate taxa). The most sensitive species included all three cyanobacterial taxa, but also the rotifer *Brachionus calyciflores* and the cladocerans *Ceriodaphnia dubia* and *Daphnia pulex*. The EC₅₀ and LC₅₀ values obtained from the laboratory toxicity tests matched well with the responses of phytoplankton, zooplankton and macroinvertebrates in two whole-lake treatments with H₂O₂. The results thus revealed a trade-off between the successful suppression of cyanobacteria at the expense of adverse effects on part of the zooplankton community. This delicate balance strongly depends on the applied H₂O₂ dosage and may affect the decision whether to treat a lake or not. Second, biological factors may affect the H₂O₂ treatment efficiency to suppress cyanobacteria. Our laboratory results show that green algae degrade H₂O₂ much faster than cyanobacteria. As a consequence, the cyanobacterium *Microcystis* was able to survive at higher H₂O₂ concentrations in mixtures with the green alga *Chlorella* than in monoculture, and the level of this interspecific protection depended on the density of green algae in the mixed cultures. Our results demonstrate that high densities of green algae in the phytoplankton community can interfere with H₂O₂ lake treatments, as they may rapidly degrade the added H₂O₂ and thereby protect the bloom-forming cyanobacteria.

Keywords: Cyanobacteria, zooplankton, green algae, interspecific protection, lake treatments

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Full-Scale Preventive Algaecide Treatment of Cyanobacterial Harmful Algal Blooms in a Reservoir Used for Potable Water Production

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Cyanobacterial harmful algal blooms (cHABs) continue to threaten freshwater systems used as drinking water sources, prompting many water treatment plants to seek methods for their treatment and prevention. Rapid mitigation of cHABs using chemical algaecides has been widely studied. However, most research has focused on responsive treatment after blooms have already developed. This study evaluates the effect of preventative low-dose algaecide treatment on water quality and cHAB control. Hydrogen peroxide-based algaecides were applied to assess full-scale treatment in a 168-million-gallon reservoir at a water treatment plant in Ohio, USA. Weekly low-dose algaecide applications were conducted during potential cHAB season (May to October) over three years. Comprehensive sample collections were performed before treatment and at 0, 24, 48, and 72 hours after application to assess changes in total chlorophyll levels, water quality parameters (pH, turbidity, and conductivity), organic matter (dissolved organic carbon, organic nitrogen, and fluorescent organic compounds), and algaecide decay. Additionally, 16S and 18S rRNA gene sequencing was performed to investigate the impact of low-dose algaecide treatment on the abundance of cyanobacteria and other phytoplankton. The results indicate that applied algaecide was completely consumed within 8 hours. The preventative treatment strategy effectively mitigated cHAB formation, while diatoms and green algae exhibited minimal changes during treatment.

Keywords: Harmful Algal Blooms, Cyanobacteria, Algaecide Treatment

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System analysis guided eutrophication control and mitigation of cyanobacterial blooms

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Eutrophication is the over-enrichment of surface waters with nutrients that may lead to harmful cyanobacterial blooms. Cyanobacterial blooms that impair ecosystem services in freshwater systems have been reported worldwide. The negative consequences of the cyanobacterial blooms underpin the need to control eutrophication and restore impacted waterbodies. Restoration of eutrophic waterbodies should always start with a system analysis, which is a thorough diagnosis of the problem. Such system analysis includes the severity of the situation, determines water- and nutrient fluxes, maps the system's biology, and identifies the use of the impaired waterbody. This diagnosis forms the basis for restoration of the impaired waterbody, identifying which buttons are best to push, and guides to the most promising interventions. The interventions will probably always consist of multiple measures targeting external and internal nutrient load, and ecosystem structure.

In our presentation, we present examples from three restoration case studies in the Netherlands of lakes varying in size from 5 to 110 hectares (more information about one case study is reported in Lürling et al. 2024). In all three cases, a system analysis guided the successive intervention composed of a package of measures. These interventions aimed at reducing the external and internal nutrient load while multiple in-lake measures were implemented to speed up recovery and reduce the undesired eutrophication symptoms. The case studies demonstrate that massive cyanobacterial blooms can be prevented by implementing a tailor-made package of measures, however in countries such as the Netherlands with strong diffusive nutrient pollution repeated maintenance interventions will be required. Therefore, adequate post-intervention monitoring is necessary to evaluate the effectiveness of the intervention, but also to determine when maintenance is required. In countries like the Netherlands, ongoing diffuse nutrient loading (mostly from agro-industrial activities) necessitates maintenance, or repeated interventions, to allow recreational activities and delivery of other ecosystem services.

Keywords: lake restoration, management, internal load, external load

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Effectiveness of algicidal bacteria in controlling harmful cyanobacteria in different bloom stages

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Cyanobacteria blooms pose increasing threats to aquatic and terrestrial ecosystem worldwide due to the adverse changes in their functioning and cyanotoxins production. Therefore, a lot of effort is put into preventing the formation of blooms and their mitigation or removal through various methods. Harmfulness of cyanobacterial blooms depends however, on various factors including stage of the bloom. In this study we investigated the impact of bioaugmentation with algicidal bacteria on phytoplankton dynamics and microbial community structure during *Microcystis*-dominated bloom stages. Laboratory microcosms experiments were conducted using field samples from MID- and POST-summer bloom periods collected from Sulejów reservoir (central Poland) representing development and late stage of the bloom respectively. During the experiment the field samples were exposed to different algicidal treatments: *Morganella morganii*, *Exiguobacterium acetylicum*, and a bacterial consortium composed by these two species with *Bacillus pumilus*.

Results showed that algicidal treatments, particularly with *M. morganii* decreased *Microcystis* abundance and changed the phytoplankton community structure, leading to pronounced proliferation of diatoms in the MID-summer microcosms. In contrast, in POST-summer microcosms representing more mature bloom periods did not show these effects. Moreover, varied intracellular microcystins concentrations were observed when different variants of algicidal bacteria were added to MID-summer microcosm. Intriguingly, the extracellular microcystins were not detected suggesting the controlling role of natural microbial communities in microcystin release. Moreover, molecular analysis demonstrated a decrease of the toxigenic genotypes in treatments with *M. morganii* and *E. acetylicum*.

Overall, this study shows great potential of algicidal bacteria in mitigation of cyanobacteria blooms and lake restoration by reducing the number of toxigenic genotypes and decreasing the contribution of harmful cyanobacteria in phytoplankton communities.

Keywords: algicidal bacteria, bloom stages, cyanobacteria bloom, *Microcystis*, *Morganella morganii*

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Application rate and temperature effects of copper- and peroxide-based algaecides on spring and fall microcystin-producing *Planktothrix* blooms

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Eutrophication of waterways has contributed to the proliferation of harmful algal blooms in aquatic ecosystems globally. Algaecides are a proven treatment strategy for bloom control; however, their efficacy can vary based on temperature, species, and application rate. A spring and fall experiment were conducted using site-collected water with associated blooms to assess efficacy of five algaecides. *Planktothrix*-dominated blooms were collected from Grand Lake St. Marys in Ohio, USA, and incubated at collection temperature as well as $\pm 5^\circ\text{C}$. Chlorophyll concentrations, cell abundance, and total and extracellular microcystins were quantified up to 72 and 96 hours after treatment (HAT) to assess the efficacy of treatment. In experiment one, dominated by *Planktothrix rubescens*, GreenClean® Liquid had the greatest decrease in chlorophyll, with up to 76% reduction at 10°C and medium application rate. Concurrently, total microcystins were reduced up to 66% from the control but showed a complete shift to extracellular form. In experiment two, dominated by *Planktothrix agardhii*, GreenClean Liquid showed the greatest reduction in chlorophyll at 20°C and medium application rate, with up to a 90% decrease compared to the control. Under the same variables, total microcystins were reduced up to 68% with a complete shift to extracellular form. These experiments showed that some algaecides were more effective at lower temperatures while others performed better at higher temperatures. For the cases listed above, SeClear and GreenClean® Liquid application rates demonstrated the greatest decrease in algal biomass at medium dose. These findings highlight the importance of considering variables such as species present, temperature, and application rate as these factors may impact algaecide efficacy and microcystin dynamics. It is crucial to understand these variables when choosing algaecide application strategies to mitigate ecological impacts.

Keywords: harmful algal bloom, cyanobacteria, cyanotoxin, treatment, management

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Hot summers raise public awareness of toxic cyanobacterial blooms

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Global warming is expected to increase the occurrence, magnitude, and duration of harmful cyanobacterial blooms worldwide. At the same time, societal demands for clean surface water are increasing with hotter and drier summers. Yet, we currently lack a global view of the impacts of warming on how cyanobacterial blooms are perceived by the public. We therefore analysed long-term data from online search engines on harmful cyanobacteria and compared these with shifts in climate change patterns¹. Our findings, covering a period of up to 20 years, reveal a globally-consistent annual pattern in online searches for harmful cyanobacteria. Interestingly, searches increased with hotter and drier summers in temperate and tropical regions, respectively. Moreover, the number of online searches, media attention and the incidence of cyanobacterial blooms in the Netherlands increased with temperature, where increases in online searches were strongest. Overall, our findings suggest that the public will become more aware of the potential problems caused by harmful cyanobacterial blooms as summers become warmer. In the future, online search patterns for harmful cyanobacteria may help to highlight regions or seasons with deteriorating water quality, especially in areas that are not monitored closely, and may thereby act as part of an early warning system for management action¹.

Keywords: CyanoHABs, Online searches, Societal impact, Global warming, Climate change

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Cyanotoxins pool size in cyanobacteria exposed to sub-lethal antibiotics concentrations

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The presence of antibiotics in freshwater ecosystems represent a problem for the environment and a health issue since sub-lethal concentrations can contribute to the diffusion of antimicrobial resistance (1,2). Among natural microorganisms species, cyanobacteria play a fundamental role as important primary producer and are considered among the susceptible species, so that can be used to determine the PNEC of antibiotics in the environment (3). Since many cyanobacteria produce cyanotoxins, some studies investigated the effect of antibiotics on cyanotoxins production, showing that it can be stimulated after exposures to sub-lethal concentrations (4,5). The main objective of this study is to determine the effect of sub-lethal antibiotic concentrations on cyanotoxins pool of monoclonal cyanobacterial strains.

After determination of the minimum inhibitory concentration over a range of environmentally relevant concentrations of antibiotics, three strains of *Microcystis aeruginosa*, one axenic, have been exposed in controlled conditions to two concentrations of erythromicine, amoxicilline and ceftazidime, epidemiologically and environmentally important antibiotics. After 14 days of exposure, total and extracellular microcystins have been measured.

Results showed a high intraspecific variability. Erythromicine has a stronger effect on PCC7806 *M. aeruginosa* than amoxicillin, and it changed the ratio between the two MC variants produced (MC-LR and [D-Asp3] MC-RR). In the erythromicine condition the intracellular content of the variants increased at the end of the experiment, even if density was reduced, suggesting the possible selection of more toxic strains in natural conditions. For strain 1450/6 amoxicillin was the most effective antibiotic in reducing density and MCs, while erythromicine and ceftazidime had no effects on density, ma slightly increased the MCs concentrations, and 1450/16 didn't show any variation between control and experimental conditions. The results highlight the important effect of antibiotics in the environment on non-target organisms like cyanobacteria, and seem to have a role in increasing the risk of exposure to cyanotoxins.

Keywords: cyanobacteria, cyanotoxins, antibiotic, microcystins

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Integrating cyanotoxin detection using LC-MS Triple Quadrupole with cell viability assessment

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Harmful algal blooms are commonly linked to the production of cyanotoxins, which are secondary metabolites produced by cyanobacteria. This study presents a sensitive and accurate method for the quantification of 12 cyanobacterial toxins (microcystins, cylindrospermopsin, anatoxin-a, and nodularin) using LC-MS Triple Quadrupole. Solid-phase extraction was performed to optimise the detection of the 12 toxins in water samples with recovery efficiencies ranging from 70-100% for the majority of toxins at pH 9. The limit of method quantification limit (MQL) for the toxins was $< 10 \text{ ng L}^{-1}$ which is sensitive enough to quantify low levels of cyanotoxins in typical water matrices. Three different species of laboratory-grown cyanobacterial strains were examined in this study, namely *Microcystis aeruginosa* (MA), *Raphidiopsis raciborskii* (RR), and *Raphidiopsis mediterranea* (RM). Cell viability was performed using a staining method coupled with flow cytometry for unicellular MA and fluorescence microscopy for the filamentous RR and RM. In MA, with ~84% viable cells ($1 \times 10^7 \text{ cells mL}^{-1}$), cyanotoxin concentrations ranged from $0.5\text{-}350 \text{ } \mu\text{g L}^{-1}$. RR and RM, with 60-70% viability ($1 \times 10^3 \text{ cells mL}^{-1}$), produced up to $200 \text{ } \mu\text{g L}^{-1}$ of cyanotoxins. Integrating cyanotoxin quantification with cell viability data enables a more comprehensive assessment of cyanotoxin risk. The integrated approach will provide valuable guidance for water treatment managers in assessing the risk of the presence of toxins post-treatment including understanding discrepancies between cell counts and toxin concentration.

Keywords: Harmful algal bloom, LC-MS, Cyanotoxins, Microcystins, Flow cytometer



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Real-time observation of modified microcystin synthesis in *Microcystis aeruginosa* and modified anabaenopeptin synthesis in *Planktothrix agardhii*

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Microcystis aeruginosa and *Planktothrix agardhii* produce microcystins (MC) and anabaenopeptins (AP) via non-ribosomal peptide synthesis (NRPS). Natural genetic variation in certain enzymatic domains reduces amino acid activation specificity, enabling the incorporation of non-natural amino acids (non-AA) into modified MCs or APs. In consequence, enabling their labeling through click chemistry reactions and spatial analysis using high resolution confocal microscopy. Here, we grew *M. aeruginosa* and *P. agardhii* under maximum growth rate conditions in the presence of non-AAs to observe timing and localization of MC/AP during modified peptide build-up (0-168 h), and decline (96 h for *M. aeruginosa*, 192 h for *P. agardhii*) after pulsed (48 h) non-AA incubation. Our results show an early heterogeneous generation of labeling signal in distinct entities, reaching saturation within 12 h for *M. aeruginosa* and 24 h for *P. agardhii*, and a steep decrease within 24–48 h. By applying specialized imaging software including machine learning, we modeled the presence of labeling signal in these entities, allowing their quantification and localization at an individual level. The modeled labeling signal represented a significant fraction of the total cellular signal intensity. For *P. agardhii*, modeled intensities constituted up to 23.3% of the total cellular intensity in 2.4% of the cell volume. While intensities in these modeled entities increased or decreased sharply during time-lapse experiments, their volumes and intracellular distribution remained comparatively stable. This relative stability of the modeled entities would support the hypothesis that they represent locations of intracellular peptide synthesis, rather than compartments for MC or AP storage. These modeled synthesis complexes show a homogeneous intracellular distribution for *M. aeruginosa*, while in *P. agardhii*, they localize near the septa between cells in the trichome. Thus, differences in the cellular localization of the NRPS might exist between unicellular *Microcystis* and multicellular *Planktothrix*.

Keywords: Non-ribosomal peptide synthesis, Click-chemistry labeling, Confocal-microscopy, Cyanotoxins, Machine Learning, Modeling, Subcellular localization

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Bioprospecting cholinergic ligands from a marine cyanobacteria of the genus *Oscillatoria*

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Anthropogenic impact on the reef ecosystems in Raivavae Island (French Polynesia) has been linked to the proliferation of benthic marine *Oscillatoria* and subsequently to human health problems following consumption of the giant clams of the genus *Tridacna* from affected areas.¹ Methanolic extracts of *Oscillatoria* cf. *bonnemaisonii*, which were collected from the reef areas surrounding the Raivavae Island, were able to block nerve-evoked muscle twitch and synaptic transmission in frog isolated neuromuscular preparations. The blockade of the amplitude of miniature-endplate potentials pointed the muscle nicotinic acetylcholine receptor (nAChR) as a potential target. Microplate receptor-binding assay, using *Torpedo marmorata* electrocyte membranes rich in nAChR of muscle type and biotinylated α -bungarotoxin as toxin tracer,² was used for the bio-guided purification of the marine cyanobacterial nicotinic ligands by preparative HPLC. High resolution mass spectrometry (HRMS) analyses of the active fraction showed a series of structure-related lipophilic compounds with overlapping elution times. NMR analyses confirmed the complexity of the mix. However, the exact masses and the fragmentation patterns obtained by HRMS allowed the identification of the bioactive molecules. Two-electrodes voltage clamp electrophysiology recordings on *Xenopus laevis* oocytes having incorporated muscle nAChR from *T. marmorata* confirmed the blocking activity of these molecules. This study resumes the state-of-the-art methodologies for the discovery of bioactive molecules and their structural and functional characterization. Marine cyanobacteria are a valuable source of novel bioactive molecules.

Keywords: Marine cyanobacteria *Oscillatoria*; nicotinic acetylcholine receptor (nAChR); bioactivity based molecular networking; toxin discovery; two-electrodes voltage clamp.

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Microcystis sp. AE03 strain in Dal Lake harbors cylindrospermopsin and microcystin synthetase gene cluster

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Cyanobacterial harmful algal blooms (CHABs) are increasing at an alarming rate in different water bodies worldwide. In India, CHAB events in water bodies such as Dal Lake have been sporadically reported with no study done to characterize the cyanobacterial species and their associated toxins. We hypothesized that this Lake is contaminated with toxic cyanobacterial species with the possibility of the presence of cyanotoxin biosynthetic genes. We, therefore, used some of the molecular tools such as 16S ribosomal DNA, PCR, and phylogenetic analysis to explore cyanobacterial species and their associated toxins. A 3-year (2018–2020) survey was conducted at three different sampling sites of Dal Lake namely, Grand Palace Gath (S1), Nigeen basin (S2), and Gagribal basin (S3). Two strains of *Dolichospermum* sp. AE01 and AE02 (S3 and S1 site) and one strain of *Microcystis* sp. AE03 (S2 site) was isolated, cultured, and characterized phylogenetically by 16S ribosomal DNA sequencing. The presence of cyanotoxin genes from the isolates was evaluated by PCR of microcystins (*mcyB*), anatoxins (*anaC*), and cylindrospermopsins (*pks*) biosynthesis genes. Results revealed the presence of both *mcyB* and *pks* gene in *Microcystis* sp. AE03, and only *anaC* gene in *Dolichospermum* sp. AE02 strain. However, *Dolichospermum* sp. AE01 strain was not found to harbor any such genes. Our findings, for the first time, reported the coexistence of *pks* and *mcyB* in a *Microcystis* AE03 strain. This study has opened a new door to further characterize the unexplored cyanobacterial species, their associated cyanotoxin biosynthetic genes, and the intervention of high-end proteomic techniques to characterize the cyanotoxins.

Keywords: Dal Lake, *Dolichospermum* sp. strain AE01, *Dolichospermum* sp. strain AE02, *Microcystis* sp. strain AE03, anatoxin-a, microcystin, cylindrospermopsin

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Portfolio of cyanobacterial secondary metabolites and their biotransformation potential in surface waters

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Cyanobacterial blooms are increasing globally and posing threats to aquatic ecosystems and human health, especially by releasing toxic metabolite[1]. Besides few widely studied microcystin, a legion of other co-produced bioactive secondary metabolites has been identified over the past decades[2,3]. However, we lack data on their fate process in surface waters, which adds difficulty in further risk assessment. First, we tracked an extensive portfolio of cyanotoxins in a five-year lake survey and identified indicator metabolites of cyanobacterial taxa[4]. Then we investigated the biotransformation potential of cyanobacterial secondary metabolites including microcystins, anabaenopeptins, cyanopeptolins and microginins with suspect screening and high resolution tandem mass spectrometry. Tests were performed with different in-situ grown freshwater biofilm communities and cyanobacterial metabolites extracted from laboratory cultures. The transformation kinetics were generally fast for cyanopeptolins and anabaenopeptins, while microcystins showed clear structure reactivity dependence. Only microcystins with the Adda moiety connecting to arginine were transformed, while others remained stable. Transformation kinetics increased and initial lag phase of transformation decreased with increasing the density of degrader community biomass and with lowering the initial concentration of cyanobacterial metabolites, suggesting potential inhibitory effect on the degraders. We identified several transformation products using in-silico prediction tools[5] for biotic reaction pathways acting on the amino acid bonds and side chains. Products were identified by annotating high quality MS2 spectra and comparison to parent ions. For example, biotransformation was evident for deamination of arginine and oxidation of tyrosine in microcystins and anabaenopeptins, and hydrolysis of the C-terminal amino acid peptide bond of anabaenopeptins, that have not been reported before. Depending on the degrading community, these transformation products are expected to be formed in surface waters when blooms subside and may be additional indicators for cyanobacteria and their risk assessment.

Keywords: Cyanobacterial toxins, LC-HRMS, persistency, transformation products, transformation pathways

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Evading toxins: microcystin persists at low temperature and light

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It is commonly thought that green, eutrophic lakes in the heat of summer are the most susceptible to cyanobacterial blooms, which actively produce cyanotoxins such as microcystin. In the winter and shoulder seasons, however, often a disconnect between toxin concentrations and the presence of toxin-producing cyanobacteria can be seen. In order to test for often high levels of cyanobacteria and their toxins in the non-productive season, we designed an experiment in the LakeLab (<https://www.igb-berlin.de/en/infrastructure/lakelab>) to elucidate the source, persistence, and degradation of microcystin (MC-RR). The objective was to determine the influence of light and temperature on MC-RR degradation rates and explore potential sources of toxins (e.g., cyanobacteria cells, water, sediment). Our experimental design consisted of four treatments applied to sediment cores: reference, spiked with MC-RR, spiked with live cyanobacteria *Planktothrix rubescence*, and water only. Duplicate treatments were incubated in the epilimnion under high light and temperature (24 hr mean: 22 °C, 1000 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) and the hypolimnion under low light and temperature (10 °C, 1 $\mu\text{mol m}^{-2} \text{sec}^{-1}$). Daily MC-RR samples were collected from the water overlying sediment cores for 20 days. MC-RR degradation rates ($\mu\text{g L}^{-1} \text{day}^{-1}$) were twice as fast in the epilimnion than the hypolimnion. The source of toxins was predominately the sediments, which had significantly higher MC-RR than the water, although it was particle-associated and not dissolved. After 20 days, high (4 $\mu\text{g L}^{-1}$) MC-RR was still detected in the spiked MC-RR sediment cores incubated in the hypolimnion, indicating that this toxin persists for at least 20 days. Our results suggest that MC-RR could be detectable in lakes much longer than blooms, with implications for conditions of low light and temperature; e.g., in hypolimnions and non-summer seasons.

Keywords: microcystin, MC-RR, *Planktothrix*, degradation rates, IGB LakeLab

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F8 -14

First study evaluating toxic effects of guanitoxin and synthetic insecticide combination on fish

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Guanitoxin (GNT) is the only naturally occurring organophosphate documented, exhibiting action potential analogous to synthetic insecticides. However, data on its toxicity in aquatic ecosystems, particularly when combined with synthetic organophosphate pesticides, remain limited. This study aimed to evaluate the toxicity of GNT extracts derived from the cyanobacterium *Sphaerospermopsis torques-reginae* in combination with the insecticides malathion (MLT) and trichlorfon (TCF) in *Oreochromis niloticus*. Fish were exposed for 96 hours to seven treatments: control, GNT extract (250 mg/L; T1), MLT (1 mg/L; T2), TCF (0.5 mg/L; T3), GNT + MLT (T4), GNT + TCF (T5), and GNT + MLT + TCF (T6). Various biological parameters were analyzed, including neurotoxicity, genotoxicity, DNA damage, and oxidative stress, and an integrated biomarker response index (IBRv2) was applied. The results demonstrated substantial acetylcholinesterase inhibition, reaching 77.2% (T1), 86.6% (T4), and 86.9% (T6) in muscle tissue and 39.0% (T4) and 42.2% (T6) in brain tissue. DNA strand breaks in the gills were approximately 7000 times higher in treatments T3, T4, and T5 emerging as one of the most toxic forms of DNA damage. Catalase activity increased in the gills (T5 and T6), while glutathione peroxidase activity increased in the liver (T3 and T4). Both enzymes serve as key components of the body's defense system, directly eliminating reactive oxygen species during respiratory metabolism. Lipid peroxidation was observed in the gills (T5), indicating disruption of gill lipid membranes. Additionally, glutathione S-transferase activity increased in the liver (T4) and gills (T5), with elevated glutathione levels in the gills (T5 and T6), suggesting an upregulated biotransformation system to detoxify chemicals. Finally, based on the IBRv2 analysis, treatments T5, T6, and T4, which represent different combinations of organophosphates, emerged as the most hazardous. These findings underscore the extensive adverse effects of these treatments, revealing complex environmental-biological interactions and highlighting significant environmental risks.

Keywords: DNA damage, organophosphates, oxidative stress, neurotoxicity, pesticides

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Crustacean zooplankton feeding on cyanobacteria and potentially toxic *Microcystis*

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Grazing by zooplankton can regulate the bloom-forming cyanobacteria but also transfer toxin-producing cells and toxic metabolites to the food web. While laboratory investigations have provided ample knowledge on zooplankton and toxic cyanobacteria interactions, information on zooplankton feeding on toxin-producing cyanobacteria in natural water bodies remains scarce. We used phytoplankton marker pigments and quantified *Microcystis*-specific *mcyE* synthase genes from the gut contents of various cladoceran and copepod taxa to assess *in situ* crustacean zooplankton feeding on cyanobacteria and potentially toxic *Microcystis* in two large shallow lakes in Estonia, Northern Europe. Our analysis showed that the crustacean taxa have markedly different algal diets, with cyanobacteria forming a significant and preferred component of the diet for some cladocerans. Toxigenic *Microcystis* was also widely ingested among the crustacean zooplankton community, with some species being more efficient at collecting toxic cells from the environment than others. The results suggest that crustacean ingestion of toxigenic *Microcystis* is not related to *Microcystis* biomass or *mcyE* gene copy numbers in the environment, but rather to the abundance and biomass of efficient grazers.

Keywords: Microcystis; grazing, crustacean zooplankton; food web; *mcyE* gene

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Plant Growth Promoting Rhizobacteria-Mediated Bioremediation for Alleviating Phytotoxicity in *Vicia faba* Exposed to Cyanobacterial Crude Extract Containing Microcystins: Human Health Risk Assessment

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Cyanobacterial blooms have been increasing in freshwater ecosystems worldwide over the last century, mostly due to eutrophication and climate change (Lakshmikandan et al., 2024). These blooms can produce a wide variety of toxic secondary metabolites, i.e., cyanotoxins, with microcystins (MCs) being the most studied due to their global distribution, ubiquity, and toxicity (Zhu et al., 2018; Wan et al., 2020). Repeated exposure to these toxins can inhibit crop growth and development, contaminate soil, which raises concerns about health risks and food safety for humans (Weralupitiya et al., 2022). The specific aim of this work was to investigate the potential role of plant growth-promoting rhizobacteria as a bioremediation tool to protect soil-plant systems from MCs and mitigate toxin-related health risks. To this purpose, *Vicia faba* plants were co-inoculated in sterile soil with MC-resistant rhizobia and rhizobacteria exhibiting growth-promoting traits in a greenhouse pot trial. The plants were exposed to MC-contaminated water at a concentration of 200 µg.L⁻¹ of MCs for one month. Growth and physiological indicators, as well as nitrogen metabolism, were assessed to investigate the phytotoxic impact of these compounds on faba bean plants in the presence and absence of MC-resistant bacterial strains. Results revealed that exposure of non-inoculated plants to these toxins led to decreased growth, impaired photosynthetic efficiency, and reduced nitrogen content in both shoot and root tissues, along with decreased activity of the nitrogen assimilatory enzyme glutamine synthetase (GS) in the leaf tissues. Conversely, inoculation with toxin-tolerant bacteria significantly improved these parameters in MC-exposed plants. The estimated daily intake values of MCs for all treatments remained below the recommended tolerable daily intake threshold of 0.04 µg.kg⁻¹ of body weight per day, as established by the World Health Organization. These values indicate a moderate-risk level, as evaluated through Risk Quotient values.

Keywords: microcystins, bioremediation, plant growth-promoting rhizobacteria, *Vicia faba*, health risk.

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Unveiling the interaction of highly toxic microcystin analogues in contact with virgin, artificially and naturally aged microplastics

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Water quality is a global environmental concern, and microplastics are contributing to the issue. It is becoming increasingly evident that microplastics can act as a vector for micropollutants, including cyanotoxins like microcystins, when co-existing in the environment [1]. This study evaluated the interaction of a mixture of three microcystins (MC-LR, -LW, and -LF, 1 µg/mL each) in contact with polypropylene, polyethylene, polyethylene terephthalate (PET), polyamide, polystyrene, and polyvinyl chloride microparticles (D50 median size < 45 µm, 2 g/L plastics). The microparticles were acquired commercially and artificially aged. Further, MC-LF was placed in contact with virgin PET (D50 81 µm) and naturally weathered PET microparticles (D50 144 µm). Microplastics were characterised in detail to ensure a reliable data interpretation. The type, weathering of the microplastics, and microcystin hydrophobicity were key factors for adsorption. Polypropylene showed the greatest adsorption of microcystin, adsorbing greater amounts of all analogues in the mixture containing three (80-100% adsorption) analogues. For virgin and aged particles, the more hydrophobic and more toxic analogues showed greater adsorption to all microplastics when compared to hydrophilic analogues (MC-LW > MC-LF > MC-LR). However, the findings suggest caution when claiming that aged microplastics adsorb more micropollutants when co-existing. Three out of the six microplastics investigated (polypropylene, polyethylene, and polystyrene) showed a decrease on all analogues adsorbed on aged microparticles. For PET, naturally aged microparticles showed the greatest adsorption of MC-LF among its weathering forms investigated. In a mixture, MC-LF did not adsorb onto virgin PET, while 8% was adsorbed onto artificially aged PET. MC-LF adsorbed 30% on naturally aged PET compared to virgin PET (11%) when individually placed in contact with microplastics. The findings indicate that highly toxic hydrophobic microcystin analogues have a higher potential to be transported into the food chain by microplastics, especially after the plastic has undergone environmental weathering.

Keywords: Adsorption, Freshwater, Cyanobacteria, Polymer, Weathering

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Extracts of the lichen *Pseudevernia furfuracea* (L.) Zopf as a source of novel anticyanobacterial agents

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Harmful cyanobacterial blooms pose significant risks to human and animal health in freshwater ecosystems. Developing effective and sustainable remediation strategies is critical to address this challenge. Lichens, unique symbiotic organisms with specialized metabolism, remain largely unexplored for their anticyanobacterial properties. In this study, we evaluated, for the first time, the anticyanobacterial potential of *Pseudevernia furfuracea* (L.) Zopf extracts from two Moroccan locations against the toxinogenic cyanobacteria *Microcystis aeruginosa* Kützings. The chemical composition of the extracts was analyzed via GC-MS, revealing phenolic cyclic compounds, primarily atraric acid and chloroatranol [1]. Antimicrobial activity was assessed using disc diffusion and broth microdilution assays, with inhibition diameters of 12.56 ± 0.82 mm and 13.66 ± 0.28 mm for extracts 1 and 2, respectively. Both extracts exhibited a minimum inhibitory and bactericidal concentration of 375 µg/ml. Growth monitoring in 100 ml of Z8 medium demonstrated a significant reduction in growth rate and cell count in treated cultures compared to controls. These findings highlight the potential of lichen-derived compounds as novel agents for mitigating harmful cyanobacterial blooms.

Keywords: *Pseudevernia furfuracea*, *Microcystis aeruginosa*, Disc diffusion assay, Broth microdilution assay, Growth monitoring

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F13 - 22

Investigation into the mode of action of aetokthonotoxin

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Aetokthonotoxin (AETX), a cyanotoxin produced by the freshwater cyanobacterium *Aetokthonos hydrillicola*, was found to be the causative agent of vacuolar myelinopathy (VM). VM is a fatal neurological disease affecting wildlife in the United States. It is transferred through the food chain to birds of prey from snails and water fowl feeding on the plant the cyanobacterium grows on.¹ The mode of action of AETX is unknown. Here, we show that AETX disrupts the coupling between the electron transport chain and ATP synthesis in mitochondria due to its physico-chemical properties. We found that AETX enhanced the oxygen consumption rate of mitochondria in mammalian cells while decreasing mitochondrial membrane potential and oxidative ATP production rate. Furthermore, we could show that AETX is able to shuttle protons across an artificial lipid bilayer membrane. As expected for the protonophore activity, the presence of the slightly acidic indole proton is essential for AETX toxicity. In summary, our results demonstrate that the mode of action of AETX is the uncoupling of oxidative phosphorylation. We anticipate our results to be a starting point for the elucidation of the adverse outcome pathway of VM.

Keywords: aetokthonotoxin, uncoupling, protonophore

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F14 -16

Prevalence of Toxic *Microcoleus* and Anatoxins in Shubenacadie-Grand Lake, NS, Canada

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In the spring of 2021, two dogs died after ingesting suspected benthic cyanobacterial mats along the shoreline of Shubenacadie-Grand Lake in Nova Scotia, Canada. Necropsy results identified anatoxins as the likely cause of death. Shubenacadie-Grand Lake is an oligotrophic lake (Surface Area: 1841 Ha, Max Depth: 45 m) that receives inputs from many streams and smaller lakes. The lake system is heavily used for recreational activities, fishing, and drinking water, and is a highly valued ecosystem. An initial study¹ focused on the prevalence, genetic characteristics, and toxicity of benthic cyanobacterial mats in a small stream and the adjacent lakeshore in the immediate area where the dogs ingested the toxic material. Toxin-producing benthic cyanobacteria, specifically *Microcoleus*, was found to be proliferating both within the stream and the lakeshore environment where the stream entered the lake. In this presentation, initial findings will be reviewed along with the results from the follow up study conducted in 2023 and 2024 which involved 18 additional sampling locations, focused on tributary outlets and popular recreational areas such as the Oakfield Provincial Park, to assess how widely distributed *Microcoleus* is throughout the lake system. Results from the 2023-2024 sampling program revealed that toxin-producing *Microcoleus* is growing at several locations throughout the lake, not just within the stream and lakeshore area where the dog deaths occurred. These findings add to a growing body of evidence demonstrating the wide distribution of *Microcoleus* within Atlantic Canadian surface waters

Keywords: benthic, anatoxin, microcoleus, oligotrophic, genetics

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First reports of nodularin production by *Nostochopsis* spp. from New York State, USA and New South Wales, Australia

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Benthic cyanobacteria are far understudied in comparison to their planktonic counterparts, yet they can also produce harmful cyanotoxins. As more attention is given to monitoring these populations, we learn more about their ecology, where we might expect them to grow, and what risks they may pose through cyanotoxin production. Globally, most of our data come from flowing systems, despite our knowledge that benthic cyanobacteria also thrive in lakes. Here, we share a comparative case study of a novel toxin producer, found in two contrasting environments – one in a small, eutrophic lake in New York State (USA), and one in a low nitrogen concentration creek in northeastern New South Wales (Australia). In 2023, dense proliferations of toxic *Nostochopsis* spp. were found at both sites. Nodularin-R was confirmed in both populations via LC-MS/MS. We applied a polyphasic approach to confirm toxigenic potential in the population from New York State and drafted the first complete genome to exist for this genus of cyanobacteria. Genome sequencing revealed the presence of the complete nodularin synthetase gene cluster in the sample from New York State, which directly matched the organization of the nodularin cassette first described in *Nodularia spumigena*¹. The population from New South Wales was identified microscopically as *Nostochopsis*, defined by its uniseriate trichomes with distinct true branching of filaments and short, lateral branches terminated by apical heterocytes². We intend to fully characterize the population found in New South Wales using the same genomic methods pending collection of more biomass. These are the first reports of any cyanotoxin production by *Nostochopsis*, a genus of benthic cyanobacteria that is reportedly used in food and consumer products in southeast Asia³ and may have important implications for public health worldwide.

Keywords: benthic cyanobacteria, nodularin, hepatotoxin, genomics

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Insights into cyanometabolite profiles in Lake Sapanca through seasons and years

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Cyanobacteria are considered the primary photosynthetic microorganisms belonging to the phytoplankton. Reported both in marine and freshwater ecosystems, cyanobacteria are typically found in drinking water reservoirs and natural lakes [1, 2]. Biodiversity is influenced by many natural and anthropogenic factors, including increasing of water temperatures, variability of pH, and level of nutrients. These conditions also favour their proliferation, producing blooms in water [3]. Cyanobacterial blooms may impact public health by reducing the efficacy of water treatment and disinfection processes or by decreasing user acceptance if they produce taste and odour compounds [4]. Moreover, cyanobacteria produce many bioactive substances, known as cyanometabolites, and certain species are also able to produce cyanotoxins, posing risks to human and animal health [5]. In Lake Sapanca, the most significant freshwater resource of the Sakarya province of Turkey, toxic *Planktothrix rubescens* blooms have been observed since the 1990s, requiring continuous monitoring studies [6]. In this frame, a study aimed at characterizing the metabolomic profile of the cyanobacterial community in Lake Sapanca through seasons and years is described. Metabolomic studies using LC-HRMS analyses enabled the detection of known cyanometabolites, and, through HRMS², the structural characterization of new compounds was achieved. Samples were collected from the three observed layers of the lake: epilimnion (surface), thermocline (10 to 18 m) and hypolimnion (after 18 m) in summer and winter from 2001 to 2022 period and differences in oligopeptide profiles are highlighted. The main microcystin variant was [D-Asp³]MC-RR, detected at the highest concentrations in the thermocline where the drinking water was withdrawn. As for cyanometabolites, e.g. Anabaenopeptin B and Oscillamide Y, an increased production was observed in winter season, with higher concentration been detected in hypolimnion. The results allowed to assess the degree of contamination of the site and to estimate the associated risks to human health and the environment.

Keywords: Cyanobacteria, LC-HRMS Cyanometabolites profiling, Drinking water, *Planktothrix rubescens*, Climate change

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Strain and environment affect toxin production by the toxic cyanobacteria, *Umezakia natans* (previously *Chrysosporum ovalisporum*)

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Managing human impacts of toxic cyanobacteria can be challenging when monitoring cell densities does not correlate well with toxin levels. Depending on the species, toxin levels may change with strain dominance and environmental conditions. This study examined the effect of both a range of environmental conditions and strain on the cylindrospermopsin (CYN) cell quotas of cultures of a nitrogen-fixing species, *Umezakia natans* (previously *Chrysosporum ovalisporum*) isolated from an urban, coastal subtropical lake. This lake is used by the public for recreation and is monitored to minimize the risk of CYN to the public. A key characteristic of this lake is the high salinity bottom waters, with surface waters being fresh. Blooms of *U. natans* began a few years ago as a result of sustained high nutrient loadings. The two strains varied in their total CYN cell quotas, with LHC02 strain having a substantially higher CYN cell quota, irrespective of the environmental conditions. Both strains were capable of growing at a wide range of salinities with CYN cell quotas being substantially higher in 10% than 70% seawater. The optimal growth rate for LHC01 was 32°C, while LHC02 grew equally well at 28 and 32°C. CYN cell quotas were highest for LHC01 at the lowest temperature tested, i.e. 18°C, and highest for LHC02 at 28°C. Both strains had a significant proportion of extracellular CYN in all treatments, and this occurred irrespective of growth phase. Overall, the experiments showed that there is a high level of variability in CYN cell quota responses between the two strains, and across the range of environmental conditions tested, and explains the poor correlation between cell densities and CYN levels. Our study points to the benefits of managing risks from blooms of toxic *U. natans* by measuring toxins rather than cell densities.

Keywords: strain, ecotype, *Chrysosporum ovalisporum*, temperature, salinity

Acknowledgements and Funding

CYN analysis was conducted by Queensland Health. This research was funded by the City of The Gold Coast.



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What if N and/or P loadings would not have been reduced? Decadal scale model simulations of MC concentrations in Lake Müggelsee

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Managing cyanobacterial harmful algae blooms (e.g. those of *Microcystis* and its toxin microcystin - MC) is an important environmental challenge. A common approach is to reduce nutrient inputs, mainly phosphorus or/and nitrogen. The success of a management action can be quantified based on phytoplankton biomass, for which observations from long term monitoring programs are often available covering the time from before to after the action, and considering long response time of lakes. However, observations of toxin concentrations are generally not available at these time scales¹. How do/did toxin concentration change with management actions? It is reasonable to assume that toxins follow the same trend as biomass, but there is evidence to the contrary due to varying MC cell quota and community composition^{1,2}. Here, models can be used in hindcast mode to reconstruct historic toxin concentrations, and they can be used to simulate the outcome of future management actions.

The shallow Lake Müggelsee in Northern Germany experienced a decline of nitrogen and phosphorus³ (~70% and ~50% less in 2014-2023 compared to 1980-1989), accompanied by a declining phytoplankton biomass (~80%). We applied an agent-based model² to Lake Müggelsee and simulated *Microcystis* biomass and toxin production (1996-2023). To calibrate the model, we used the weekly monitoring data, published MC concentrations⁴ and additional observations (i.e. MC concentration and toxigenic fraction) in 2022/2023. The model could reproduce the data of individual years as well as long-term averages. We then increased nutrient loads to represent historic conditions to estimate past MC concentrations and how phytoplankton would have developed when nitrogen and/or phosphorus would not have been reduced. Then we predicted the outcome of an expected future scenario, namely the 75% reduction in inflow due to the stop of coal mining in the watershed⁵.

Keywords: Microcystis, microcystin, long-term monitoring, modelling, nutrient reduction

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High-frequency monitoring of lentic water bodies with autonomous aquatic drones guided by AI techniques and dynamical models

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Robotized small catamarans (named aquatic drones here-after) equipped with vertically-movable multi-parametric probes are useful to obtain 3D information related to the environmental conditions and development of cyanobacteria blooms. Their autonomy behaviour, supported by different Artificial Intelligence (AI) path planning techniques and the simulations provided by dynamical models, can help us to increase or modify the frequency of different water-body monitoring tasks focused on cyanobacteria presence and abundance. To illustrate the benefits of this approach, we will present several of our AI-based path planning strategies. In particular, we will explain how the flexibility of Evolutionary Algorithms (EAs) allows us to optimize 3D monitoring trajectories that the drone (horizontally, at the water surface) and probe (vertically) can follow to efficiently monitor regions where it is expected (according to the simulations) to find high concentration of cyanobacteria, and how the learning capabilities of Gaussian Process (GPs) let us adapt the displacements of the drone to the information provided by several dynamical models (before the monitoring mission starts) and by the probe (during the development of the mission). During the presentation, we will also show the main characteristics of our in-house autonomous aquatic drones and how both AI techniques can use the information provided by the simulations of different dynamical models, including EEMS, SPH, COMSOL, and our own models. All in one, we will show how the combined background in cyanobacteria ecology, robotics, automation, simulation and AI-based path planning, is helping us to develop new tools for High-frequency monitoring of lentic water bodies.

Keywords: water quality, cyanobacteria, model-based simulation, path planning, unmanned vehicles

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Biotic interactions shape the realised niche of toxic cyanobacteria

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Cyanobacterial blooms pose a significant threat to freshwater ecosystems, driven by eutrophication and climate change. While traditional models aiming at these occurrences primarily focus on abiotic factors, our study highlights the critical role of biotic interactions in shaping cyanobacterial growth dynamics over temperature and phosphorus levels. Using five years of high-frequency data from a eutrophic lake, we employed state-space reconstruction techniques to investigate the complex interplay between cyanobacterial daily net growth rates and co-occurring plankton taxa. Results show that biotic interactions can change the magnitude and shape of cyanobacterial growth response to temperature and phosphate levels and that both zooplankton and phytoplankton interactions can significantly alter the temperature and phosphorus requirements for a positive net growth in all studied cyanobacteria. This can have significant impacts on our understanding and prediction of cyanobacterial blooming. Our findings suggest that incorporating biotic factors into bloom mechanistic or forecasting models is crucial for accurate understanding, predictions and effective management strategies.

Keywords: biotic interactions, realised niche, empirical dynamic modelling

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Identification of ecologically relevant microcystin degraders in Lake Zurich

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Rare bacteria that rapidly respond to substrate pulses have higher and more diversified functional potential than abundant oligotrophs. In Lake Zurich, the filamentous cyanobacterium *Planktothrix rubescens* will cause such seasonal maxima in available organic matter during the breakdown of their metalimnetic summer population due to abiotic stressors. The onset of autumnal thermal mixing entrains *P. rubescens* filaments in the turbulent upper water layers, leading to annual blooms, but also exposing them to higher shear forces and unfavourable irradiation levels. One compound released by disrupting cyanobacterial cells is the bioactive secondary metabolite microcystin (MC), a recalcitrant heptapeptide toxic to most eukaryotes, including humans. However, concentrations of MC in Lake Zurich are undetectably low, despite the size and seasonal fluctuations of the *P. rubescens* population, suggesting efficient microbial MC biotransformation. Applying a most-probable number approach, we found that the estimated concentrations of MC degraders in Lake Zurich were seasonally and spatially highly variable, ranging from <1% of the total bacterial community in depths mostly void of *P. rubescens* during lake stratification to up to 10% throughout the upper water column after autumn mixing. Over this latter period, we enriched the abundant fraction (>1%) of the lake community in batch cultures. In these experiments, lysed axenic *P. rubescens* filaments were replaced by pure MC as substrate to promote the formation of simplified MC degrading consortia. We performed flow cytometry and MC measurement for all cultures before conservation for subsequent sequence and bioinformatic analysis. The number of communities obtained reflected our MPN estimates, with highest growth success after onset of autumn mixing. Surprisingly, we detected MC removal for only every third community after 11 inoculation days. Planned work includes the long-read sequencing of 16S rRNA genes from selected enrichments by Oxford Nanopore MinION to identify those bacteria involved in or profiting from MC biotransformation.

Keywords: *Planktothrix rubescens*, microcystin, biotransformation, rare biosphere, Lake Zurich



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Algal-rithm: Automated Classification of Harmful Microalgae using Convolutional Neural Networks

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Harmful algal blooms (HABs) are a widespread issue that can cause economic disturbance and contaminate drinking water through the release of toxins, harmful secondary metabolites, and the formation of scum. Existing monitoring techniques are arduous and expensive, resulting in poor bloom characterization. HABs have become of increasing concern due to anthropogenic eutrophication increasing the frequency of bloom events. Convolutional neural networks (CNNs) have been successfully applied for automation in various fields. Applying CNNs to automate HAB characterization could help lower costs and shorten the decision-making process. A collection of a diverse, abundant dataset of key HAB species will be fundamental in realising automation. The dataset will concentrate on filamentous HAB species, as the variability in trichome length and cell number presents challenges for both classification and enumeration. A 600-image dataset of *Dolichospermum flos-aquae* CCAP 1403/13E and *Nodularia spumigena* PCC 73104 was collected. Six model architectures, three custom architectures (ThreeConv3x3, ThreeConv4x4 and TwoConv3x3) and three existing architectures (AlexNet, VGG16 and EfficientNet), two filters (Morphological, High Pass) and two optimizers (SGD, Adam) were trialled in combination to assess dataset collection methodology and outline initial classification accuracy. The combination AlexNet, Morphological and Adam achieved the highest average accuracy of 87.89% and a Matthews correlation coefficient of 0.76. The results highlight the potential for automated HAB characterization and validates the methodology used for dataset collection.

Keywords: Artificial Intelligence; Cyanobacteria; Image Classification; Harmful algal blooms

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Ultrasensitive Non-Competitive Microcystin-LR Assay Using a Two-Nanobody Sandwich Format with a positive readout

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The detection of microcystin-LR (MC-LR), the most prevalent and highly toxic variant of microcystins, is critical for rapid risk assessment of cyanobacterial blooms. Fast and straightforward methods like immunoassays are essential for this task, but due to the small size of this toxin existing test used competitive formats, which are counterintuitive and lack sensitivity. To overcome this limitation, we employed an innovative immunization strategy using the immunocomplex formed by MC-LR with a high-affinity nanobody that assures the stability of the complex. Through subtractive phage display selection, we isolated a novel nanobody that specifically recognizes this immunocomplex, enabling the ultrasensitive detection of MC-LR at concentrations as low as tens of ng/L. The assay's specificity was validated using an extensive panel of MC-LR congeners. This non-competitive format generates a positive signal, simplifying interpretation and facilitating its application in various platforms. We explored mix-and-read and lateral flow configurations, demonstrating the assay's potential for point-of-care use.

Keywords: Nanobody, Risk assessment, microcystin LR

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Characterization of Cyanobacteria and Cyanotoxins in a National Park in Nova Scotia, Canada

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There are over 46 freshwater lakes in one of Nova Scotia's (Canada) most renowned national parks, known as Kejimikujik National Park (KNP) and National Historic Site. During the summer months many people enjoy the recreational activities offered by KNP, which include a multitude of hiking trails, campgrounds, and canoeing routes. The recreational activities in the park are centred around the lakes and interconnected rivers, which also support a diverse assemblage of aquatic organisms. The lakes in the park are remote and primarily undisturbed, however cyanobacteria blooms have still been observed. There is a general lack of understanding as to what drives cyanobacteria dynamics in minimally disturbed lakes. Additionally, oligotrophic lakes in Canada have been experiencing an increase in reported bloom sightings. There are many environmental factors which might contribute to the proliferation of cyanobacteria in KNP, including water temperature, mixing regimes, flushing rates, nutrient abundance, and light availability. This study aims to assess the factors driving the presence of cyanobacteria and cyanotoxins in KNP. Preliminary MiSeq 16S sequencing results indicate that cyanobacteria are present in small abundances (<5% relative abundance) in some of the lakes in the park, with visual reports of planktonic and benthic cyanobacteria blooms. Potentially toxin-producing cyanobacteria of the order Nostocales have been identified in several lakes. Increased temporal and spatial sampling have been employed on lakes identified as possessing Nostocales. Additionally, solid phase adsorption toxin trackers (SPATT) were deployed in several locations across the park. Cyanobacteria were primarily present in clear, oligotrophic lakes, possessing total phosphorus (TP) concentrations between 4 to 10 µg/L, and total organic carbon (TOC) concentrations between 2 to 5 mg/L. The findings from this study will not only lead to a better understanding of the presence of toxin-producing cyanobacteria in KNP, but also in other remote, understudied lakes throughout Atlantic Canada.

Keywords: Cyanobacteria, Cyanotoxins, Freshwater, Oligotrophic, Nostocales

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The Development of QPCR Assay for the Detection of Anatoxin and Guanitoxin Producing Cyanobacteria

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Timely and accurate identification and reporting of Harmful Algal Blooms is critical for managing their threats. The current Phytoxigene Quantitative PCR (qPCR) assay has been in use to for screen for potential risk of the toxins Microcystin/Nodularin, Cylindrospermopsin and Saxitoxin, plus total cyanobacteria by the 16SrRNA gene since 2016. Testing with toxin analysis has previously demonstrate that there is a very high correlation between toxin gene detection and toxin production. The results from these studies indicate that this method can predict HAB risk and simplify the process of toxin analysis. Anatoxin- is a secondary, bicyclic amine alkaloid and cyanotoxin with acute neurotoxicity and can cause death in all mammals, with dog deaths attracting the most attention. The toxin is produced by multiple cyano-bacteria genera throughout the world, most commonly by benthic organisms including Microcoleus, Anabaena, Oscillatoria and Aphanizomenon. Guanitoxin, formerly known as Anatoxin-a(s), is a neurotoxin most commonly produced by the benthic cyanobacteria. A lesser understood toxin as it is more labile, genetic screening for a gene responsible for its's production will provide a better understanding of its scope and subsequent risk. Phytoxigene has developed a multiplex qPCR assay that will detect the presence of Anatoxin and Guanitoxin producing genes required for Anatoxin and Guanitoxin synthesis, along with an Internal Amplification Control to check for environmental inhibition. The process and challenges to develop the assay will be outlined.

Keywords: Anatoxin, Guanitoxin, PCR, monitoring



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Introducing BCCM/ULC: A public culture collection for ex situ conservation of cyanobacterial diversity and support of taxonomic and genomic studies

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Cyanobacteria are a phylum of morphologically diverse photosynthetic bacteria. Their long and complex evolutionary history has contributed to the successful colonization of a wide range of habitats from polar to temperate and tropical regions.

The BCCM/ULC is a young public collection, currently hosting more than 500 cyanobacterial strains, of which approximately 140 derive from polar, subpolar, and alpine environments, followed by tropical, subtropical and mediterranean biotopes as well as strains of Belgian origin. An ISO certificate covers the public deposition and distribution of strains, as part of a multi-site certification for the BCCM consortium.

All strains are studied by applying a polyphasic approach workflow (i.e., morphological, molecular, and ecological data). Furthermore, the collection includes more than 20 strains that are the reference (or 'type') for newly described taxa including *Plectolyngbya*, *Shackletoniella*, *Timaviella*, *Parakomarekiella*, *Petrachloros*, *Leptochromothrix*, *Vermifilum*, *Tigrinifilum*, *Affixifilum*, *Sirenicapillaria*, *Ophiophycus* and *Floridanema*. Recently, a set of taxonomically interesting strains of rock-inhabiting cyanobacteria from Finland as well as strains from freshwater, marine and terrestrial habitats from Florida, USA (Berthold–Laughinghouse Culture Collection) have been deposited into the collection.

Additionally, an integrated approach that combines whole-genome sequencing, metagenomics, phylogenomics and phylogenetic placement is applied to study taxonomically interesting morphotypes and bioactive metabolite-producing strains. Recently, the collection has developed a suite of highly reproducible genomic workflows (GEN-ERA; <https://github.com/Lcornet/GENERA>), coded in Nextflow and based on Singularity containers, that meet the requirements of open science and FAIR practices.

Keywords: cyanobacteria, polyphasic approach, taxonomy, culture collections

Acknowledgements and Funding

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Genomic approach on the taxonomic diversity and biosynthetic gene clusters of cyanobacteria with insights on strains microbiome and virome

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Cyanobacteria genome analysis can improve our understanding of their taxonomy through a multilocus approach that can better explain evolutionary lines. Furthermore, cyanobacteria genomics will allow for a better characterization of their potential for metabolite production. Genomic sequencing of uni-cyanobacterial strains can also unveil their microbiome and virome composition, which are often overlooked. Recently, more than 100 strains of cyanobacteria from the Bank of Algae and Cyanobacteria of the Azores (BACA) were sequenced by Illumina. Bioinformatics processing followed a custom-made python pipeline designed to analyse the cyanobacterial genomes, their microbiome and virome. This approach allowed the assembly of high-quality cyanobacterial genomes that can be applied in taxonomical studies, increasing the data available for several genera and species and fulfilling significant gaps in taxonomical studies and more robust analysis. Apart from reporting several novel taxa that are important genetic resources for biotechnological applications, the deep sequencing of the BACA cyanobacterial strains allowed the discovery of the microbiome and virome associated with the cyanobacteria strains. AntiSMASH and BIG-SCAPE analysis of cyanobacteria genomic data revealed a high diversity of biosynthetic gene clusters (BGCs), creating opportunities for further works on metabolite research in specific genera with high genetic diversity and higher number of BGCs. The study of cyanobacteria maintained in culture collections such as BACA, which still have much of their genetic diversity unstudied, can be a critical source of data for taxonomical studies and the search for novel bioactive metabolites.

Keywords: phylogeny, genomes, BACA, antiSMASH, BIG-SCAPE

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Seasonal population dynamics of *Microcystis aeruginosa* and associated cyanophages in the river Moselle

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Ecosystems worldwide are threatened by an increasing amount of cyanobacterial blooms that are fueled by eutrophication and warming waters in consequence of the climate crisis, endangering surrounding environments¹. Since 2017, cyanobacterial blooms of *Microcystis aeruginosa* are repeatedly observed in the river Moselle between France, Luxembourg and Germany². In potential regulation mechanisms for these harmful algae blooms, biological control mechanisms via e.g. phages specifically infecting cyanobacteria are discussed³. This study investigates the population dynamics of *M. aeruginosa* and cyanophages that are known to specifically infect this species (Ma-LMM01-like phages) in the Moselle. Throughout 2020 to 2023, populations of *M. aeruginosa* and associated phages (intracellular) were quantified at the river mouth in Koblenz, Germany, using Droplet Digital PCR (ddPCR). Additionally, both populations were quantified in associated water bodies in the catchment in Germany, Luxembourg and France in a sampling campaign in 2024. Seasonal dynamics revealed a significantly correlated co-occurrence of both populations with high abundances during late summer. The spatial analysis indicated a widespread presence of cyanophages in the catchment. Additionally, various protocols for effective phage isolation were tested to enable future assessment of free phage particles in the water column, as the previously applied in-house standard method only allowed isolation of particle-associated phages. Filtration using magnesium chloride (MgCl₂) coated membranes proved as an efficient method for phage isolation from the water column. Preliminary application of MgCl₂ filtration in the Moselle revealed a more complex development of the Ma-LMM01-like phage population in association with *M. aeruginosa* than previously indicated with the in-house standard protocol.

Ma-LMM01-like phage infection is characterized by their strict strain-specificity and infection may lead to strain-level shifts within their host population⁴. In future analysis, examination of the *M. aeruginosa* strain succession through genotyping may provide a more conclusive understanding of the previous findings in the context of environmental pressures.

Keywords: Harmful algal blooms, phage-host dynamics, Ma-LMM01-like phages, ddPCR, phage-isolation

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Temporal changes in the genome and associated micro-biome in lab-grown marine Cyanobacteria over a decade reveal adaptive selection

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Primitive photosynthetic organisms known as Cyanobacteria have been adapted and living on Earth for approximately three billion years, inhabiting a variety of environments. From the Bangaram island of Lakshadweep, India, we identified a new strain of Cyanobacteria with profound acid neutralizing activity. Large scale genome comparison with polyphasic approaches placed this strain as a novel species of *Leptolyngbya iicbica* LK (Dutta, Subhajeet, et al., 2024). High throughput genome sequencing of this organism at periodic intervals over a decade reveals temporal distribution of the associated bacterial populations. Additionally, core bacterial populations belonging to the class Bacteroidia and Alphaproteobacteria was found. We have used genomics and transcriptomics data to investigate the potential metabolic interactions of LK with the associated bacteria to understand the intercommunication between the organisms under different abiotic stress conditions. Complete genome of LK and the associated bacteria was sequenced thrice and a chromosome level assembly of LK was obtained from the third time sequenced data. We have investigated the marine coastal eco-genomic properties of the LK and compared them with the taxonomically close organisms from marine and fresh water habitat. We have detected genomic rearrangements and variants with a variation rate of one variant per 478,221 bases and six InDels (four insertions and two deletions) over a decade. The identified variants have 89 total predicted impacts, of which 4 are high, 2 are low, 2 are moderate, and the rest 81 are Modifiers. Additionally, we found two IS-element induced genome-wide structural alterations. Interestingly, similar IS-element mediated changes and variations were also found in the associated bacterial genomes, indicating co-adaptation. This suggests that random variations, the mobility of IS-elements, and structural alterations of the genome over time may aid in adaptation in lab conditions.

Keywords: Novel species, Core bacterial populations, Genomic variants, IS-elements, Adaptation

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Interaction between freshwater cyanobacteria and cyanophages; new tools for investigation of the infection cycle and its dynamics

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Cyanophages, viruses infecting cyanobacteria play an important role in aquatic ecosystems, regulating cyanobacterial populations and influencing a genetic diversity and biogeochemical cycles. The involvement of cyanophages to reduce cyanobacterial blooms has been considered as a part of lake management strategies. However, the lack of deep knowledge on the cyanophages - cyanobacteria interactions of in freshwater ecosystems limits their potential use.

In the present research we aimed at adapting novel approaches to understand the cycle and dynamics of cyanophage LMM01 infecting freshwater cyanobacterium *Microcystis aeruginosa* NIES-298. We utilized standard methods like chlorophyll fluorescence, cell count and virus titre measurement to compare infected and uninfected cultures and additionally, we employed new methods: MPI (Molecular Profile of phage Infection) [1] and VirusFISH [2].

The MPI method bases on a qPCR technique to quantify separately non-adsorbed phage genomes and virions, solely virions or non-adsorbed virions, which allows following the subsequent steps of infection cycle. This highly sensitive and precise method can detect low levels of viral nucleic acids, but lacks the ability to resolve single-cell differences or phage localization.

VirusFISH involves fluorescently labelled nucleic acid probes that are designed to bind specifically to viral RNA/DNA sequences. This technique allows to study the distribution and localization of viruses within infected cultures. It has the potential to detect multiple viruses or nucleic acid targets in the same sample, which could offer insights into co-infections or interactions between viruses.

Both methods allowed for a more detailed description of the infection process, which was prolonged compared to that one previously described [3]. Adsorption and phage DNA multiplication, observed rapidly after infection, occurred initially only in a small cells fraction and progressed over time. The occurrence of several infection cycles is postulated. In conclusion, a combination of these tools may serve for better understanding the course of a phage infection in different ways, thus providing a broader picture of this process.

Keywords: cyanobacteria, phages, VirusFISH, MPI, qPCR

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Defining Seasonally Variant Modules of Biosynthesis Encoded by Bloom-Forming Cyanobacteria *Microcystis* & *Dolichospermum* in Western Lake Erie

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Cyanobacteria that proliferate in harmful algal blooms (cyanoHABs) are known to produce at least 2,425 different metabolites. However, hepatotoxic microcystins account for 90 percent of the research on this subject while representing only 13 percent of their total documented chemical potential. In temperate and sub-tropical lakes and reservoirs globally, *Microcystis* and *Dolichospermum* often dominate and co-occur within cyanoHABs, with numerous strains of each genus typically co-occurring. Metabologenomics research shows that these different strains encode the synthesis of unique repertoires of secondary metabolites via diverse suites of biosynthetic gene clusters (BGC) within their genomes¹. However, little is known about how the composition of BGCs and metabolites change in response to varying biotic and environmental conditions (nutrients, temperature, etc.) across bloom seasons and between years. Here, we used western Lake Erie, a basin plagued by toxic cyanoHABs, to investigate the spatiotemporal distribution of cyanobacterial BGCs and metabolites and their relations to biotic and abiotic conditions. We identified chemotypes, distinct modules of BGCs with co-varying abundance patterns, for *Microcystis* and *Dolichospermum*. Each chemotype consists of unique sets of BGCs, with the composition of each chemotype partially reflecting the BGC makeup of individual strains from the two genera. We infer that the chemotypes reflect seasonal variations in the composition of cyanobacterial strains within the bloom rather than differential expression by a consistent consortium. The range of molecules putatively produced by these chemotypes may offer significant advantages for nutrient acquisition and grazer defense, extending beyond the effects of individual metabolites within each chemotype. Studying cyanoHABs through this lens can lead to further insights into the ecological function and chemical ecology of cyanobacterial secondary metabolites. Moreover, this research can provide a blueprint to begin studying the risks of co-varying cyanobacterial metabolites towards humans, wildlife, organisms within the water column, and the environment broadly.

Keywords: Microcystis, Dolichospermum, Biosynthetic Gene Clusters, Harmful Algal Blooms, Multi-Omics

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Conserved Marker Genes Reveal *Microcystis* Strain Diversity and Dynamics in Western Lake Erie

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Microcystis, a dominant genus in Lake Erie harmful algal blooms, exhibits significant strain-level diversity, influencing its population dynamics and toxin production. To identify phylogenetic markers of *Microcystis* strain diversity, we constructed a pangenome using available reference genomes and compared phylogenetic trees built from each of 2,351 individual core genes to an established phylogeny and assessed the ability of these core genes to predict whole genome content and bioactive compound genotypes. We developed primers suitable for current Illumina-based amplicon sequencing with near-complete coverage of available *Microcystis* genomes and demonstrated that they outperform existing options for assessing *Microcystis* strain composition. We further determined that genetic markers can be used to infer *Microcystis* gene content and phenotypes such as potential production of bioactive compounds, although marker performance varied by bioactive compound gene and sequence similarity.

We applied these markers to a seven-year time series (2015–2021) of Lake Erie harmful algal bloom samples, uncovering temporal and spatial patterns of *Microcystis* strain diversity. Our analyses revealed distinct patterns in strain composition correlating with bloom progression and environmental factors, including nutrient availability and temperature, with an increased diversity of microcystin producing strains during early bloom periods. Notably, previously undocumented strain variants were identified, underscoring the value of this marker for uncovering novel diversity. This work provides a framework for strain-level monitoring of *Microcystis* blooms and offers a scalable approach for linking genetic diversity to functional traits, with potential to improve toxin production forecasts and HAB management.

Keywords: *Microcystis*, omics, marker genes, strains, population dynamics

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Environmental factors influencing cyanobacteria dynamics in contrasting urban lakes

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Urban freshwater lakes are subject to enormous pressure from anthropogenic activity within their watersheds. Untreated stormwater, wastewater effluent, and road salt all contribute to the degradation of urban lakes, shifting relatively pristine water bodies to heavily degraded stormwater ponds in a matter of decades. The Halifax Regional Municipality (HRM) is the largest population centre on Canada's east coast and home to > 1000 lakes. Its lakes, scattered throughout urban, suburban, and rural communities, are heavily used by residents for recreational purposes. Since 1980, a synoptic water quality survey of 51 HRM lakes has been conducted on a decadal basis. The survey, taking place in the spring immediately after ice-out, has documented changes to water chemistry and trophic status over a 40-year period corresponding with rapid urbanization. Strong increases (> 50%) in conductivity, iron, sodium, chloride, and calcium have been observed over this period (Doucet et al., 2023; Bermarija et al., 2023). In the most recent synoptic water quality survey (2021), additional water samples were collected from 24 of the 51 lakes for complementary 16S rRNA MiSeq next-generation sequencing. Here, we examine the cyanobacterial and bacterial community composition across these contrasting urban lakes and explore how both lake basin and watershed characteristics shape these communities, with a particular focus on small, stormwater-impacted waterbodies.

Keywords: urban development, environmental, lake management

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Genetic divergence of non-toxic and toxic *Microcoleus* leading to vitamin auxotrophy

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Microcoleus autumnalis are filamentous, benthic cyanobacteria found in freshwaters worldwide¹. They are well known for producing anatoxins, which have caused animal mortalities, and pose human health risks¹. During proliferation, *Microcoleus* forms visible mats, which can contain two distinct types of *Microcoleus*, with anatoxin production limited to toxic strains^{1,2}. We aimed to characterise the genomes of toxic and non-toxic strains to identify differences that could explain how both strains coexist. Sequenced and analysed genomes revealed that non-toxic and toxic strains constitute separate clades, and all toxic strains have substantially smaller genome sizes (~1 Mbp)³. Toxic strains were missing several genes and pathways, including those involved in thiamine (vitamin B1) biosynthesis and salvage, despite thiamine being an essential cofactor for several metabolic processes³. The absence of thiamine biosynthesis genes has been identified in individuals from other bacterial communities, where sharing of thiamine, or its precursors, is thought to occur⁴. The localisation of toxic strains alongside non-toxic strains in aquatic mats suggests that toxic strains could gain thiamine from non-toxic strains to support growth. Laboratory studies have been undertaken to explore this predicted thiamine auxotrophy and thiamine sharing between strains to better understand the links between predicted capacity from genomes and the physiological outcomes in strains. This loss of metabolically essential genes by toxic strains could be explained through the lens of the Black Queen Hypothesis, where loss of essential genes can occur if their products can be gained from other organisms⁵. This study provides insights into the evolutionary divergence of non-toxic and toxic *Microcoleus*. Additionally, findings indicate that metabolites provided by other co-occurring bacteria, including members of the non-toxic clade, could be key to the survival of toxic strains and the occurrence of toxic proliferations.

Keywords: Genetics, anatoxin, thiamine, benthic

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Genetic and metabolic diversity of cyanobacteria on the rock-water interface in Alpine mountains and effects on the local environment

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Subaerial dark-colored surfaces, called *Tintenstrich* communities (translates to “ink stripes”), extend on rock surfaces in semi-aquatic environments.¹ These communities are widespread in high mountain areas, including much of the Alpine region, and are mainly composed of free-living cyanobacteria, also associated with lichen-forming fungi.² Cyanobacterial toxins are a growing concern for environmental and public health in surface waters worldwide;³ and it also plays key roles in ecological relationships, often through the production of bioactive compounds. However, their role at the land-water interface as in *Tintenstrich* communities in the Alpine regions are widely unknown. Therefore, we explore the genetic and metabolic diversity of cyanobacteria across the Swiss Alps. We describe, for the first time, the relative abundances of cyanobacterial families and highlight that many remain unclassified, emphasizing potential uniqueness of these Alpine ecosystems. We further establish that toxin-encoding genes involved in the biosynthesis of anatoxins, microcystins, and nodularins commonly occurred in cyanobacteria from *Tintenstrich*. Metabolites themselves were frequently identified in *Tintenstrich* biomass for the class of anatoxins and anabaenopeptins, and rarely for microcystins and nodularins. We further explore effects on local herbivores. We evaluate tolerance and avoidance towards cyanotoxins through feeding experiments with *Chondrina avenacea* and *Cantareus aspersus*, a specialist for *Tintenstrich* and a generalist snail, respectively. Depending on the cyanotoxins used to tint food the response of these grazers varies. Additionally, we assess the possibility of mobilization of cyanobacteria from rock surface with the runoff water and of regrowth in downstream surface waters. Our study presents the first empirical data on toxin producing cyanobacteria in Alpine *Tintenstrich* and provides first insights into ecological interactions and environmental dynamics within these communities.

Keywords: Cyanobacteria, *Tintenstrich*, cyano-lichen, anatoxins, microcystins

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Genomic insights into adaptations to survival and toxicity of cyanobacteria in hot springs: a case study comprising cyanobacterial genomes from Europe, Iceland and Central Asia

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Extreme environments, like thermal springs, are full of unknown microorganisms with unique adaptations. In this study, we focused on the functional genes of several cyanobacteria thermophilic species, some isolated and sequenced by our team and some being reference genomes. Animals and humans use thermal springs for bathing and recreation; thus, the information about potential cyanotoxin biosynthesis by thermophilic cyanobacteria is relevant, along with the identification of thermophilic adaptations. We isolated cyanobacteria strains from thermal springs from Tajikistan, Greece, Iceland, and Poland. We assembled six whole genomes from those isolations - three genomes of recently described new genera and species (Jasser et al. 2022), two Nostocaceae species from the cold desert in Pamir, and the first whole genome of *Calothrix thermalis* isolated from a hot spring in Iceland.

A comparison of newly assembled six genomes with reference genomes of several thermophilic and non-thermophilic cyanobacteria species has not fully confirmed shorter genomes in thermophilic species - *Calothrix thermalis* and *Thermoleptolyngbya hindakii* had similar size or longer genomes than closely related non-thermophilic species. However, *Hillbrichtia pamiria* had a noticeably smaller genome than closely related taxa. Earlier studies suggested that in extreme environments, the production of cyanotoxins is limited (Khomutovska et al. 2020); thus, we expected that toxin-coding genes, as toxins are secondary metabolites, would not be present in thermophilic genomes. This was true for *H. pamiria*, as we could not identify any genes related to microcystin or cylindrospermopsin. Moreover, we observed a general reduction in gene clusters encoding secondary metabolite production in thermophilic genomes compared to non-thermophilic ones. However, in *C. thermalis* we discovered whole microcystin and cylindrospermopsin clusters. The cylindrospermopsin operon was also identified in *Thermoleptolyngbya hindakii*. Our results confirm that some thermophilic cyanobacteria species have toxin-encoding genes and are part of discovering genomic adaptations in such environments.

Keywords: Thermophilic cyanobacteria, thermal springs, extremophiles, cyanotoxins

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Effects of cyanosphere bacteria on the growth of the anatoxin-a producing *Microcoleus*

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Toxin producing cyanobacteria have become increasingly problematic over the years, threatening ecosystems and killing domestic animals. Cyanobacteria live in close association with other microorganisms such as heterotrophic bacteria¹. Long-lasting and ecologically significant interactions, such as cross-feeding² and phyllosymbiosis³, have been described between planktonic cyanobacteria and some heterotrophs. By contrast, very little is known about such interactions in benthic cyanobacteria. One of the most problematic benthic species, *Microcoleus anatoxicus*, has a significantly reduced genome compared to the rest of the *Microcoleus* genus. As *M. anatoxicus* misses some essential genes for the biosynthesis of vitamins⁴, it has been proposed that it could obtain key metabolites from interactions with other microorganisms. In this study, we address this hypothesis experimentally by studying interactions between an axenic *M. anatoxicus* strain and heterotrophic bacteria isolated from two different sites in the Gorges de l'Areuse (Neuchâtel, Switzerland). One site was dominated by a toxic *M. anatoxicus* strain, the other by a non-toxic Oscillatoriales (genus *Potamolinea*). Isolated heterotrophs and cyanobacteria were co-cultured to assess their effect on cyanobacterial growth. Positive and negative effects were observed, and further investigations will be conducted to identify the molecular mechanisms underlying these effects. By shedding light on biotic factors that stimulate the proliferation of toxic cyanobacteria, our research should provide crucial insights about the ecology of harmful algal blooms.

Keywords: anatoxin-a, benthic toxic cyanobacteria, cyanosphere, *Microcoleus anatoxicus*

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Digging deep in the toxicity of *Trichormus variabilis*: toxicity and toxin production

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Cyanobacteria are distinguished by their ability to biosynthesize a wide array of compounds with diverse biological activities, which hold potential applications across various industries, including food production and medicine. Many of these compounds belong to cyanotoxins, renowned for their potent biological effects. In this research, we used the strain *Trichormus variabilis* TAU-MAC 1614 during a two-week co-cultivation experiment with the aquatic plant *Lemna trisulca*. It was observed to completely inhibit the plant's growth, induce approximately a fivefold increase in carotenoid content within plant tissues, and alkalize the growth medium to a pH exceeding 10. Extracts of *T. variabilis* also caused a prolonged reduction in the photosynthetic efficiency of *L. trisulca* and an increase in cellular respiration rates. In the Thamnotoxkit F toxicity test, the LD50 of the extract was determined to be 0.5–1 mg of dry mass/ml. Similarly, in the Algaltoxkit F test, high concentrations of the extract inhibited the growth of the green alga *Selenastrum capricornutum*. The observed toxic effects are likely attributable to the presence of microcystins, which are hepatotoxins, characteristic of cyanobacteria. Analyses using the LC-MS technique confirmed the presence of at least five microcystin variants in methanolic extracts from *T. variabilis* cells and the culture medium. Further studies, employing advanced analytical techniques and bioinformatics approaches, are ongoing to enhance understanding of the potential mechanisms of toxicity and elucidate the structures of the identified compounds.

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Native Fish Species (*Cnesterodon decemmaculatus*) as a Bioindicator of *Microcystis* Bloom Toxicity

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Due to the increasing impact of *Microcystis* blooms in freshwater bodies of temperate regions of South America, microcystins (MCs) levels usually reach seasonal peaks of 20,000 µg/L or higher. This poses a significant risk to humans and animals through direct contact, consumption of contaminated water, or contaminated food. *Cnesterodon decemmaculatus* is a native species from southern South America, previously identified as a useful tool for evaluating water quality (De La Torre et al., 2005; Saona et al., 2015.). To assess the potential of *Cnesterodon decemmaculatus* as a bioindicator of the toxic effects of *Microcystis* blooms in water ecosystems, we evaluated the effectiveness of histopathology and immunohistochemistry for detecting these effects. For this purpose, fish were exposed *in vitro* to three concentrations of a *Microcystis* sp. bloom (containing 55, 550 and 5500 µg/L MCs) over 96 hours (n=10 in duplicate for each dose). The concentration of total MCs in the bloom was analyzed by ELISA and the predominant chemical variants identified by MALDI-TOF were MC-LR and MC-RR. Immunohistochemistry was performed with an *in vitro* biotinylated single chain antibody previously selected by our group (Pérez-Schirmer et al., 2017) and developed with HRP-Streptavidin (Thermo) and DAB (Sigma) as substrate. The estimated LC50 value for MCs in this bloom was 650 µg MCs/L (15,5 g bloom/L). Even at the lowest concentrations tested, histopathology revealed significant effects in various tissues, such as gill edema, disruption of liver architecture, intestinal necrosis, and loss of cellular cohesion. Immunohistochemistry, clearly showed that MCs predominantly accumulated in digestive system cells, mainly in the liver and intestine. Due to its ubiquity in aquatic ecosystems, omnivorous diet, and well-documented biology, *C. decemmaculatus* shows strong potential as a sentinel species for MC exposure.

Keywords: microcystin, fish, histopathology, immunohistochemistry

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F42-68

Comparing Aquatic Environmental DNA and Sedimentary DNA to Investigate Cyanobacterial Community Dynamics Across a Trophic Gradient

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The Masurian and Suwałki Lakelands of north-east Poland contain thousands of lakes with diverse morphologies, land-use histories, and varying trophic states. While many of the lakes experience intense eutrophication due to anthropogenic activity, others have relatively high water quality.

As a result of increasing temperatures and anthropogenic stressors, various forms of freshwater biomonitoring show that toxigenic and non-native cyanobacterial species are increasing. Because of the high-effort nature of traditional microscopy, aquatic Environmental DNA (eDNA) metabarcoding as a form of biomonitoring is now commonplace. While aquatic eDNA is a robust and complementary method to microscopy, it has been demonstrated that recent sedimentary DNA (the upper 1-2 cm; sedDNA) can also be a powerful tool to monitor fish, plants, and a variety of organisms including benthic and planktonic microorganisms. In addition, sediments can concentrate, accumulate, and preserve eDNA, and may provide a more representative picture than a 'snapshot' from a single water sample.

Therefore, we will present a comparison between aquatic (eDNA) and sedimentary (sedDNA) cyanobacterial metabarcoding data from an approximately 400 BP long fragment of the V3 - V4 regions of 16S rRNA, and traditional planktonic microscopic analysis from more than 20 lakes from the Masurian and Suwałki Lakelands, which are representative of a range of trophic states. These data will be assessed in terms of the detection of invasive and/or toxigenic species, the presence of cyanotoxin biosynthesis genes (microcystins (*mcy*), saxitoxins (*sxt*), cylindrospermopsin (*cyr*), anatoxins (*ana*); which have been amplified and sequenced separately from 16S rRNA), coherence between eDNA and sedDNA, and various measures of taxonomic composition across the trophic gradients. Our aims are to determine whether, on a single sampling day at each lake, water samples or recent sediment samples are coherent and complementary in assessing potentially toxic and/or invasive planktonic cyanobacteria and community composition.

Keywords: microcystins, metabarcoding, environmental change, eutrophication

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F43 - 001

Exploring the Ecotoxicity of Metal-Organic Frameworks for Sustainable Water Treatment and Cyanobacterial Bloom Management

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The increasing demands of modern society have led to rapid industrial growth, resulting in the discharge of wide variety of pollutants, such as toxic metal ions, dyes, antibiotics, and various industrial wastes, into the environment. These pollutants pose significant risks to water resources, threatening human health, ecosystems, and the environment. Therefore, innovative management strategies should be developed to enhance the water quality. There has been an increasing interest in the use of metal-organic frameworks (MOFs) in circular economy applications related to the protection of the environment and agriculture. MOFs are a novel class of crystalline porous materials composed of metal ions coordinated with organic linkers. These materials exhibit remarkable porosity and a large surface area, which make them highly promising for a wide range of applications including water treatment. The increasing prevalence of cyanobacterial blooms in eutrophic water presents an urgent environmental issue, necessitating effective strategies for their management. With these objectives, the project CIRQUA (Integrated Approaches at Local Scale for Enhancing Water Reuse Efficiency and Sustainable Soil Fertilization from Wastewater's Recovered Nutrients) aims to improve nature-based solutions, focusing on constructed wetlands (CWs) for wastewater treatment and water recovery in rural areas. MOFs have garnered attention for their potential in addressing such challenges (Fan et al., 2019; Li et al., 2022). However, the release of such materials into the aquatic environment raises concerns about their ecological impact (Ruyra et al., 2015; Yuan et al., 2022). In this study, total of 10 MOFs were synthesized and evaluated for their toxicity against the microalga *Pseudokirchneriella subcapitata* and the microcrustacean *Daphnia magna* and the fish *Danio rerio* (zebrafish). Also the preliminary results for ecotoxicity of UiO-66-NH₂ was evaluated, specifically its effects on the growth, viability, and toxicity of the cyanobacteria *Microcystis aeruginosa*.

Keywords: Circular economy, Cyanobacteria, Microcystis

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Inhibitory effect of aqueous extracts of *Pistia stratiotes* and *Pontederia crassipes* on cyanobacteria *Microcystis aeruginosa* and the defensive role of its microbiota associated

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The allelopathic properties of macrophytes, such as *Pistia stratiotes* and *Pontederia crassipes*, offer a sustainable approach to cyanobacterial bloom control. This study investigated the effects of aqueous extracts from these macrophytes on *Microcystis aeruginosa*, focusing on physiological impacts and the role of its associated bacterial communities. At a concentration of 4.0 g L⁻¹, both extracts inhibited cyanobacterial growth, with *P. stratiotes* achieving complete inhibition and *P. crassipes* reducing growth by 60%. Photosynthetic activity was suppressed more effectively by *P. stratiotes* (99% vs. 12% for *P. crassipes*), associated with the downregulation of the *psbA* gene. Oxidative stress was induced, with increased reactive oxygen species (ROS) and peroxiredoxin gene (*prxA*) transcripts, alongside enhanced superoxide dismutase (SOD) activity in *P. stratiotes* treatments. In parallel, the extracts altered the composition of bacterial communities associated with *M. aeruginosa*. *P. stratiotes* extract favored genera such as *Shinella* and *Flavobacterium*, while *P. crassipes* promoted *Enterobacterales*. These bacterial communities were used to supplement new cultures of *M. aeruginosa* and when these cultures were exposed to extracts again, there was mitigated effect on cyanobacterial inhibition (reduced inhibition to 40% for *P. stratiotes* and 12% for *P. crassipes*). The associated bacterial exhibited increased metabolic activity, degrading allelochemicals and supporting antioxidative defenses. The protective role of heterotrophic bacteria highlights their importance in allelopathic interactions, influencing both the efficacy of macrophyte extracts and the resilience of *M. aeruginosa*. This integrative approach underscores the potential of macrophyte extracts as biocontrol agents while revealing the critical role of microbial communities in modulating allelopathic effects.

Keywords: Cyanobacteria, allelopathy, gene expression, cyanosphere, oxidative response

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Scalable Hydrogen Peroxide Dosing Solutions for Tackling Toxic Cyanobacteria

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Hydrogen peroxide (H₂O₂) offers a promising, eco-friendly approach to managing cyanobacteria and associated toxins in water systems¹, particularly waste stabilization ponds. This study assessed H₂O₂ efficacy in controlling surface toxic cyanobacterial blooms and sedimented sludge cyanobacteria at large wastewater treatment and recycling plants. Trials using granular and aqueous H₂O₂ forms targeted varying dosages (4–26 mg/L) and contact times, with sampling from pond surfaces and sludge layers in laboratory, pilot and full-scale. Novel Multiplex Tandem PCR methods were used to explore toxin production capacity during the trial.

Surface bloom treatments yielded significant reductions in cyanobacterial cell viability (50–80%) and total cell counts (30–50%), with cyanotoxins reduced from 200–40 µg/L to undetectable levels. H₂O₂ degradation occurred rapidly, with concentrations falling below detection limits within 96 hours. However, cyanobacteria in sedimented sludge exhibited greater resistance, with maximum 60% reduction in cell viability, highlighting the need for tailored strategies to address sludge-associated cells.

Field trials demonstrated the importance of environmental factors such as UV exposure and mixing in enhancing H₂O₂ efficacy. While cyanobacteria were selectively targeted over eukaryotic algae, microbial community shifts and potential increases in opportunistic bacteria underscore the need for comprehensive post-treatment monitoring. Advanced genomic tools are used to evaluate ecosystem-wide impacts and resistance risks.

This research showcases the scalability challenges and highlights the need for optimized dosing strategies in dynamic field conditions. Ongoing work focuses on expanded full-scale trials, with onsite H₂O₂ production, to refine application methods, ensuring effective H₂O₂ delivery across diverse water types, including treated wastewater, recycled water, and recreational water. These findings provide actionable insights for water utilities to implement sustainable cyanobacteria and toxin management strategies, addressing both surface blooms and sediment challenges.

Keywords: Treatment, Oxidation, Hydrogen peroxide, Scalability

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From Lab to Lake, Evaluating Efficacy of Emerging Nanobubble Ozone Technology (NBOT) for Cyanobacterial Harmful Algal Bloom (CHAB) Control

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New and effective Cyanobacterial Harmful Algal Bloom (CHAB) treatment strategies are needed to address negative impacts to fisheries, recreational areas, and drinking water sources while minimizing adverse effects on nontarget organisms. Nanobubble ozone technology (NBOT) is an emerging, innovative, CHAB control strategy that encapsulates ozone within nanobubbles, unlocking ozone's potential for use in natural waters. Replicated lab and mesocosm NBOT experiments and full-scale field trials were conducted 2021 - 2024. Lab studies confirmed high densities of nanobubbles were produced and ozone was released from nanobubbles over time. Replicated mesocosm experiments evaluated efficacy of NBOT at multiple doses and compared NBOT to traditional algaecide CHAB treatments. Algaecides had severe negative impacts on the zooplankton community compared to minimal non-target impacts from NBOT. NBOT treatments resulted in up to 99% reduction in CHABS and 92% reduction in microcystins and affected dissolved organic carbon characteristics and nitrogen form. Oxygen only nanobubble treatments were ineffective at CHAB control, demonstrating that ozone is a critical treatment component. Multi-month NBOT trials were conducted at Lake Sylvan and Grand Lake Saint Marys West Beach, both located in Ohio, USA. At Sylvan Lake, cyanobacteria and cyanotoxin concentrations decreased when ozone dose was increased, and recreational advisories were avoided. Cyanobacteria and toxin concentrations were higher during the Grand Lake Saint Marys beach NBOT trials and treatment effects were localized. Recreational advisories at the treatment beach were reduced in the first year, but subsequent shorter duration trials on a higher biomass bloom were ineffective. A short-term exploratory stream trial was also conducted. Our findings reveal the importance of three design components critical for CHAB treatment: 1) applied ozone:DOC in the water body, 2) water body volume to treated volume per time, and 3) spatial coverage of treatment. Future studies include evaluation of NBOT for benthic CHAB and marine HAB control.

Keywords: Ozone Nanobubble Treatment, CyanoHAB Control

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Uncovering the cytotoxicity of cyanopeptides in *Microcystis* extracts on three fish cell lines

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Cyanobacteria are well-known for their toxicity to animals and humans, causing both acute and chronic health effects¹. Microcystins (MCs), a class of cyanopeptides mainly produced by *Microcystis*, are of particular concern as involved in poisonings of humans and wildlife. As a result, many countries have established regulatory limits for MCs in drinking and recreational waters². Beyond MCs, other cyanopeptides (e.g. aeruginosins, microginins, microcyclamides) produced by many cyanobacterial genus demonstrated enzyme inhibition^{3,4}, cytotoxicity, sublethal and lethal effects^{5,6}. However, the toxicity of these cyanopeptides remains incompletely determined, and no regulations currently address them.

This study aims to characterize the toxicity of complex metabolic extracts from cyanobacteria strains producing various cyanopeptide families, with or without MCs, to identify the most toxic extracts. Acute toxicity was assessed on three fish cell lines - common carp brain (CCB), carp leukocyte culture (CLC) and rainbow trout liver Waterloo 1 (RTL-W1) exposed to extracts from 18 pre-selected *Microcystis* strains exhibiting diverse cyanopeptidome profiles. Cell mortality and cell concentration were assessed via flow cytometry following 24-hour and 48-hour exposures.

The results revealed light to moderate cytotoxicity, with mortality rates ranging from 5% to 46% compared to 89% mortality in the positive control. Effects were slightly more pronounced at 48h for both MC-containing and non-MC-containing extracts (e.g. micro/aeru-cyclamides, microginins). Extracts inducing cell mortality also reduced cell concentration, while others altered cell concentration without observable cell death, suggesting impacts on cell proliferation. The CLC and RTL-W1 cell lines displayed greater sensitivity compared to the CCB cell line.

These results will be integrated with data obtained from mammalian cell lines and medaka embryos exposed to the same 18 cyanobacterial extracts using a Weight-of-Evidence procedure to identify the most toxic extracts. Following an Effect-Directed Analysis approach, further cytotoxicity analysis with fractions of the most toxic crude extracts will be conducted.

Keywords: cellular bioassays, cytotoxicity, cyanotoxin, *Microcystis*, weight of evidence

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Application of non-thermal plasma processes as a green approach for the treatment of cyanobacteria and cyano-metabolites of health concern

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Cyanobacterial proliferations in surface freshwater bodies are becoming more frequent and severe [1], raising concerns for drinking water supplies and public health due to their potential toxicity [2]. To safeguard consumers health from cyanobacterial contaminations, water treatment plants commonly use cheap chemical oxidants, such as chlorine, as a pre-treatment step of raw water may be required under regional or national regulations. Nevertheless, the efficacy (cell inactivation and cyanotoxin degradation) and safety (release of dangerous disinfection by-products) of such oxidants has been questioned, and improved alternatives have been explored. In this context, non-thermal plasma (NTP) represents an innovative and green approach for water disinfection, as it generates a mixture of highly reactive species when in contact with water (e.g., O₃, OH•), while only requiring a gas such as air and electricity to be produced [3]. It was found that NTP technology is able to effectively inactivate various microorganisms and degrade persistent organic contaminants [4], thus has a great potential for disinfection of water contaminated by toxic cyanobacteria.

This work aims at exploring NTP as a feasible treatment for cyanobacteria-laden waters, contributing to the development of an efficient, scalable and "green" NTP-based technology for drinking water treatment. Here, different plasma reactors were designed and tested on freshwater spiked with assorted cyanotoxins, and on cultures of the toxic cyanobacterium *Microcystis aeruginosa*. NTP treatments were conducted in triplicate at increasing time exposures versus untreated controls. Physico-chemical parameters, cell viability, and changes in the cyano-metabolites profile were monitored. Photosynthetic efficiency of *M. aeruginosa* was impaired by NTP, while LC-HRMS analysis revealed complete cyanotoxins degradation after few minutes of treatment.

These results suggest that NTP could be a powerful disinfection tool for the treatment of waters contaminated by harmful cyanobacteria and set the base for further research.

Keywords: non-thermal plasma; LC-HRMS; drinking water treatment; cyanotoxins degradation; culture experiments

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Contamination of irrigation water with cyanobacterial toxins (microcystins): assessment of their phytotoxicity and health risk, case study Lalla Takerkoust Lake, Marrakech, Morocco

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In many countries located in semi-arid areas, irrigation with raw water from lake-reservoirs is a common agricultural and horticultural practice, and an important tool for farmers to improve crop yields. This kind of surface water bodies can contain potentially toxic bloom-forming cyanobacteria, and its use as a source for irrigation water can enable a transfer of cyanobacterial toxins (cyanotoxins) into the crop plant. When these toxins are accumulated in crop plants it poses serious human health risk when they enter the food chain. Located 35 km south-west of Marrakech city in Al Haouz province, Lalla Takerkoust lake-reservoir was constructed for electricity production, potable water supply, and farmland irrigation. Over the past few decades, toxic bloom-forming cyanobacteria have been occurring during summer-autumn seasons in Lalla Takerkoust lake-reservoir and have been dominated by microcystin producing cyanobacterium *Microcystis aeruginosa*. In the Lalla Takerkoust agricultural perimeter, we detected the bioaccumulation of MCs in edible fruits exposed to environmentally realistic concentrations of MCs. The health-risk level for humans was high when contaminated fruits were consumed during bloom formation and decay periods (up to a 53-fold increase compared to the TDI = 0.04 µg kg⁻¹). In the same way, the risk level was also high for farm animals (up to a 19-fold increase compared to the TDI = 2.3 µg L⁻¹). To deal with such a biohazard, we explored the role of rhizospheric microorganisms as potential bioremediation biosystems in mitigating MC accumulation and phytotoxicity in edible crops. Therefore, greenhouse trials were conducted with crops in microorganism-free and microorganism-rich soils. MCs impaired plant growth and physiological performance, including photosynthesis and nitrogen assimilation, in microorganism-free soil. However, plant growth microorganism-rich soils remained intact, and the disrupted physiological functions were restored. Moreover, MC bioaccumulation and health risk were reduced in plants grown in microorganism-rich soil.

Keywords: cyanotoxins, irrigation water, bioaccumulation, crop products, health risk



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Low cyanobacteria concentration but high risks derived from multitoxicity

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Water reservoirs in Mediterranean and southern European regions face significant challenges from cyanobacterial blooms due to prolonged high temperatures. This study investigated the dynamics of cyanobacterial communities in a Central Spain drinking-water reservoir over 21 months (March 2023–December 2024). An array of methods was employed, including microscopy, PCR-based screening, metabarcoding, and fluorometric analyses. Sampling spanned a longitudinal gradient from the reservoir's tail to the water intake point.

Our results revealed well-established cyanobacterial blooms, with low cyanobacterial chlorophyll *a* concentration (average values around $\leq 10 \mu\text{g/L}$, occasionally reaching values $>30 \mu\text{g/L}$). Morphological analyses and 16S rRNA metabarcoding identified six dominant cyanobacterial genera: *Planktothrix*, *Raphidiopsis*, *Anabaenopsis*, *Dolichospermum*, *Microcystis* and *Aphanizomenon*; they are recognized as widespread cyanotoxin producers commonly. PCR screening detected cyanotoxin biosynthesis genes associated with microcystins (*mcyE*), saxitoxins (*sxtA*), anatoxins (*anaF*) and cylindrospermopsin (*cyrJ*). Cyanotoxin quantification revealed co-occurrence of the hepatotoxins microcystins and the neurotoxins saxitoxins. Among the cyanobacterial assemblage, we identified amplicon sequence variants (ASVs) and morphotypes corresponding to *Umezakia/Chrysosporum ovalisporum*, a primary cylindrospermopsin producer in Mediterranean Europe. The detection of cylindrospermopsin and related biosynthesis genes will be discussed, contributing to our understanding of this underreported toxic species in the region. The presence of cyanobacterial communities capable of synthesizing the four types of toxins may create a special risk situation that is difficult to predict. Any change in environmental conditions (e.g. nutrients ratios) may modify the succession of toxin-producing taxa with a consequent risk to the system and human health. These findings underscore the critical relevance of robust, long-term monitoring programs to mitigate the risks posed by cyanobacterial blooms in essential water supply systems.

Keywords: cyanotoxins, metabarcoding, water quality, microcystins, saxitoxins, Mediterranean, monitoring

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Management and communication of cyanobacteria risks in recreational waters, an international comparison

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The World Health Organisation (WHO, 2021) provides high-level guidance for the management of cyanobacteria risks in recreational waters and recommends interventions and strategies that may be used when risks are high. The WHO guidance was intended to be nationally or locally adapted according to circumstances. Risk assessment, management and communication vary widely across the world. The aim of this study was to gain an understanding of the diverse approaches being used globally and to learn from the experiences and strategies of different countries, particularly around risk communication. Ultimately, we hope to use this knowledge to promote best practice. Previously, the management of cyanobacteria risks in the recreational waters of 17 countries was summarized via surveys of ICTC participants (see Ibelings et al. 2014). We have updated and extended this work using an online survey distributed via relevant mailing lists and email addresses collected from literature or reports. The survey covered both planktonic and benthic cyanobacteria. In this presentation we will summarize the results of our survey with reference to the current WHO guidance. We highlight the wide variety of management and communication approaches of interest to recreational water users, water body managers and researchers concerned with improving the management of cyanobacteria risks in their local area.

Keywords: Recreational guidelines, risk communication, sampling approaches

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Microbial Community Response to Hydrogen Peroxide Treatment: a microcosm experiment in an eutrophic pond

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Hydrogen peroxide (H₂O₂) offers a promising, environmentally friendly approach to cyanobacteria control since leaves no harmful residues and selectively targets cyanobacteria (Piel et al. 2019; Keliri et al. 2021). This study investigates the efficiency of H₂O₂ on *Planktothrix agardhii* bloom and its impact on prokaryotic communities. To understand the effects of a high concentration treatment on non-target microbial communities, H₂O₂ was added at concentrations of 2 mg/L and 10 mg/L in 50 L microcosms, with six replicates and three were left untreated as controls. The experiment lasted for five days. After 24 h, no H₂O₂ was detected in the H₂O₂-treated sets, which seems consistent with other researches (Matthijs et al. 2012; Mikula et al. 2012). 2 mg/L H₂O₂ caused a significant reduction in chlorophyll-*a* content, with a decrease of 55% and 78% within 4 and 12 hours, respectively. 10 mg/L H₂O₂ caused cyanobacteria population to collapse in 4 hours by a decrease of 95.7% in chlorophyll-*a* (Bauzá et al. 2014). The prokaryotic community was analysed by 16S rRNA gene-targeted amplicon sequencing using MinION™ (Oxford Nanopore Technologies). At the beginning of the experiment, Cyanophyceae dominated the community, representing 74% of the total reads. Twelve hours after 2 mg/L H₂O₂ addition, the abundance decreased to 14%, and a significant decrease was observed at 48 hours, with the abundance dropping to 2%. A similar pattern was observed in the 10 mg/L H₂O₂ treatment, where the abundance decreased by almost half, then to 5%, and dropped to less than 1% after 72 hours. Pseudomonadota and Bacillota species took advantage of this situation and dominated the community. In particular, in the 10 mg/L H₂O₂ treatment, Bacillota members (genus: *Exiguobacterium*) constituted the majority of the community after 24 hours (>80%). The findings suggest that H₂O₂-based algaecides are effective for managing cyanoHABs and emphasize their impact on non-target microorganisms.

Keywords: Cyanobacterial bloom, 16S rRNA sequencing, cyanoHAB management, hydrogen peroxide, *Planktothrix agardhii*

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Effect of Nanobubbles on Ozone Delivery and Harmful Algae Mitigation

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Nanobubble (NB) technology presents a promising solution to the pressing challenge of harmful algal blooms (HABs), which demand immediate and effective intervention. Traditional ozone (O₃) treatments, such as standard bubbling methods, have shown efficacy in controlling HABs but face limitations in gas delivery efficiency and dosing precision. This study investigates the potential of NB technology to enhance O₃ delivery and improve HAB mitigation in both freshwater and saline environments. Our findings demonstrate that NB-assisted O₃ treatments significantly outperform conventional methods by rapidly elevating O₃ levels and dissolved oxygen in water. Notably, NB treatments required between 4 to 20 times less oxidant concentration than environmentally friendly alternatives like hydrogen peroxide to effectively reduce two key HAB species: *Microcystis aeruginosa* in freshwater and *Karenia brevis* in seawater, respectively. Even at low O₃ concentrations (<1.5 ppm), the NB treatment induced notable oxidative stress and photosynthetic disruption in these algal cells, highlighting its enhanced efficiency. Our findings show that O₃ in NBs form exhibits enhanced reactivity, resulting in a quicker breakdown in solution while increasing its oxidation potential. By maximizing gas-liquid interactions and achieving more uniform distribution in aquatic environments, NBs precise dosing and may reduce unintended impacts on non-target species. This positions O₃ NB treatments as a more sustainable alternative, reducing greenhouse gas emissions commonly linked to traditional ozonation methods. Additionally, the rapid oxygen release from NBs helps maintain healthy dissolved oxygen levels, which are vital for sustaining aquatic ecosystems. This study underscores the transformative potential of NB-assisted O₃ treatments as an environmentally sustainable strategy for managing HABs. Future research and practical implementation are vital to fully unlock the benefits of this innovative technology in addressing the growing threat of HABs.

Keywords: Hydrogen peroxide, Dissolved oxygen, Oxidative stress, *Microcystis aeruginosa*, *Karenia brevis*,



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Evaluation of ultrasound for management of harmful algal blooms in sequential drinking water reservoirs

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Ultrasound is an emerging technology to manage cyanobacteria harmful algal blooms (cHABs). It involves ultrasonic transducers placed in reservoirs that emit ultrasound throughout the cHAB season. Various mechanisms have been proposed, including targeting gas vesicles that regulate algal buoyancy, potentially collapsing them or inducing cavitation. Despite laboratory findings, field operation effects remain unclear. A field study was conducted over two seasons in the reservoirs of Del-Co Water Company in Delaware, Ohio, USA from May to October of 2023 and 2024. This study used ultrasound signals, algae type, turbidity, chlorophyll-a, phycocyanin, and remote sensing observations using PlanetScope images from reservoirs with and without ultrasound deployed to understand field operation. The ultrasound signal attenuated with distance with attenuation coefficients about ten times greater than pure water. Spatially, no significant water quality differences were observed in reservoirs with ultrasound, but variations across connected reservoirs indicated the potential influence of detention time. No significant differences were observed in algae relative abundance over time in ultrasound treated reservoirs compared to chemically treated reservoirs. Within the ultrasound treated reservoir, no clear impacts of ultrasound on algae abundance by distance from the ultrasound units were observed. Field evaluation suggested that known mechanisms such as cavitation and gas vesicle collapse are unlikely to affect cHABs at measured sound pressures and frequencies. Overall, the ultrasound treated reservoirs did not show significant differences compared to chemically treated reservoirs.

Keywords: ultrasound, harmful algal bloom management, sound attenuation, cyanobacteria

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A Strategy combining Education, Citizen Science, and Digital Tools to Manage Toxic Benthic Cyanobacteria

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Toxic benthic cyanobacteria are an emerging environmental health risk that has been largely neglected in many regions. We are using Switzerland, in which the problem was just recently recognised, as a test field to develop methods to raise awareness in the public and to support authorities on the monitoring and decision-making concerning the management of areas of heightened risk, such as those with previously recorded blooms. As the reporting of these potentially harmful cyanobacteria grows, traditional responses like area access restrictions might result on an socioeconomic impact that is disproportionate in relationship to the risks. Our work focuses on supporting authorities in the development of risk management strategies using multiple complementary tools. First, we explore educational approaches that combine the didactic use of games and similar playful activities with service learning to transfer critical information to children, who, in turn, are expected to relay this knowledge to their families¹. Additionally, we are testing the use of digital tools and citizen science to facilitate environmental monitoring and decision-making processes. To further improve risk management, our work also involves testing the use of digital epidemiology tools such as social media and google trends to define periods of accrue need for information to the public, or the use of real time geolocalization data to refine risk mapping and develop more effective informational tools for the public. For all of the above, the methods to evaluate the outcome and effectiveness of the approach pose many challenges, but also provide learning opportunities to refine strategies for improved impact. Through these efforts, we aim to promote a more proactive community response and improve awareness to the issue of toxic blooms.

Keywords: communication, risk, public awariness

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Digital tools for sustainable management of water resources

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Global warming induced by anthropogenic climate change has intensified the hydrological cycle and challenges not only on water quantity but also on quality. These challenges include polluted runoff from urban areas and man-made systems, running into receiving water bodies such as rivers and lakes, causing eutrophication problems, increased harmful algal blooms and high pathogen loads. This paper aims to provide a comprehensive overview of cutting-edge digital tools for sustainable water resource management, highlighting both hardware and software solutions as well as integrated systems. Our review targets both microbial and cyanobacterial bloom issues in sustainable water management. Our key research focus areas include:

Real-Time Monitoring: Online sensors and portable water quality monitoring systems for immediate data collection.
Remote Sensing and Imaging: Use of satellites, drones, and open-source microscopes for water quality and biodiversity analysis.

Integrated Digital Platforms: Catchment-scale water quality platforms, featuring advanced data analytic tools, processed-based and data-driven AI modelling tools, visualization tools.

With the rapid digitalization of water management, this review not only identifies research gaps but also emphasizes practical applications, empowering citizen and scientists and enhancing societal impact in sustainable water management.

Keywords: Online sensors, Remote sensors, Processed-base modelling, AI techniques, Integrated digital platform.

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Lessons learned – One year of monitoring toxic benthic cyanobacteria in a mesotrophic lake

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There is a growing global concern about benthic toxic cyanobacteria, in particular anatoxin-producing *Microcoleus* sp. The proliferation of this species on submerged aquatic vegetation in lakes shows that it is not restricted to hard substrates and flowing waters as its preferred habitat. In the dimictic mesotrophic Lake Tegel (Berlin, Germany), the highest abundances of *Microcoleus* sp. were found in association with the aquatic moss *Fontinalis antipyretica*. This rootless macrophyte can detach from the lake bottom and be washed ashore, which is what happened when fatal dog poisonings were reported from this lake.

A one-year monitoring of macrophyte-associated benthic cyanobacteria was carried out at Lake Tegel in 2023-24. The toxin content of biofilms of randomly collected *Fontinalis antipyretica* rake samples from 3 transects and 4 depths was determined and tested for correlation with physical and chemical water parameters as well as with microscopy data. Anatoxin correlated significantly with Secchi depth, dissolved oxygen, conductivity, total phosphorus, sulphate and *Microcoleus* sp. abundance, but not with depth, temperature, pH, nitrogen and *Oscillatoria* sp. abundance.

Additional video camera screening revealed patchy distribution and dynamic changes in benthic cyanobacterial HABs. A commercial immunological anatoxin-a quick test kit has proved valuable and soft measures such as olfactory factors or the general appearance of blooms can already indicate high toxin levels.

The factors identified as potentially promoting the growth of anatoxin-producing *Microcoleus* in Lake Tegel will be tested experimentally in the recently launched joint research project DIVATOX-2.

Keywords: Anatoxin, *Microcoleus*, benthic, macrophytes, lakes

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Ultrasound is of limited use for the mitigation of cyanobacterial bloom development in slow-flowing waters

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As a highly populated area, Flanders face significantly human pressure, which makes it particularly prone to profound cyanobacterial bloom-formation. While in the past this symptom was mainly restricted to small, shallow and hypertrophic fish ponds, blooms are increasingly becoming omnipresent, including deeper lakes and slow-flowing waterways such as canals. Increasing restrictions on the use of such contaminated waters, combined with a rising demand for water due to climate change, place water managers under severe pressure to develop rapid solutions to combat cyanobacterial blooms. The use of ultrasound, claimed to prevent and repress cyanobacterial bloom-formation, is increasingly applied but its effectiveness and potential ecological impact are rarely investigated under real field conditions. To this end, a study was conducted in a recreational section of a canal that is prone to yearly bloom-formation by toxic strains of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. Before the blooming season, 7 commercially-available ultrasound transmitters were installed in the recreational section, constantly providing 40 kHz acoustic soundwaves over a total surface area of approximately 50.000 m² between May to October 2023. The effect of the ultrasound application was investigated by comparing the community dynamics of phytoplankton, zooplankton, macro-invertebrates, fish and key physico-chemical parameters, including potential metal and nutrient release from the sediments in the ultrasound treated location with those in two adjacent locations in the same canal without the deployment of ultrasound. The analysis revealed that the treatment had no profound effect on cyanobacterial bloom development. Neither could adverse effects on other foodweb compartments, physico-chemical variables nor the sediment layer be detected. Our results strongly suggest that ultrasound is not effective in open areas with a rather constant influx of cyanobacteria-loaded water such as parts of canals and other slow-flowing waters. Mitigation measures should instead focus on nutrient reduction and the avoidance of thermal pollution.

Keywords: ultrasound, cyanobacteria, foodweb, blooms, canal

POSTERS





P1 -19

Cyano-metabolites in Lake Marathonas (Greece) by LC-MS untargeted analysis and molecular networking in GNPS

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Cyanobacteria produce a plethora of secondary metabolites (more than 3000 reported so far), several of them being bioactive or toxic. The main route of exposure to toxic cyano-metabolites (cyanotoxins) is drinking water consumption, when source waters are affected by toxic cyanobacteria. Determination of cyano-metabolites is usually carried out by targeted LC-MS/MS analysis for a limited number of compounds, for which commercial standards are available. High-resolution mass spectrometry (HRMS) offers much more valuable data, due to the accuracy of mass measurements and data-dependent acquisition, however, annotation of metabolites is challenging due to the limited MS2 spectra available in public libraries. In this study, we carried out a preliminary evaluation of the presence of cyano-metabolites in Lake Marathonas, a water reservoir of Athens (Greece), using untargeted LC-HRMS. Data analysis was carried out using the CyanoMetDB list of cyano-metabolite-structures, the open MS2 spectral libraries and molecular networking workflows in GNPS, and MetFrag for in silico fragmentation. Results showed that the use of molecular networking combined with the above tools, greatly enhances the potential to annotate cyano-metabolites, mainly via "annotation propagation" in the networks. In samples taken from Lake Marathonas in 2010 and 2020, 19 additional cyano-metabolites (mainly peptides) were annotated at various confidence levels, as compared to previous targeted analysis results. The preliminary results support the future development of efficient LC-HRMS data analysis workflows with computational tools now included in GNPS2, which will contribute to better understanding of the occurrence of cyano-metabolites in lakes and water reservoirs.

Keywords: Cyanobacteria, cyanotoxins, LC-HRMS, GNPS, GNPS2

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P2 -78

Reactivity of cyanobacteria metabolites with ozone: Multi-compound competition kinetics and transformation products

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Water suppliers need to account for cyanobacteria blooming events by better understanding the efficiency of treatment strategies, such as ozonation, to abate cyano-metabolites and their associated toxicity. However, of the over 3084 cyano-metabolites currently known (CyanoMetDB)^{1,2}, the ozonation of less than a dozen metabolites have been extensively studied. This is due, in part, to the absence of commercially available purified reagents for most cyano-metabolites. Instead, these compounds can currently only be obtained as complex mixtures extracted from laboratory-grown cyanobacteria, which makes it impossible to perform studies with individual compounds. To overcome this obstacle, a multi-compound O₃ competition kinetic method was developed to determine second-order rate constants for the reactions of O₃ with 31 cyano-metabolites extracted from two cyanobacterial strains (from $<10^2$ to $7.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) and representing a wide range of cyano-metabolite structures.³ This method uses sixteen competitors that have been validated to simultaneously cover apparent second-order O₃ rate constants between 10^2 - $10^7 \text{ M}^{-1} \text{ s}^{-1}$. In addition, ozonation products of selected cyano-metabolites were investigated by LC-HRMS/MS, combining mechanistic information of O₃ reactions and suspect screening with MS² fragmentation fingerprint match between cyano-metabolites and their potential ozonation products. This study is a step forward towards better understanding the transformation of the large variety of cyano-metabolites during ozonation.

Keywords: water treatment, transformation products, ozonation, suspect screening, non-target screening

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P3 - 9

Towards a better evaluation of fish contamination: application of a validated UHPLC-MS/MS method for microcystins analysis

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The prevalence of cyanobacterial blooms in freshwater systems worldwide is well known, and they become more and more present in countries with temperate climates, due to global climate change. Moreover, some blooms contain toxin-producing cyanobacteria species. Among them, the hepatotoxic microcystin congeners (MCs) are commonly observed in European blooms. Yet, the dissemination of these MCs in the ecosystem, food web and human food chain are insufficiently studied. A lack of suitable and sufficiently robust analytical methods for different matrices should still be addressed. For instance, for fish, multiple studies report the accumulation of MCs in diverse species, without accounting for the possible differences in the matrix during the development and validation of the analytical method. Accurate assessing the accumulation of MCs in different fish species is vital to evaluate the effect of the blooms on the ecosystem and the potential human health risk after consumption.

Therefore, a method was validated to enable robust MCs analysis in multiple fish species from freshwater ecosystems. After multiple optimization steps due to the differences in matrix effect, the method was validated for eight MCs and nodularin. Method validation included five fish species, commonly consumed in Europe. The method was developed to determine MCs concentration both in muscle and liver. Thereafter, it was applied to multiple fish samples, collected from Belgian waters.

A concentration of up to 88.3 $\mu\text{g kg}^{-1}$ sum of MCs was found in perch and sander liver tissue. Moreover, a perch muscle sample contained 6 $\mu\text{g kg}^{-1}$ MC-LR, while MC-RR was only detected, below the limit of quantification. Consumption of contaminated fish muscle could contribute to human exposure to MCs or accumulate in the food web. There is still a need for comprehensive research on the potential harm to humans and the ecosystem from cyanotoxins, using a One-Health approach.

Keywords: Microcystin, ecosystem, Fish, Food web



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Cyanobacterial toxins and peptides in Lakes Vegoritis and Great Prespa, Greece

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Bioactive secondary metabolites of cyanobacteria, including cyanotoxins and cyanopeptides such as anabaenopeptins, aeruginosins, microginins, and aeruginosamides, can be present in freshwater systems. The adverse effects of cyanotoxins on public health and aquatic ecosystems are well-known, while other cyanopeptides are understudied. The aim of the present study was to investigate the co-occurrence of cyanotoxins and cyanopeptides in Lakes Vegoritis and Great Prespa, Greece, which are used for irrigation, fishing and recreational activities, during a 6-year monitoring period (2018-2023). An extended dual-cartridge SPE, LC-MS/MS method [1, 2] for the simultaneous determination of multi-class cyanometabolites, targeting 18 cyanotoxins (12 microcystins, nodularin, 3 anatoxins and 2 cylindrospermopsins) and 10 cyanopeptides (3 anabaenopeptins, 4 microginins, 2 aeruginosins and aeruginosamide) was validated and applied. Cyanotoxins belonging to cylindrospermopsins and microcystins along with cyanopeptides from all the targeted classes were detected in Lake Vegoritis. The maximum cyanotoxin concentrations were 0.73 µg/L sum of CYNs and 1.02 µg/L sum of MCs, with CYN, MC-LR and MC-RR being the most frequently occurring toxins. Anabaenopeptins B and F were the most frequently detected cyanopeptides, present in almost all samples from Lake Vegoritis with sum concentrations up to 10.8 µg/L, while microginin T1 was the most abundant, reaching 47.0 µg/L. In Lake Great Prespa, only anabaenopeptins were detected in all the samples, with a sum concentration up to 1.5 µg/L. In contrast, MC-LR was detected in 17% of samples, with concentrations up to 0.04 µg/L. These results contribute to the enrichment of the database on toxic cyanobacterial blooms in Greek freshwater bodies [3], as this is the first report of the co-occurrence of cyanobacterial toxins and peptides in these two lakes. The findings further emphasize the need for evaluation of the co-existence of cyanotoxins along with less studied bioactive metabolites for comprehensive risk assessment.

Keywords: cyanotoxins, cyanopeptides, Greek lakes, LC-MS/MS

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P5 -10

Moonlighting activity of threonine synthase in cyanobacterial cell death

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Threonine, an essential amino acid for translation, exhibits toxicity towards slow-growing cyanobacteria. This prompted us to perform transcriptomic and proteomic analyses using *Microcystis aeruginosa* cells treated with threonine, revealing a noteworthy upregulation of *thrC*, annotated as pyridoxal 5'-phosphate (PLP)-dependent threonine synthase. PyMoL-based structural prediction and *in vitro* biochemical assays unveiled the moonlighting functions of ThrC in both threonine biosynthesis and deamination. The production of α -ketobutyrate from threonine, both *in vivo* and *in vitro*, provided strong support for the role of ThrC as a threonine deaminase. The kinetics of ThrC as a threonine synthase using O-phospho-homoserine were consistent with the Michaelis-Menten kinetics of ThrC in *Thermus thermophilus*, albeit with a slightly lower V_{max} value. However, the *in vitro* deaminase activity of ThrC, exhibiting sigmoidal kinetics, suggests the potential production of α -ketobutyrate under threonine oversupply conditions in slow-growing cyanobacteria. The phylogenetic lineage of our ThrC is positioned distinctively apart from canonical threonine synthase and threonine deaminase. Gene duplication and subsequent divergence of *thrC*, followed by gene deletion, could have contributed to the possession of moonlighting ThrC protein in aquatic bacteria including cyanobacterial lineages. Metabolomic data revealed that the presence of threonine and α -ketobutyrate disrupted the balance of amino acids (alanine and methionine) and DNA biosynthetic pathways via feedback inhibition. Interestingly, the addition of exogenous alanine or methionine could alleviate threonine toxicity. Our data revealed that threonine does not uniformly support the growth of freshwater cyanobacteria to the same extent, but rather can be toxic to certain groups within this bacterial family.

Keywords: toxic cyanobacteria; threonine metabolism; threonine toxicity; threonine deaminase; collateral response

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MC-LR and AFM1 in milk: quantification potential and toxicological analysis

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Biotoxins are an increasing problem in human foods. Climate change helps toxins to emerge in new (northern) regions while increasing their occurrence of events in already vulnerable regions. This is specifically true for mycotoxins and cyanotoxins, as their origin organisms benefit from a warmer climate, and they seem more and more present in areas where it was not the case, some decades ago. A major data gap still exists concerning the co-occurrence of these toxins and their combined toxicological effect on humans.

Therefore, our study evaluated the occurrence of a common mycotoxin (Aflatoxin-M1) and eight microcystins in milk. Initially, an analytical method was established to assess all toxins with one validated method. This method was used to analyze 113 milk samples collected from different Belgian areas. Additionally, the potential synergistic effects of the toxins on mitochondrial dysfunction in HepG2 cells were evaluated at sub-cytotoxic levels.

Both toxin groups were not detected in the milk samples. However, the inhibition of cell viability and a hyperpolarization in the mitochondrial membrane potential was observed during toxicological tests after the combined exposure of HepG2 cells to 100 ng mL⁻¹ AFM1 and 1000 ng mL⁻¹ MC-LR for 48h. Additionally, extracellular flux analysis revealed inhibitions of several key parameters of mitochondrial function.

Keywords: Milk, Protein interaction modeling, Microcystins, Aflatoxin M1, Toxicology

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Application-ready quantitative methodologies for cyanotoxins in environmental and food matrices

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Toxic cyanobacteria blooms are prevalent throughout the world. Therefore, human exposure to toxins produced by cyanobacteria is to be expected. The current lack of reliable quantitative data from research and monitoring in different matrices is restricting the implementation of risk assessment methodologies to evaluate the health impact on human and animal populations. Uncovering the relevant exposure routes for risk assessment requires ready-to-use analytical methodologies specific for each matrix to quantify these harmful compounds. Indeed, these methodologies require adaptations depending on the targeted matrix and complexity of the analyzed toxin mixture. Furthermore, crucial analytical parameters, like limits of detection and quantification, reproducibility and measurement uncertainty, have to be safeguarded and well-defined to ensure the reliability of the method.

As microcystins are currently the most observed cyanotoxins in Europe, multiple methods were developed to quantify eight microcystins and nodularin in different environmental and food matrices using a UHPLC-MS/MS approach. Methods were developed to evaluate the toxins in environmental water and collected cyanobacteria biomass. Moreover, methods for drinking water and food matrices (specific methods for fruits and vegetables, culinary herbs, algal-based food supplements, fish, and milk) were also developed.

After these methods were successfully developed and validated, they are now application-ready for monitoring and research. Dealing with the differences in matrix effect through adaptation of pH, dilution of extracts, the application of solid phase extraction and optimization of the UHPLC gradient was vital to successfully develop methods in the different matrices. The finalized methods are applicable for the quantification of toxin levels corresponding to relevant (suggested) guideline values, like those provided for drink and recreational water [1], and food supplements [2].

Keywords: UHPLC-MS/MS, microcystins, food, water, monitoring

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Development of Targeted and Non-Target Methods for Multiclass Analysis of Cyanotoxins

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Cyanobacteria produce many secondary metabolites that can impact toxicological effects of cyanotoxins but their impacts on cyanobacterial proliferation is not known. Multiclass cyanotoxin analysis (e.g. microcystins, anatoxins, cylindrospermopsins) is challenging due to varying properties, large numbers of analogues, and a lack of standards for many compounds. Incorporating anatoxins into these methods is particularly difficult due to their polarity, small size and therefore high chemical background at low m/z. Recently, several new anatoxin analogues, including reduction products and conjugates, were identified and detected in samples from Atlantic Canada that are not considered in existing methods. Here, new multiclass LC-MS/MS methods with improved class coverage and selectivity for anatoxins for target and non-target analysis (NTA) have been developed. A targeted method was established using scheduled selected reaction monitoring scan mode on a triple quadrupole mass spectrometer and was optimized to detect 17 microcystins, 17 anatoxins, 3 cylindrospermopsins, nodularin, and aetokthonotoxin, which is a newly emerging cyanotoxin linked to mass mortalities of bald eagles in the Eastern United States. Method performance was validated with respect to limits of detection, linear range, accuracy, and precision for each class of toxins. This method was then modified for NTA to allow for detection of previously unknown toxin analogues and other secondary metabolites using data dependent high-resolution MS/MS acquisition, which allows for simultaneous collection of quantitative full scan and structural MS/MS data. Method parameters were optimized to prevent oversampling of abundant ions while still collecting MS/MS data for closely eluting isomers. This data was used to establish automatic data processing methods using commercially available metabolomics software to detect analytes of interest by database searching and characteristic fragmentation. These methods will lead to improved analysis of cyanotoxins and other secondary metabolites to study drivers of benthic cyanobacterial proliferation and assess threats to human and animal health.

Keywords: Cyanobacteria, cyanotoxins, LC-MS/MS, multiclass analysis

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Harnessing the biosynthetic capabilities of *Microcystis* freshwater cyanobacteria through a multi-omics and synthetic biology approach

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Microcystis, a genus of cyanobacteria commonly associated with harmful algal blooms (cHABs), is a rich source of bioactive secondary metabolites. These organisms encode various biosynthetic gene clusters (BGCs) that produce metabolites through diverse pathways, many of which remain cryptic, lacking identified biosynthetic products. To investigate the biosynthetic potential within cHABs, we utilized the Western Lake Erie Culture Collection (WLECC), a set of 21 *Microcystis* isolates from Western Lake Erie, focusing on two key BGCs: *pmnT3* and micropeptin (*mcn*).¹

The *pmnT3* BGC contains two modular polyketide synthases, a nonribosomal peptide synthetase-like module, and a type III polyketide synthase gene.² While the product of this BGC remains unidentified, its inverse regulation relative to the highly hepatotoxic microcystin biosynthetic genes is of particular interest.³ To identify the metabolic products of PmNT3, we employed the Chassis-independent Recombinase-Assisted Genome Engineering (CRAGE) system in collaboration with the Joint Genome Institute to heterologously express the products of PmNT3.⁴ We successfully integrated the 56 kb BGC into a host vector under an inducible T7 RNA polymerase promoter to enable controlled expression and facilitate the identification of its metabolites. Combined with mass spectrometry, NMR analysis, and pathway engineering, this approach aims to uncover cryptic metabolites, expanding chemical space and revealing potential therapeutic or toxic compounds.

In addition, we explored the micropeptin (*mcn*) BGC, harbored in several WLECC *Microcystis* isolates, which produces anti-inflammatory compounds.^{5,6} Genomic analysis revealed that some isolates with the *mcn* gene cluster also encode a flavin-dependent halogenase (McnD), indicating the potential for strain-specific halogenation. Although halogenated micropeptins have not been detected in the isolates or field samples, we hypothesize that halogenation could enhance their therapeutic potential by improving lipophilicity and target binding properties.

These studies aim to expand our understanding of *Microcystis*' biosynthetic potential and unlock novel therapeutic metabolites.

Keywords: Biosynthetic Gene Clusters, Heterologous Expression, Halogenation, Microcystis, Metabolites

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From a Single Toxin to a Toxin Network: New Insights into Aetokthonotoxin Derivatives

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Cyanotoxins exhibit a remarkable chemical diversity, showing most distinct structures.¹ This diversity, driven by different environmental and ecological factors, plays a critical role in their impact on aquatic ecosystems and human health.^{1,2} The cyanobacterium *Aetokthonos hydrillicola* has recently become famous as the "eagle killer" producing the biindole alkaloid aetokthonotoxin (AETX), a pentabrominated neurotoxin causing Vacuolar Myelinopathy.³ The toxin is transported via the food chain, posing risks to wildlife and potentially human health.³ HPLC-HRMS² analysis of extracts of environmental samples of the cyanobacterium revealed the presence of AETX derivatives and biosynthetic intermediates. Mass spectrometry, with its high-throughput capabilities and enhanced by computational approaches, is the optimal method for streamlining and accelerating the discovery of natural products and their derivatives.⁴ We aimed to use these approaches, e.g. Classical Molecular Networking for obtaining an overview over the chemical space around AETX, MassQL for in-depth analysis,⁶ and Feature-Based Molecular Networking for studying structural diversity.⁷ We identified in total 43 biosynthetic intermediates and derivatives of AETX. Intriguingly, we detected several iodinated derivatives, a rare halogenation in specialized metabolites of freshwater organisms. Supplementation studies with KBr, KI or both were utilized to investigate the production of these derivatives. After large scale cultivation, five AETX derivatives were isolated, and their structures were elucidated using NMR spectroscopy. Based on these data, the chemical structures of the additional derivatives detected in the extracts by HPLC-HRMS² could be proposed. We conclude that in addition to bromide, iodide is also accepted as a substrate by the halogenases involved in AETX biosynthesis, AetF and AetA. Moreover, cytotoxicity assays of the isolated derivatives showed that they differ markedly in their cytotoxicity.

This study demonstrates the diversity of natural AETX derivatives, and the indisputable advantages of the MS-based tools mentioned.

Keywords: Aetokthonotoxin, Biosynthetic Intermediates, High-Resolution Mass Spectrometry, Feature-Based Molecular Networking, MassQL

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A Novel Lateral Flow Assay that Detects Acetylcholine Receptor Ligand Toxin Anatoxin-a (ATX-a)

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Attogene has developed a unique, robust, stable, and scalable competitive lateral flow assay capable of detecting the acetylcholine receptor ligand toxin Anatoxin-a. We have developed methods to stably assemble a functional recombinantly expressed acetylcholine receptor (AChR) surrogate to the human $\alpha 4\beta 2$ and $\alpha 7$ nicotinic acetylcholine receptor onto colloidal gold. The complex of AChR colloidal gold was next embedded into a release matrix pad and dried. These AChR bound gold release matrix pads were applied to backing cards containing nitrocellulose sprayed with a specially designed nicotine conjugate (test line) and an independent control line. A wick and sample pad were applied to the backed cards and then cut into strips. Using these strips, we demonstrate that in the presence of anatoxin-a and dihydroanatoxin-a the AChR bound gold specifically reduces its ability to associate with the nicotine conjugate (test line), resulting in a diminished test line signal. We have evaluated this response to be both concentration dependent and isomer specific. On the other hand, the test line was not diminished by microcystin or other non-AChR ligands. Attogene has also demonstrated this test is able to detect AChR ligand toxins present in *Oscillatoria brevis* (UTEX B 1567), *Anabaena flos-aquae* (UTEX 2557), *Anabaena subcylindrical* (UTEX LB2382), UTEX 2497, UTEX 1611, UTEX 3013 *Anabaena* algae cultures. The test is stable, robust and will be offered in lab and field deployable formats for customers to perform rapid screening and easy to use analysis of Anatoxin-a in the low ppb range.

Keywords: Acetylcholine Receptor, Anatoxin-a, Dihydroanatoxin-a, Lateral Flow

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Detection and quantification of cyanotoxins in the mucilage of natural *Microcystis* colonies

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In the natural environment, *Microcystis* colonies are embedded in a mucilaginous matrix composed of Extracellular Polymeric Substances (EPS). While most studies distinguish between the intracellular and extracellular forms of microcystins (MCs), intracellular MCs measurement typically performed by filtration quantify only free MCs, excluding protein-bound form. Here, we investigated the detection and quantification of MCs in the mucilage of a *Microcystis*-dominated bloom, employing methods to separate and quantify EPS attached to cells. The lowest particulate MC concentration was measured on the filter by classical filtration ($112.44 \pm 4.94 \mu\text{g L}^{-1}$), representing free particulate MC only. Centrifugation followed by lyophilization of the pellets slightly elevated particulate MC concentrations ($153.98 \pm 15.65 \mu\text{g L}^{-1}$). However, fixation and solubilization led to a further increase in particulate MC concentrations, ranging from 1.6 to 1.9 times higher than those obtained using classical filtration, depending on the duration of solubilization. Extended solubilization (10 to 60 min) and centrifugation to separate cells from mucilage demonstrated a significant contribution (up to 72%) of MCs bound to proteins or mucilage/attached EPS, indicating that these methods (lyophilization, centrifugation, fixation and heating) allow quantification of MC forms not captured by simple filtration. We also observed a shift in MC variants between the intracellular form and the mucilage. We discuss the impacts of mucilage-associated MCs on MC quotas, on the health of exposed/consuming organisms and on the degradation of MCs by heterotrophic bacteria.



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Hapalosins are produced by terrestrial symbiotic *Nostoc* sp. KVJ10

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In this study we show that a terrestrial cyanobacterium *Nostoc* sp. KVJ10 is producing hapalosins, cyclic depsipeptides previously shown to possess multidrug resistance reversing activity (1). *Nostoc* sp. KVJ10 was isolated from symbiotic organs of the hepatic *Blasia pusilla* L. from the subarctic island of Kvaløya, Troms, Norway (2). Analysis of the genome suggested presence of hapalosin operon. Indeed, the cyanobacterium constitutively produced three variants of the compound, one described earlier from *Haplosiphon welwitschii* (1), and two novel variants. Gene expression studies showed that the *hapA* was higher expressed under diazotrophic conditions, and its expression was influenced by the presence of other *Nostoc* strains. Three compounds with masses 489 Da, 501 Da and 503 Da were purified from the cell extracts. Structure elucidation performed by UHPLC-MS² and NMR confirmed that all those compounds were three variants of hapalosins. The HPLC fraction containing hapalosins showed antibacterial activity against *Streptococcus agalactiae*.

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Gene copy numbers impact the accumulation of cyanophycin in toxic and non-toxic *Microcoleus*

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Microcoleus is a genus of benthic cyanobacteria, globally distributed in freshwater ecosystems such as rivers and lakes. It can be problematic due to the production of anatoxins, which cause animal deaths. Unlike many cyanobacteria, which thrive in eutrophic waterbodies, *Microcoleus* typically proliferates in clean water with low phosphorus and elevated or slightly elevated nitrogen. Aquatic *Microcoleus* species are unable to fix atmospheric nitrogen, and genomic evidence suggests they employ other diverse strategies to obtain this nutrient¹. This study investigates the role of cyanophycin, an intracellular nitrogen and carbon storage molecule in *Microcoleus*, which may contribute to differences in ecological success among toxic and non-toxic species. Cyanophycin is an insoluble nitrogen-rich granule, which accumulates in cells when exogenous nitrogen is in excess, and conditions are otherwise unfavourable. Its synthesis and degradation are regulated by the *cphAB* genes, which encode cyanophycin synthetase (CphA) and cyanophycinase (CphB). Genomic evidence indicates that non-toxic strains have more copies of cyanophycin gene clusters (*cphAB*) than toxic strains². It is hypothesized that these extra copies enable enhanced synthesis and degradation and thus confer an advantage to non-toxic strains. To test this hypothesis, cyanophycin accumulation in toxic and non-toxic strains of *Microcoleus* was compared. Strains were starved of nitrogen and then cultured in MLA media with and without nitrate supplementation (plus/minus chloramphenicol to inhibit cyanophycin degradation)³. Granule formation was confirmed with transmission electron microscopy (TEM) and light microscopy using Sakaguchi staining⁴. Granules were only present in cultures provided with nitrate, and TEM indicated that the average granule number per cell was higher in non-toxic (2.82 ± 0.98 SD) than toxic (1.35 ± 0.58 SD) strains. Findings provide additional evidence that non-toxic *Microcoleus* have a greater capacity for intracellular nitrogen storage. This study will further explore the mechanisms of nitrogen and carbon storage in the ecological success of *Microcoleus*.

Keywords: Cyanobacteria, Intracellular granules, Nitrogen-storage

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Immunoreagents and immunoassays for the rapid analysis of dihydroanatoxins

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The most relevant anatoxins due to their toxicity and presence in environmental samples are anatoxin-a, homoanatoxin-a, dihydroanatoxin-a, and dihydrohomoanatoxin-a. These cyanotoxins are alkaloids with a characteristic 9-azabicyclo[4.2.1]nonane skeleton. This structure is substituted at C-2 with an acetyl group (COCH₃) in anatoxin-a and a propionyl group (COCH₂CH₃) in homoanatoxin-a. Both cyanotoxins are structurally related to their hydrogenated analogues between C-2 and C-3, i.e., dihydroanatoxin-a and dihydrohomoanatoxin-a. The main exposure route for humans and animals to these toxic compounds is through contaminated water, either via intentional or accidental ingestion; although intoxication through the consumption of dietary supplements containing cyanobacteria is also feasible [1]. The high acute oral toxicity of dihydroanatoxin-a and dihydrohomoanatoxin-a, as well as their presence in environmental samples, particularly during the spring [2], suggest that they represent relevant, emerging contaminants requiring close attention from organizations responsible for ensuring human health and animal welfare.

Currently, the recommended analytical technique for determining these cyanotoxins in samples is liquid chromatography coupled with high-resolution mass spectrometry. Nowadays, antibody-based techniques are considered complementary analytical strategies to instrumental methods when a high number of analyses must be performed in a short time, or when on-site testing and/or analysis in low-resource settings is needed. Recently, our research group published the development of the first immunoreagents specific of anatoxin-a [3,4]. These antibodies exhibit high specificity for anatoxin-a and homoanatoxin-a, yet, despite their relative structural similarity, they do not recognize dihydroanatoxin-a or dihydrohomoanatoxin-a. The aim of the present project is to synthesize functionalized derivatives (haptens) of dihydroanatoxins and to generate the first antibodies specific to these cyanotoxins. The obtained antibodies will enable the development of sensitive immunoassays for the detection of these new contaminants in environmental samples.

Keywords: Cyanotoxins; Hapten; Antibody; Immunoassay; ELISA

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Searching antibacterial activity of cyanobacteria: a contribution to the Blue-Green Age of antibiotic discovery?

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Antibiotic resistance is one of the most serious public health problems today. The reduction in the effectiveness of antibiotics against the growing number of resistant bacteria has not been accompanied by the development of new antibacterial drugs, creating a very upsetting scenario [1]. Returning to nature to discover new antibacterial compounds is a potential solution and most studies on new antibiotics have been conducted on plants, bacteria and fungi [1,2]. Several antibacterial compounds against pathogenic bacteria have also been identified in distinct cyanobacterial genera such as *Nostoc*, *Lyngbya*, *Fischerella*, among others [1,2]. However, their activity spectrum is often low and some are toxic for mammalian cells [2]. Besides, the potency and toxicity of many of them was not determined and the need to test other species and strains is recognized [2].

The Estela Sousa e Silva Algae Culture Collection (ESSACC) from INSA is a repository of 159 cyanobacterial strains belonging to 16 genus and 23 species, mainly isolated from Portuguese freshwater reservoirs, where cyanobacterial blooms are recurrent. ESSACC strains have been screened for their antimicrobial activity against relevant clinical and veterinary bacteria. Briefly, cyanobacterial biomass is produced in 5L cultures, and then concentrated by centrifugation and lyophilization. Dry biomass is extracted sequentially with distinct solvents (n-hexane, dichloromethane:methanol (2:1), methanol 70% and water). The resulting extracts are dried and redissolved in DMSO 25%. The antibacterial activity of the cyanobacterial extracts is evaluated by the Disk Diffusion Assay. We tested 42 cyanobacteria strains of 18 genera, corresponding to 277 extracts, against 60 pathogenic bacteria of 8 genera. We identified an inhibitory effect on the bacterial growth in 20% of the cyanobacterial strains. The bioactive cyanobacterial compounds are currently being characterized by LC-MS/MS.

Keywords: cyanobacteria, freshwater, culture collection, antimicrobials, pathogenic bacteria

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Microcystin production in *Scytonema cf. javanicum* from semi-arid river basins as adaptation to global warming scenarios

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Some species of cyanobacteria can produce toxins, secondary metabolites with adverse effects on various forms of life, including humans. The synthesis of cyanotoxins is not specific, but their production is regulated by environmental factors, as the same species may or may not produce them, depending on the environment in which it grows.

Scytonema cf. javanicum, which was found in different semi-arid watercourses, was subjected to different environmental conditions linked to climate change, such as increased nitrogen concentration, high ultraviolet radiation, increased salinity, and high temperature, in order to determine their effect on microcystin synthesis.

Scytonema cf. javanicum mats were incubated for 10 days in BG11 medium with different ammonium concentrations (0 mM NH₄Cl, 50 mM NH₄Cl, 500 mM NH₄Cl) and different values of temperature (20 °C, 30 °C, 40 °C, conductivity (2.09 mS/cm, 4.11 mS/cm, 8.17 mS/cm), and radiation (PAR=50.30 W/m², UVA=6.44 W/m², UVB=1.51 W/m²) with a 16:8 light:dark photoperiod except those receiving UVB with only 4:20 light:dark cycle.

Microcystins were extracted, analysed and identified according to an adaptation of the method of Faassen and Lüring (2013). Five types of microcystins were tested: MC-LR, MC-LW, MC-YR, MC-LF, MC-RR and only microcystin LW was detected in the samples examined. Principal component analysis showed that both nitrogen concentrations and salinity values conditioned LW microcystin synthesis by *Scytonema cf. javanicum* more actively than temperature and ultraviolet radiation.

These results suggest that extreme conditions in semi-arid river basins may lead to an alteration of the normal patterns of phytoplankton and phytobenthos succession, causing various effects that could induce significant changes in photoautotroph community structure.

Keywords: cyanobacteria, microcystin, *Scytonema cf. javanicum*, climate change, semiarid river

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Development and certification of a dietary supplement reference material for multiple classes of cyanobacterial toxins

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Algal dietary supplements are frequently prepared using cyanobacterial biomass obtained from non-axenic open air production lakes. Studies have shown that source materials and final products can contain cyanobacterial toxins that present a risk for human health [1] and some of these toxins are regulated in certain jurisdictions. Reference materials are therefore required to assist in the development and validation of analytical methods for raw material and product testing for both research and regulatory purposes.

Two dietary supplement certified reference materials (CRM) were prepared. The first using a typically-consumed non-toxic cyanobacteria (*Aphanizomenon flos-aquae*) in order to provide a negative matrix material (CRM-Cyano-A). The other was prepared by blending *Aphanizomenon flos-aquae* with a variety of toxic cultured cyanobacterial species to give CRM-Cyano-T, a material with a toxin profile including microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs) and saxitoxins (STXs). The blended matrix was freeze-dried and homogenized, then aliquoted into 0.6 g units. For characterization and certification, a novel multi-class liquid chromatography-mass spectrometry method (LC-MS/MS) [2] was used to evaluate homogeneity and stability of all toxin classes present. Strategies were developed for accurate value assignment of the various toxins, including optimized extraction and class-specific detection methods. Quantitation approaches included internal and external calibration with matrix effect compensation using matrix matched calibration, isotope dilution or standard addition. Certified values and associated uncertainties were assigned for analytes where calibration CRMs are available, with concentrations ranging from 1.3 (GTX3) to 74.9 (CYN) µg/g. Information values will be provided for additional cyanotoxins and secondary metabolites of interest.

Produced in compliance with international standards on reference materials, CRM-Cyano-T will play a role in development and validation of methods for nutritional supplement inspection and environmental monitoring.

Keywords: (reference material, cyanobacteria, cyanotoxins, characterisation, certification)

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P19 - 105

Feasibility studies on the production of calibration solutions for guanitoxin

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Guanitoxin (GNT) is a potent natural neurotoxin first identified in Canada and isolated from *Anabaena flos-aqua* (*Dolichospermum*) in the 1980s [1,2]. Access to GNT producing cultures or toxic field material is limited, and has impeded work on isolation of GNT for reference material development. The absence of GNT reference materials has significantly hampered work on the development of methods for sample screening and identification, and as result there is a poor understanding of GNT distribution in the environment.

In 2014, a cyanobacterial field sample was collected from Utah Lake (Utah, USA) following human and animal fatalities. While GNT was not formally identified as the cause of poisoning, it was detected using a recently developed liquid chromatography-tandem mass spectrometry (LC-MS/MS) multi-class method [3]. The identity was confirmed by high-resolution tandem-mass spectrometry, showing Δ 1.5 ppm mass error for $[M+H]^+$, and product ion spectra comparable to those in the literature [4].

Towards production of reference materials for GNT, an extract of the same field sample was taken through a series of purification procedures, including size exclusion chromatography, weak cation exchange and semi-preparative HPLC, to isolate GNT and associated decomposition products. The purified material was characterized by LC-HRMS/MS, and small-scale feasibility studies used to establish stability at a range of pH and storage conditions. Purified GNT from isolation on a larger scale will be used to produce a stock solution for structural confirmation. Quantitative NMR of the stock followed by accurate dilution will be carried out to prepare a calibration solution reference material for GNT. This will allow for quantitative LC-MS method to be established for GNT, which will aid in the identification and monitoring of GNT in environmental and drinking water samples.

Keywords: Guanitoxin, Anatoxin-a(s), isolation, stability, reference materials

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P20 - 137

Verification of Broad Cross-Reactivity of an ELISA for Microcystins using Nineteen Quantitative Reference Materials

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Microcystins are cyclic peptide toxins produced by cyanobacteria, and found all over the world. They are found in drinking water, recreational water, and can contaminate food such as vegetables and food supplements. Poisoning episodes resulting in severe liver damage in humans and animals may occur. Analysis is complex due to more than 300 reported variants of microcystins. Availability of standards is very limited, and few of these are genuinely quantitative. Therefore, a rapid and cost-effective analytical method that can capture all microcystins in one analysis is to safeguard public health and livestock is highly desirable.

The cross-reactivity to various microcystins is therefore an important factor in immunoassays when the analyte is such a large group of structurally similar peptides. Reliable standards are lacking for most of these 300. Most standards are purified intended for LC-MS analysis, and are often poorly quantified¹. In ELISA, even traces of other microcystin analogues may give rise to a higher cross-reactivity in the assay. Recently, a wide range of quantitative standards of microcystins and nodularins were reported as part of the validation of an LC-MS/MS method², but these standards are also ideal for validation of alternative methods, including ELISA.

An existing microcystin-ELISA³ was modified, resulting in excellent cross-reactivity to microcystins and nodularins. Our cross-reactivity studies demonstrate that the resulting ELISA exhibited broad specificity towards all available reference materials of microcystins and nodularins tested. Notably, the limit of quantitation for total microcystins in drinking water was 0.04 µg/L, well below the World Health Organization's maximum recommendation of 1 µg/L for long-term exposure. This ELISA provides an excellent research tool, quantifying total microcystins across diverse matrices, including drinking water, cyanobacterial cultures, tissue extracts, urine and algal bloom material. Moreover, its utility extends to the detection of metabolites and conjugates of microcystins. Thus, this microcystin-ELISA offers a sensitive and swift analytical tool for reliably screening large sets of diverse sample types.

Keywords: cross-reactivity, microcystin, ELISA, LC-MS, reference materials

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One-step solid phase extraction method for simultaneous targeted and untargeted analysis of cyanotoxins and other micropollutants in water samples

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This paper describes an one-step solid phase extraction method for the isolation of cyanotoxins, pesticides, polycyclic aromatic hydrocarbons (PAHs) and various other contaminants from environmental water samples. The proposed method simplifies the extraction of multiple micropollutants from a single sample by a less laborious extraction workflow than conventional methods. A general-purpose HLB cartridge (200 mg/6 mL) is used for the selective isolation of these compounds from water samples lakes, rivers, drinking water etc. The conditioning of the cartridges is carried out initially with dichloromethane followed by methanol and water for optimal analyte retention and extraction. After sample loading, the cartridges are dried under nitrogen flow to remove any residual water. The initial extraction is performed using methanol, which isolates a range of contaminants, including cyanotoxins, pesticides and other organic compounds. The second phase of extraction targets PAHs and is carried out by washing the cartridges with dichloromethane and collecting the sample in a different vial. Both extracts are completely dried under nitrogen flow and they are reconstituted using different solvent mixtures: 70:30 methanol/ water mixture and acetonitrile respectively. The extracted fractions are analyzed using high-performance liquid chromatography with fluorescence detection (HPLC-FLD) for targeted analysis of PAHs and ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-QToF-MS) for both targeted (DDA and DIA) and untargeted analysis. This method fulfills all the requirements specified by ISO/IEC 17025 and has been validated to ensure its reliability and accuracy. The mean recoveries are quite satisfactory, ranging from 50-110 % for pesticides, cyanotoxins and PAHs that were analysed. This multi-extraction method provides an efficient approach for the simultaneous isolation of a wide range of environmental contaminants, achieving high recovery and reproducibility. The use of advanced chromatography and mass spectrometry enables the sensitive detection and quantification of analytes, supporting both targeted and untargeted analytical workflows.

Keywords: solid phase extraction, cyanotoxins, pesticides, PAHs



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Unexpected occurrence of marine secondary metabolites in inland waters of post-mining lakes in north-west of Czechia

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Tropical oceans are well-known hotspots of mat-forming filamentous cyanobacteria from the order Oscillatoriales (sensu lato), the producers of a wide range of secondary metabolites. Among them bioactive oligopeptides and depsipeptides are of special interest. The dolastatins and their derivatives, a group of highly cytotoxic oligopeptides were as well for forty years regarded to be only of marine origin. Recently, dolastatin 10 derivatives aetokthonostatins were discovered to be produced by the invasive cyanobacterium *Aetokthonos hydrillicola* occurring in fresh-water reservoirs of subtropical United States¹. The discovery triggered the question, whether there can be other producers of such compounds inhabiting further fresh-water ecosystems.

Our metabolomic investigation (HPLC-HRMS/MS with GNPS comparison) of algal mats of inland waters of post-mining lakes in the north-west of Czechia determined production of a rich spectrum of cyanobacterial secondary metabolites. The most prominent group were oligopeptides known to be produced by tropical marine Oscillatoriales, including dolastatin 1, 14 and G derivatives. Their presence was confirmed by the MS/MS spectra (fragmentation analysis).

Originally, dolastatins were described from community samples and producers identified as taxa of the genera *Lyngbya* and *Symploca*. However, the genera are highly polyphyletic due to frequent morphological convergence in the oscillatorialean group. By recent metagenomic research, the actual producers of dolastatin 10 – *Caldora penicillata*² and dolastatin 11 derivatives lyngbyastatins – *Okeania* sp.³ were identified with the phylogenetic position retrieved. Such information for dolastatins 1, 14 and G and their derivatives is still missing. Complementary analysis of V4-V5 16S rRNA amplicons was conducted here and identified 30 amplicon sequence variants (ASVs) belonging to the order Oscillatoriales. Their phylogenetic analysis found previously undetected lineages e.g. from the marine groups Sirenicapillariaceae and Vermifilaceae. Present and following research can lead to the determination of the actual producers of less examined dolastatin derivatives and tracing of their impact on the fresh-water environment.

Keywords: algal mats, cyanobacteria, cytotoxicity, dolastatins, oligopeptide

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Paper Spray High-Resolution Tandem Mass Spectrometry (PS-HRMS/MS) for Rapid, Multi-class Cyanotoxin Analysis

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Traditional methods for cyanotoxin analysis rely on complex sample preparation and time-intensive chromatographic techniques, limiting their accessibility and cost-effectiveness for widespread monitoring. Here, we present a rapid (~2 minute/sample) paper spray-high-resolution tandem mass spectrometry (PS-HRMS/MS) method for the simultaneous, multi-class, quantitation of cyanotoxins requiring minimal sample preparation.

An in-house paper spray source was designed using a commercial electrospray source and 3D-printed parts, for use with Orbitrap mass spectrometers. Sample preparation consisted of freeze-thaw cell lysis, followed by spiking a 250 µL water sample with 5 µL of an isotopically labelled internal standards at 20 µg/L. Aliquots (10 µL) were applied onto VeriSpray paper strips and eluted with 90:10 MeCN/H₂O with 0.01% formic acid at 90 µL/min. Optimized mass spectrometry conditions allowed quantitation of five microcystins, nodularin, anatoxin and homoanatoxin, along with representative isotopically labelled analogues. A 2-min instrument method using product ion scans for each precursor mass allowed for selective detection of targeted analytes.

Method limits of detection were between 0.2 µg/L (hATX) and 2 µg/L ([Dha⁷]MC-LR), meeting regulatory standards for recreational water. These could be further improved for analysis of drinking water by using repeated sample spotting to achieve a linear increase in signal. Accuracy was assessed by analyzing 12 strains of laboratory cultured cyanobacteria with diverse toxin profiles over a wide range of concentrations, including two non-toxic strains used in dietary supplements (*Aphanizomenon* sp. and *Arthrospira platensis*). Excellent agreement was observed with LC-HRMS (R^2 of 0.98) and no significant false positives or negatives. Preliminary testing of environmental water samples (N = 30) also showed good agreement with LC-MS/MS, but interferences between some microcystins congeners highlight areas for further method refinement.

The developed methodology offers rapid analysis and the potential for cost-effective sample shipment on-paper, which could significantly improve access to cyanotoxin testing in the future.

Keywords: Cyanotoxins, Anatoxin-a, Microcystins, Mass Spectrometry, Direct Ionization

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Growth and cylindrospermopsin production in *Chrysosporum ovalisporum* under varying salinity conditions

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Cylindrospermopsin (CYN) is a potent cyanotoxin able to interfere with the cytochrome P450 system, impair protein synthesis and even damage DNA, generating hepatotoxic, neurotoxic and cytotoxic effects in mammals ¹. *Chrysosporum ovalisporum* is a potentially invasive cyanobacterium considered one of the most relevant CYN producers in the Mediterranean, tropical and subtropical regions ². Yet environmental factors are known to affect synthesis and extracellular release of CYN, there is no information on the effect of salinity on CYN production and release in this species. *C. ovalisporum* UAM292 was subjected to 3 salinities (BG11 medium supplemented with 0, 10 and 20 g NaCl L⁻¹) under which growth, total CYN and extracellular CYN (% of total) were analysed in semi-continuous cultures. *C. ovalisporum* survived up to 10 g NaCl L⁻¹, without statistical differences between 10 and control (0 g NaCl) regarding growth rate (0.21-0.22 day⁻¹), total CYN (5.6-6.5 µg mg⁻¹ dry weight) and extracellular CYN share (10-14% of total CYN). Chlorophyll *a* was significantly lower at 10 g NaCl L⁻¹ indicating a possible oxidative stress that was counteracted by *C. ovalisporum* and hence not reflected in growth. These preliminary laboratory results suggest that despite being a freshwater species, *C. ovalisporum* might potentially thrive in certain parts of the freshwater-marine interplay such as the mesohaline sections of estuaries, producing and releasing similar amounts of CYN as in its native freshwater habitat. Therefore, this risk should not be overlooked for water managements especially in estuaries with economical relevance (e.g., mollusc farming) located downstream of freshwater reservoirs affected by *C. ovalisporum* blooms.

Keywords: *ovalisporum*, cylindrospermopsin, salinity, estuaries, extracellular

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P25 - 212

An SPE-UPLC-MS/MS analytical method for microcystins monitoring in the raw and drinking water of the city of Thessaloniki

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Microcystins (MCs) are a group of heptapeptidic cyclic cyanotoxins produced by photosynthetic prokaryotic organisms called cyanobacteria. They are widespread in fresh waters and are known for their hepatotoxicity as well as their cancer promotion potential in case of chronic exposure of humans to low microcystin concentrations in drinking water.¹ The unusual Adda β -aminoacid, the cyclic structure and the non-esterified Glu residue present in these molecules, are often associated with their toxicity.²

MC-LR, being the most toxic among this group, has a guideline value in potable water of 1 $\mu\text{g/L}$ according to World Health Organisation.³ The same MC-LR guideline value is also included in the recent European Drinking Water Directive 2020/2184.⁴

Human exposure to microcystins may occur through a direct route such as drinking water¹, through recreational activities³ or hemodialysis⁵, or through an indirect route such as food.

Thus, systematic measurement and monitoring⁶ of MC-LR concentration in water resources and potable water is necessary especially when the water source is surface water as in the city of Thessaloniki where more than half of the drinking water comes from Aliakmonas river. The main aim of this study is the development and validation of a sensitive, multi-microcystin detection method for the systematic monitoring of four microcystins (LR, RR, YR and LA) in the drinking and raw water (both surface and ground water) of Thessaloniki. The need for increased sensitivity and elimination of matrix interferences led to the incorporation of an automated solid phase extraction pretreatment step to the method before the analysis of the samples with an Ultra-performance liquid chromatograph coupled to tandem mass spectrometer (UPLC-MS/MS). A quick and simple method with a reporting limit of 0,1 $\mu\text{g/L}$ for both matrices and all four microcystins fully validated according to ISO 17025:2017 in both levels, the level of limit of quantitation and the EU DWD parametric value of 1 $\mu\text{g/L}$.

Keywords: Microcystin, MC-LR, monitoring, UPLC-MS/MS, water samples

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Focusing on the removal of taste and odor compounds: MIB and Geosmin monitoring in raw and drinking water of Thessaloniki

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Global climate change effects, for example rising water temperature and human activities including agricultural nutrient loads, enhance more frequent bloom events of taste and odor (T&O) producing cyanobacteria, in water sources. Geosmin and 2-methylisoborneol (MIB) are the main cyanobacterial T&O compounds and can result in complaints from consumers at levels as low as 5-10 ng/L due to their earthy and musty odor.^{1,2} Since producers of T&Os, such as cyanobacteria, have seasonal fluctuations in many reservoirs, it is expected that occurrence of T&Os will also show seasonal changes.^{3,4}

Almost 60% of Thessaloniki drinking water is supplied by Thessaloniki Water Treatment Plant (TWTP) where Aliakmonas river water is treated. TWTP has a several step treatment process that includes pH reduction/pre-ozonation/coagulation/sand filtration/ozonation/GAC filtration/pH adjustment and chlorination. Back in 2008 the first T&O bloom event was observed and ever since both T&O compounds are systematically monitored by an accredited according to ISO 17025:2017 HS-SPME-GC-MS analytical method in raw and potable water. The limit of quantitation for both compounds and both matrices is 1.65 ng/L, and the laboratory reporting limit is 5 ng/L.

Regarding the results of the systematic monitoring of raw and drinking water of Thessaloniki, a seasonal variation of T&O compounds concentration is observed with peak concentrations (even above 100 ng/L) from September to January depending on the weather conditions. MIB's concentration is always larger than geosmin's ones in the same sample. During the several treatment steps of the TWTP process, almost 50% of these compounds are removed by pre-ozonation and the rest is removed by sand filtration probably due to biodegradation that occurs in the sand filters. During the last monthly 5-year monitoring, only three times MIB was detected in very low concentrations (5-10 ng/L) after sand filtration while no geosmin was ever detected in that treatment step.

Keywords: MIB, geosmin, taste and odor, HS-SPME-MS/MS, water samples.

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Lifetime and Degradation Products of Anatoxins in Long-Term Storage

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Anatoxin-a (ATX) is a potent neurotoxin produced by a variety of planktonic and benthic genera of cyanobacteria in both freshwater and estuarine systems that has been associated with canine and livestock casualties. The WHO has drinking water and recreational water recommendations for ATX as do many states in the US. The U.S. EPA has not set formal guidelines in part due to concerns about ATX stability in long-term toxicology studies. ATX and homoanatoxin (HTX) are unstable in aqueous solution during long term storage however the details of storage conditions and how that affects sample analysis results are poorly understood. Here we do a retrospective reanalysis of historical samples received by the laboratory over the past 10 years. These samples were lyophilized immediately upon receipt, extracted in 50% methanol/water acidified with 1% acetic acid and analysed by LC-MS/MS within one month of receipt. Part of the initial extract was also stored in cryovials at -20°C and -80°C for reanalysis at a later time. We observed transient increases of ATX concentrations with storage over short time scales as well as the degradation rates of ATX, HTX, dihydroanatoxin-a (dhATX) and dihydrohomoanatoxin-a (dhHTX) after longer time periods. Initial results suggest that after two years stored at -20°C, a major part of the ATX in these samples had degraded. These results have important implications for use quantifying uncertainty in measured concentrations of historical voucher samples and to examine the role of anatoxin as a causative agent in animal fatalities.

Keywords: Anatoxins, degradation, stability, long-term storage, analytical chemistry

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Synthetic Approach to Anatoxin-a and Related Congeners for Use as Certified Reference Materials

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Anatoxin-a is a highly neurotoxic alkaloid produced by several freshwater cyanobacteria species. Initially called "Very Fast Death Factor", anatoxin-a and congeners' presence in drinking water sources have been associated with widespread dog, livestock, and wildlife fatalities. The anatoxin family is highly toxic owing to the compounds' potency as neuromuscular blocking agents acting on the nicotinic acetylcholine receptor. While it was originally thought that variants of anatoxin were not of any biological concern, recent work has shown that homoanatoxin-a (the homologated form of anatoxin-a) and their dihydro derivatives are equally if not more toxic than the parent compound. The lack of high-quality analytical standards for these compounds remains a key limitation for robust detection methods, however, leading to inadequate toxicological studies and health advisory guidelines. Our goal for this project is the synthesis of substantial amounts of several anatoxin congeners including anatoxin-a, homoanatoxin-a, and their dihydro derivatives for use as certified reference materials. This will lead to improvements in the monitoring of and response to harmful algal blooms by expanding analytical detection methods of anatoxin-a and its congeners.

Keywords: harmful algal blooms, anatoxin-a, Nicotinic acetylcholine receptor, cyanobacteria, microcystins

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First Report of Canine Poisoning by Microcystins in Norway

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In August 2021, 3 dogs died and one dog became ill but eventually recovered, after swimming in Lake Akersvannet, Norway. Symptoms were severe vomiting and lethargy starting 4–5 h after the swim. The lake was subject to a *Microcystis aeruginosa* bloom at the time, and contained microcystins by ELISA. Water samples were collected and sent for chemical analysis. Samples of tissues were provided from the deceased animals by the responsible veterinarians after necropsy, and a series of urine samples were also obtained from the dog that survived. The tissue samples were homogenized and extracted with MeOH. The water samples, urine, and most of the tissue extracts were positive for microcystins by an ELISA with broad and excellent cross-reactivity for microcystins.^{1,2} These samples were then analysed with an untargeted LC–HRMS/MS method for microcystins that included mercaptoethanol derivatization, capable of detecting a wide variety of known and novel microcystin congeners.^{2,3} Only MC-LR was detected in the water, and in extracts of the liver, bile, small intestine, kidney, and spleen, but not in the urine, with highest levels in the liver and bile. Nor were any microcystin metabolites or conjugates detected by LC–HRMS/MS. The results are consistent poisoning by MC-LR produced by the cyanobacterial bloom in Lake Akersvannet. This is the first report of poisoning of dogs by cyanotoxins in Norway. This is also the first report from Norway in which only MC-LR was present in a cyanobacterial bloom; usually a more complex array of microcystins is present—often in the absence of detectable MC-LR. The urine of the surviving dog contained MCs weeks after the poisoning episode.

Keywords: Microcystin; Norway; dogs; poisoning

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Metabologenomics of *Raphidiopsis* species

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Raphidiopsis is a globally distributed bloom-forming cyanobacterium in freshwater systems. Although *Raphidiopsis* is a well-known cylindrospermopsin and saxitoxin producer, it also contains biosynthetic gene clusters (BGCs) to produce other secondary metabolites, including potential cyanotoxins. However, classification of these BGCs remains difficult as their products are undescribed. As (meta)genomic sequencing expands at a rapid rate, it is crucial to begin characterizing these unknown BGCs to facilitate fundamental research into their diversity and function within microbial communities. To do this, we used an integrated 'omics approach, leveraging metabolomics and genomics (i.e., metabologenomics) to characterize three strains of *Raphidiopsis* isolated from freshwater lakes in Florida, USA. Additionally, we coupled these data with genomic analyses of publicly available *Raphidiopsis* genomes. We identified BGCs using antiSMASH and characterized with BiG-SCAPE and minimum information about biosynthetic gene clusters (MiBiG) database. Screening of secondary metabolites from the three strains was undertaken using HPLC-MS/MS and analyzed using Global Natural Products Social Molecular Networking (GNPS) and cyanometDB. The outputs from these programs were used to link the BGCs with products using NPLinker and NPOMix. We identified nine uncharacterized BGCs shared among the three strains, which also occur in several other *Raphidiopsis* genomes. However, we only identified the cylindrospermopsin BGC in one of our analyzed strains. We herein explore the secondary metabolite diversity produced by these strains, and putatively link them to their associated BGCs. This research highlights the need to explore and identify unannotated BGCs and their secondary metabolites from cyanobacterial cultures.

Keywords: Biosynthetic Gene Clusters (BGCs), Secondary metabolites, (meta)genomic sequencing, Cyanotoxins, Cyanobacteria



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Quantification of gene involved in anatoxin A production and diversity of cyanobacteria in a French lake

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Blooms of cyanobacteria have been observed in the swimming area of a French lake (Saint-Cyr, 86) few years ago. *Oscillatoria* have been identified based on microscopic observation. Therefore, as anatoxin A could be produced by this bacteria, anatoxin A have been search and detected by ELISA. In order to better understand this phenomenon, we decided to use molecular methods to detect a gene responsible for anatoxin A synthesis and estimate cyanobacteria diversity in the lake. A PCR method has been designed on anaC gene (1) but to our knowledge no qPCR method was described.

In this study, we designed new qPCR primers, based on *anaC* gene sequences, to detect this gene in various cyanobacteria genera. The method was first validated using a collection strain of *Oscillatoria*. Then, qPCR allowed to detect this gene in several samples taken from the lake. The gene was more present in sediment as compared to water. Besides, a PCR amplicon, obtain from the lake samples, was sequenced and its sequence was 100% similar to sequences of *Oscillatoria* in databases. It confirmed that this toxin is likely produce by this genus in the lake.

Diversity of cyanobacteria was assessed by 16S rRNA gene amplicon sequencing. More than twenty genera were detected in sediments as well in water. *Cyanobium* sequences were in majority (64%). Also, sequences from *Oscillatoria* were present (4%), as well as other toxin producing genera such as *Pseudanabaena* and *Microcystis*.

In conclusion, we designed a new qPCR method to quantify anatoxin A-related gene, we confirmed that the toxin is likely produced by *Oscillatoria* in this lake and described an important diversity of cyanobacteria within the lake, including *Oscillatoria*.

Keywords: anatoxin, qPCR, diversity

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Analytical approaches for better mitigation of human and animal health threats from cyanobacterial toxins

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Cyanotoxins are well known to affect the health of humans and animals through drinking water, contaminated food stuffs and recreational/dermal exposure. Whilst detailed legislation exists describing risk management approaches for human health threats from naturally occurring marine biotoxins, relatively few guidelines are in place for mitigating against risks from the large range of cyanotoxins that have been reported in environmental samples to date.

This presentation will focus on a tool kit of analytical methods applicable to analysis of surface waters, algal/bacterial cells and aquatic foodstuffs for cyanobacterial toxins. Current monitoring in the UK is only conducted in direct response to reported blooms and takes place only through use of light microscopy, which is unable to determine levels of toxicity threat. This can result in significant risks to human and animal health as well as economic impacts when compliance decisions are taken without toxin confirmation. This talk will discuss the availability of current, validated techniques as well as other research tools, laboratory and field based, which are required in order to successfully mitigate against cyanotoxin outbreaks, within a rapid timeframe.

Specific case histories and key risk scenarios will be given to exemplify such approaches in the real world. This will include data that demonstrates rapid uptake and slow depuration of microcystins and nodularins in shellfish, as well as the occurrence of a range of neurotoxin analogues confirmed in biological matrices through both targeted LC-MS/MS and screening LC-HRMS methods. Approaches will be proposed for standardisation of such methods in both the laboratory and the field, including the preparation and analysis of matrix reference materials, with the ultimate aim to reduce the effects of cyanotoxins upon both human and animal health, as well as reducing socioeconomic impacts on industry.

Keywords: cyanotoxins, LC-MS/MS, analysis, method validation, risk mitigation

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Intra-specific response in akinete production under stress conditions in *Raphidiopsis raciborskii*

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Raphidiopsis raciborskii is a potentially toxic cyanobacterium, well known for its rapid spread, invading and expanding in many temperate aquatic ecosystems. One mechanism of spread is through the akinete, specialized cells that can resist unfavorable conditions for decades. The aim of this study was to evaluate the intra-specific variability in akinete production within four genetically different strains (MEL07, 26D9, 27F11, ZIE11). We imposed nitrogen-free conditions as a trigger for akinete production. The experiment ran for 30 days at 20°C (control) and 28°C (treatment) with a 12:12 h photoperiod. All four strains started the akinete production within the first days and increased their abundance towards the end of the experiment. We found strain-specific differences in the akinete abundance among strains and temperatures: in MEL07 the overall abundance of akinete was low, and at 28°C many akinete remained immature. Strains 27F11 and 26D9 produced a higher overall number of akinete, however, at the lower temperature. The strain ZIE11 responded similarly at both temperatures. All strains grew well and reached a higher biomass at 28°C than at 20°C. Despite the nitrogen-free conditions, the C:N ratio was constant among strains and temperatures suggesting substantial nitrogen fixation. The different response of the strains in the production of akinete demonstrates the high degree of phenotypic plasticity of *R. raciborskii* allowing for invading into various local environments. The role of akinete is still an understudied part of the cyanobacterial life cycle, but may be pivotal for the spread of *R. raciborskii*.

Keywords: akinetes, nitrogen limitation, temperature, stressor, *Cylindrospermopsis*.

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Seasonal succession of phytoplankton and driving factors of cyanobacteria blooms in shallow urban ponds in Brussels

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Small, shallow urban lakes play a crucial role in urban areas, providing important ecosystem services, such as water quality regulation, biodiversity support, and recreational opportunities, which are closely linked to the biomass and composition of phytoplankton communities. In several Brussels ponds, cyanobacteria blooms are recurrently observed, raising concerns due to their negative effects, including increasing turbidity, decreasing diversity and possible toxin production. To gain insights into the dynamics of phytoplankton succession and the drivers of cyanobacteria occurrence in urban water ecosystems, this study investigates the seasonal succession of phytoplankton groups (cyanobacteria, chlorophytes, cryptophytes, diatoms, chrysophytes, euglenophytes, and dinoflagellates) related to change of morphological and biogeochemical conditions. A chemotaxonomic analysis of phytoplankton communities in 20 ponds, conducted over four seasons within a year, revealed significant inter-pond variations in total phytoplankton biomass. Cryptophytes and chlorophytes dominated in most ponds, while a subset exhibited elevated cyanobacteria biomass, particularly during summer. High cyanobacteria biomass was associated with reduced macrophyte cover, long water residence times, and high turbidity. The two-year (bi)monthly analysis of four selected ponds provided a more detailed view of temporal variations in phytoplankton dynamics and amplitude of cyanobacteria blooms. When present, cyanobacteria blooms are dominated by genera *Dolichospermum*, *Microcystis*, and *Aphanizomenon*, identified via 16S rRNA sequencing with cyanobacteria-specific primers.

Keywords: cyanobacteria – urban ponds – phytoplankton – chemotaxonomy – seasonal succession

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***Gloeotrichia echinulata* genome sequences from the US indicate a tight species cluster of cyanotoxin-negative and geosmin-positive genomes**

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Gloeotrichia echinulata is a member of the Nostocales that is often associated with toxic HABs in the United States. Little is known about the properties of *Gloeotrichia echinulata* relevant to public health and the provision of clean drinking water (1). One report suggested that *Gloeotrichia echinulata* in a NE USA lake was a possible microcystin producer, although the source of detected microcystin was not verified. We have assembled genome sequences of *Gloeotrichia echinulata* derived from populations in three lakes in the Pacific Northwest and two lakes in the New England (NE) region of the US. The samples come from diverse geographies, from three different watersheds in the Pacific NW and from two different states in New England. The genomes are very closely related, with 98-99% pairwise genome-wide average nucleotide identity, well above the 97% cutoff between species. The genomes share many characteristics, including the absence of genes for the synthesis of the classic cyanotoxins (microcystin, cylindrospermopsin, anatoxin-a, saxitoxin, guanine toxin) and the presence of geosmin synthase. Variation in gene content or sequence often falls into two clusters comprised of the NW and NE genomes, although there is some overlap and the NW and NE genomes do not fall into clearly separate subclades in the phylogenomic tree. Although the current sample is small, it appears that *Gloeotrichia echinulata* in the US may exist as a tightly related clade with predictable properties that are important to public health and to drinking water utilities

Keywords: nontoxigenic cyanoHAB, geosmin producer; complete genome sequences

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More than just disorder - metabolite diversity of *Microcystis* strains shows tight correspondence to genotype and may contribute to ecotype specificities

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Microcystis is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide. This species remarkably produces numerous bio-active accessory metabolites, which are believed to be potentially involved with different ecological and/or physiological processes. Their genuine contribution to the evolutive success of *Microcystis* blooms remains undetermined.

To better depict the potential relation between the local genetic diversity of blooming *Microcystis* populations and the respective associated chemical diversity, we conducted a joined genomic and metabolomic analysis of 65 *Microcystis* strains collected from various lakes from France and European countries. Interestingly, both core- and noncore-gene phylogenetic analysis place 59 of these strains in 12 distinct genetic clades of at least 2 genomes, being widely distributed along the whole *Microcystis* phylogeny and presenting specific signatures of accessory metabolite biosynthesis. The chemical analysis of metabolite diversity produced by these strains, cultured under lab conditions, reveals the production of stable metabolite corteges, beyond little variations along replication, growth phases and culture conditions. Indeed, these strains belonging to 12 different genotypes correspond to 13 distinct metabolotypes according to an accurate one-metabolotype-for-one-genotype rule. This observation reveals that *Microcystis* collected from certain environments present a large set of genetic and subsequent corresponding metabolotype diversity, whereas all strains originating from certain other lakes present a net genetic uniformity. Overall, our investigations reveal that the production of accessory metabolites constitute well conserved chemical traits across the different *Microcystis* genetic clades, suggesting these molecules may be involved in key adaptative and selective processes, that still remains unspecified.

Keywords: cyanobacteria, accessory metabolites, diversity, ecology, cultures.

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The global rise in filamentous, benthic *Microcoleus* proliferations – a review of their ecology, toxin production and research needs

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Over the last two decades, the distribution, intensity, and frequency of *Microcoleus* proliferations in rivers, lakes, and reservoirs has increased markedly worldwide. *Microcoleus* often produces anatoxins (anatoxin-a, homoanatoxin-a, dihydroanatoxin-a, and dihydrohomoanatoxin-a), and ingestion of toxic mats has caused numerous dog fatalities globally and raised significant human health concerns. This global consortium came together to summarise current data on the distribution, taxonomy, toxin production, toxicity, ecology, environmental drivers, biotic interactions, and knowledge gaps related to *Microcoleus*. Anatoxins from benthic Oscillatoriales taxa have been documented in over 16 countries. These occur in diverse environments, from cobble-bedded streams to large sandy rivers, to reservoirs including those which are becoming more oligotrophic, and lakes, where proliferations are sometimes associated with macrophytes. Genomic studies have revealed multiple anatoxin-producing *Microcoleus* species/strains exist, with the most common species/strains exhibiting a global distribution. Anatoxin concentrations display substantial spatial and temporal variability both within and between waterbodies. Studies into drivers of proliferation are still relatively limited. However, research in streams and rivers shows that proliferations are most likely when there is some enrichment of dissolved inorganic nitrogen but when water-column dissolved reactive phosphorus is very low. Metagenomic studies are providing many new insights into the potential drivers, showcasing the unique strategies *Microcoleus* employs to access and store nutrients. Significant knowledge gaps remain, particularly regarding the factors driving the apparent global increase in *Microcoleus* proliferations, effects on the aquatic foodweb and potential control measures. Collaborative international efforts, such as this consortium, provide a valuable platform for sharing knowledge and techniques, accelerating research in this fast-moving field.

Keywords: benthic cyanobacteria, monitoring, anatoxins, *Microcoleus*, phenology

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Evaluating the *nifH* gene marker in cyanobacteria taxonomy: fragment length and pseudogene presence

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Cyanobacterial taxonomy, which continues to present a major challenge, has seen significant advancements in recent times owing to the use of molecular sequence data of appropriate markers. Many cyanobacterial species can fix atmospheric nitrogen, while it constitutes a characteristic of the order Nostocales, the taxonomy of which is further complicated by its notable diversity. The *nifH* gene, a structural gene of the main nitrogenase enzyme, is a widely used molecular marker of diazotrophic organisms, especially cyanobacteria. Despite this, our study showcases that the majority of cyanobacterial *nifH* sequences (86.8% of 6870 records) found in databases consist of a 350bp fragment of the 891bp gene. Phylogenetic analysis using the fragment and the complete gene revealed that the latter is a more accurate marker. Additionally, in 492 annotated genomes of five major Nostoclean genera (*Anabaena*, *Nostoc*, *Calothrix*, *Aphanizomenon*, *Dolichospermum*), an abundance of sequences characterized as "*nifH* pseudogenes" was found. These findings underline the need for further study of the *nifH* genes in cyanobacterial genomes, and the update of phylogenetic analysis towards the use of the complete gene, instead of the fragment.

Keywords: *nifH*; gene markers; pseudogenes; nitrogenase; phylogeny



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Investigating the ecology and temporal dynamics of *Microcoleus* species in a Swiss river

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Toxic benthic cyanobacteria, particularly *Microcoleus* species, have been implicated in domestic animal fatalities, primarily affecting dogs, worldwide¹. Research has focused on tools enabling toxin detection, genetics, or monitoring schemes for these toxic species²⁻⁴. However, the ecology of *Microcoleus* cyanobacteria remains underexplored. Therefore, this study aims to better understand the benthic microbial communities involving *Microcoleus*, potential interactions between species, and abiotic factors that might drive community structure. Over the course of a year, we collected benthic sediments from a Swiss river associated with canine intoxication. We extracted total environmental DNA and sequenced the microbial communities to identify dominant cyanobacteria. Additionally, we investigated potential anatoxin-a production using an Oscillatoriales-specific PCR targeting the *anaC* gene. Our results revealed that microbial communities appeared to be influenced more by site-specific factors rather than temporal changes. Bacterial communities were predominantly composed of cyanobacteria and proteobacteria. *Microcoleus* species were consistently present across all sites, showing significant correlations with other cyanobacteria, such as *Potamolinea*, and proteobacteria, such as *Rhodobacter*. Eukaryotic communities were mainly dominated by algae, with relatively high abundances of diatoms, rotifers, and nematodes. *Microcoleus* species also presented significant correlations with eukaryotic organisms, particularly *Pinnularia* diatoms. Certain abiotic factors, such as conductivity, sodium and calcium ions, followed similar seasonal trends as *Microcoleus* dynamics. Interestingly, no positive signal for *anaC* was detected in the benthic communities, suggesting the potential presence of both toxic and non-toxic species co-occurring. This research enhances our understanding of the ecology of *Microcoleus* species in lotic systems. However, further studies are necessary to improve species identification resolution, distinguishing between toxic and non-toxic *Microcoleus* species, to gain a deeper understanding of these cyanobacteria.

Keywords: *Microcoleus anatoxicus*, benthic, ecology, communities, anatoxins

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Cyanobacteria community status of two natural lakes in Greece

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Cyanobacteria are among lake environments' most abundant microalgae taxa (1). This study analysed an eight-year (2016-2023) time series of cyanobacteria data from two natural Greek lakes (urban Lake Pamvotida and Lake Zazari, the latter being under pressure from livestock farming and agriculture). According to the Water Framework Directive, the data were obtained from the national water monitoring network. The objectives of the present study were a) to investigate the composition of cyanobacteria taxa in both lakes, b) to present the inter-annual variations of cyanobacteria biovolume and c) to make a comparison between the two lakes. In Lake Pamvotida the maximum number of cyanobacteria taxa per sample were 18 (17/9/2020), and in Lake Zazari 19 taxa (18/7/2017). The genera *Aphanocapsa*, *Chroococcus*, *Pseudanabaena*, *Dolichospermum*, *Microcystis* and *Merismopedia* were the most common cyanobacteria taxa in both lakes. In the majority of the samples, in both lakes, the proportion of cyanobacteria was over 50% of the total phytoplankton biovolume, while in some cases it reached a percentage of about 95%. During the period of study, an increase in cyanobacteria biovolume was observed from the beginning towards the end of the summer season each year. Even though Lake Pamvotida is an urban lake, it displayed lower phytoplankton and cyanobacteria biovolume values than Lake Zazari. As cyanobacteria dominance is one of the most important and detrimental ecological effects of eutrophication (2), water quality monitoring can improve our knowledge of the status of cyanobacterial composition in Greek lakes.

Keywords: Cyanobacteria, Greek lakes, Phytoplankton biovolume, monitoring

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New taxa of filamentous rock-inhabiting cyanobacteria from Finland

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Cyanobacteria represent a group of photosynthetic bacteria found in a variety of habitats ranging from terrestrial and aquatic to extreme environments. Caves, cave-like environments, and rocks represent examples of such habitats. Cyanobacteria thriving in aquatic and soil habitats have been extensively studied. However, the number of studies dealing with terrestrial rock-inhabiting cyanobacteria is limited. The aim of this work is to investigate the diversity of epilithic filamentous cyanobacteria from non-studied terrestrial habitats in Finland.

Environmental samples were collected from different locations in Southern Finland including the historically and culturally important Suomenlinna Fortress (UNESCO) using sterile tools. Strains were isolated and studied by applying a polyphasic approach workflow, which included morphological (light microscopy), molecular (16S rRNA, 16S-23S ITS) and ecological data.

Thirty strains of filamentous cyanobacteria were isolated for the first time from wet and dry rocks as well as other sciophilic habitats in Finland. Nine of these strains belong to the order Nostocales and resembled *Nostoc*, *Calothrix* and *Roholtiella* whereas the remaining 21 strains were morphologically close to homocytous filamentous genera such as *Leptolyngbya*, *Pseudanabaena*, *Shackletoniella*, *Phormidesmis*, *Phormidium*, *Kamptomena* and *Microcoleus*. Based on 16S rRNA phylogenetic analysis, the strains shared 92–99.9% sequence similarity with previously described cyanobacterial taxa. These results combined with the study of ITS secondary folding structures, indicate the existence of at least 12 new taxa. Two new *Pseudanabaena* species, *P. epilithica* and *P. suomiensis* have already been described by applying a polyphasic approach workflow. These findings contribute to expanding cyanobacterial diversity and highlight the importance of studying terrestrial epilithic cyanobacteria.

Keywords: cyanobacteria, polyphasic approach, rocks, Suomenlinna Fortress, Finland

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Genomics at the forefront: A new dawn in the delineation of cryptic cyanobacterial species

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Cyanobacterial taxonomic research has been constantly refined over the years with the advent of new technologies and has now entered the fascinating 'genomics era'. The introduction of genomic data contributes to achieve a phylogenetically stable classification system and to determine the breadth of cyanobacterial metabolisms. In this work, we highlight the significance of using genomic data in the taxonomic studies of cyanobacteria through two scenarios.

In the first scenario, we demonstrate the advantage of a combined genomic and morphological investigation in the characterization of cryptic Nostoclean strains. A toxic cyanobacterium, *Kaarinaea lacus* PCC 9237 gen. nov., sp. nov., showed interesting morphological attributes such as unusual heterocyte patterning, trichome fragmentation at the site of multiple contiguous heterocytes and occasional proheterocyte division, a trait last reported in 1970s. The genomic analysis indicated the involvement of unknown gene(s) in heterocyte patterning and differentiation. Furthermore, the genomic investigation for biosynthetic gene clusters (BGCs) revealed a significantly different heterocyte glycolipid BGC, a rarely reported *pk2*-like-cluster and an anachelin-like-cluster in the strain.

In the second scenario, using the example of the order Pseudanabinales, we demonstrate the importance of genomic analysis in delineating cryptic species of *Tumidithrix* that cannot be distinguished by 16S rRNA gene and ITS rRNA analyses. Furthermore, the multilocus phylogeny confirmed *Limnothrix redekei* (type species) to be a member of *Pseudanabaena sensu stricto* (type species - *Pseudanabaena catenata*). In this work, we address the taxonomic complexity of *Limnothrix* and demonstrate the need for deep reclassifications.

Keywords: genomics, cryptic species, proheterocyte division, heterocyte glycolipid, *Limnothrix*

Acknowledgements and Funding

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Metagenomics study of extremophile cyanobacteria biodiversity in Bolivian Altiplano environments

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The Bolivian Altiplano exhibits extreme conditions for life, such as high altitudes, high UV-radiation, low precipitation, freezing temperatures, and saline and alkaline conditions (Fornari et al., 2001). Despite that, cyanobacterial mats thrive in these environments, but their diversity is completely unknown. This paper aimed to elucidate the morphological and 16S rRNA gene diversity of cyanobacterial mats from the Bolivian Altiplano, and their associated microbiomes. For that, eleven samples were collected. From these environmental samples, the 16S rRNA amplicons were sequenced using Illumina NextSeq. Their taxonomy was assigned using SILVA database. To obtain a more precise taxonomic classification of the Cyanophyceae taxa, a 16S rRNA Maximum Likelihood (ML) phylogeny and 16S rRNA identity comparisons were performed (Strunecký et al. (2023)). The morphological analysis was conducted under stereomicroscope and revealed seven morphotypes related to Nostocaceae, Coleofasciculaceae, Rivulariaceae and Microcoleaceae. The sequencing generated 310 16S amplicon sequence variants (ASVs), of which 134 were classified into 32 taxa within Cyanophyceae. From these sequences, the ML phylogeny and the 16S identity comparisons revealed forty-two different genera, distributed across nine orders. In addition, thirty ASVs were distributed across sixteen clades, which were not related to already known cyanobacterial genera and are potentially new taxa. The associate microbiome was composed by Alphaproteobacteria, Bacteroidia, Gammaproteobacteria, Clostridia, Cyanophyceae and Campylobacteria. The functional analysis revealed that the major functions of the microbiome encompassed phototrophic and chemoheterotrophic metabolisms, sulfur respiration, nitrogen fixation, nitrate and nitrite metabolism and fermentation. There was a prevalence of nitrogen-fixing cyanobacteria, alongside the presence of bacteria with bioremediation potential, such as *Shewanella* and *Pseudomonas*.

Keywords: bacteria; biodiversity; microbiome; extremophiles; functional analysis

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São Miguel Island as a Hotspot for Cyanobacterial Diversity: A Polyphasic Taxonomic Study

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Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC) is a biological resource centre located at Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), holding approximately 1.200 cyanobacterial strains, and serving as a valuable source for the scientific community¹. However, sampling campaigns and the isolation of new species and genera from diverse environments are crucial for enrich the culture collections^{2,3}. The aim of this study is to document the biodiversity of cyanobacteria genera isolated from diverse aquatic and terrestrial ecosystems, including extreme environments of São Miguel island. Cyanobacteria are considered a rich source of secondary metabolites with significant biotechnological potential, but they also produce potent cyanotoxins that pose risks to human health. Here, we employed a polyphasic methods, comprising 16S rRNA gene Maximum Likelihood and Bayesian Inference phylogenies, 16S rRNA identity (p-distance), 16S-23S ITS secondary structures, morphological and habitat analyses^{2,3}. The presence of microcystin, cylindrospermopsin, anatoxin, and saxitoxin also be assessed for all strains⁴. Samples were collected in 2021 from 63 different locations on the island, resulting in a total of 86 strains. Seventeen strains were identified as belonging to the Nostocales order, fourteen to Nodosilineales, thirteen to Leptolyngbyales, and forty-two strains were distributed across other seven orders⁵. Our results also identified eight potential new genera that can be described in the future. Also, six potential new species can be described. Thus, this work is a showcase of the crucial role of polyphasic approaches in the modern taxonomy of cyanobacteria, which provides an excellent framework to be applied, where not only the new biodiversity would be discovered but also the correct classification would be achieved.

Keywords: Cyanobacteria, Taxonomy, Azores Island, New Genus, New Specie

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Associations of invasive cyanobacterial species and phytoplankton community structure with abiotic influences in post-glacial temperate lakes under the climate change

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Eutrophication, driven by the growing human population, intensive agriculture, industrialization, and the effects of global warming, is causing significant negative changes in freshwater resources worldwide. One of the most concerning consequences is the replacement of native species by invasive ones, which can spread through various mechanisms [1]. Climate change is further exacerbating this issue by enabling many species to expand their ranges from lower to higher latitudes, thereby altering their geographic distributions [2]. Among the tropical invasive species identified in temperate Europe, *Raphidiopsis raciborskii*, *Chrysosporum bergii*, and *Sphaerospermopsis aphanizomenoides* present significant threats. Of these, *R. raciborskii* is the most concerning, having already become highly invasive in Western Poland. This freshwater, planktonic, filamentous, potentially toxic, nitrogen-fixing cyanobacterium is a major focus of concern [3]. This study aimed to investigate the presence of invasive cyanobacterial species in the post-glacial lakes, analyze the composition of phytoplankton communities, and assess the impact of environmental variables on the distribution of invasive cyanobacteria. Conducted in August 2023 across twenty-five lakes, the study spanned a geographical gradient from central Poland to the northeast, encompassing various depths, sizes, and trophic statuses. Our analysis identified *Raphidiopsis raciborskii* in five lakes, *Chrysosporum bergii* in three lakes, and *Sphaerospermopsis aphanizomenoides* in one lake. However, the biomass of all these species was low. In the second sampling performed during the summer of 2024, the biomass of the invasive cyanobacterial species was higher, although it remained lower compared to levels observed in Western Poland. The greatest phytoplankton biomass was found in the highly eutrophic Lake Suskie, followed by Lake Sztynorkie, where *R. raciborskii* reached its maximum abundance. Various environmental parameters were examined to assess their relationships with invasive species, while toxin analyses based on eDNA were also conducted, with statistical analyses performed to interpret the data.

Keywords: Biodiversity, environmental parameters, invasive Cyanobacteria, phytoplankton structure, *Raphidiopsis raciborskii*

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CyanoSeq: a curated reference database of cyanobacterial 16S rRNA sequences

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Since the advent of next generation sequencing methods (NGS), metabarcoding studies using targeted genes have become the most common approach for studying microbial community diversity. NGS methods provide insights not captured by traditional methods, allowing for increased resolution of microbial communities. However, diversity assessments require reliable reference databases for accurate assignment of taxonomic composition. While curated databases exist for bacteria, the Cyanobacteria remain poorly curated within them. The taxonomic rankings of Cyanobacteria provided within them are often incorrect as they are: not comprehensive, deficient in many well-described cyanobacteria, and do not resolve polyphyletic genera. The combination of uncomprehensive and incorrect taxonomy within current databases can lead to incorrect taxonomic assignment of metabarcoding reads resulting in misinterpretations of the cyanobacterial community. To ameliorate these issues, we present CyanoSeq¹ (<https://github.com/flefler/CyanoSeq>), a curated database of cyanobacterial 16S rRNA sequences for taxonomic assignment of metabarcoding reads. CyanoSeq is assembled from 16S rRNA sequences found within NCBI, with their taxonomies curated from cyanobacterial taxonomic literature as well as a systematic assessment of uncharacterized cyanobacterial sequences. In addition to curated cyanobacterial sequences, CyanoSeq is composed of plastid, bacterial, and Vampirovibriophyceae 16S rRNA sequences for more robust phylogenetic interpretations. CyanoSeq currently contains 4174 curated cyanobacterial sequences along with 123 chloroplast and bacterial sequences for use in classifying reads from metabarcoding studies.

Keywords: Database, metabarcoding, systematics

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Insights into the structure and function of a *Dolichospermum*-dominated cyanoHAB in Lake Okeechobee, Florida, USA

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Lake Okeechobee is a large eutrophic subtropical lake in Florida, USA, which frequently experiences cyanoHABs dominated by *Microcystis* and/or *Dolichospermum*. In May 2022, a *Microcystis*-dominated cyanoHAB occurred in the northwest region of the lake and reached its maxima in June. By August, the bloom had decreased in severity, which corresponded with decreased inorganic nitrogen availability and a shift in dominance to diazotrophic cyanobacteria (e.g., *Dolichospermum*, *Raphidiopsis*). While several known toxigenic taxa were identified, only microcystin was sporadically detected. During bloom attenuation, we sampled three different locations in August and September to gain insights into the community composition and their associated functional roles via metagenomic sequencing coupled with long term monitoring data. Results showed that in addition to aforementioned taxa, metagenome assembled genomes (MAGs) of several bloom forming genera (e.g., *Cuspidothrix*, *Sphaerospermopsis*) were recovered, along with a diverse bacterial assemblage. Annotation of functional pathways within the MAGs revealed enriched pathways within the bloom-forming cyanobacteria, primarily nitrogen cycling genes (e.g., nitrogen fixation). This, coupled with low concentrations of labile nitrogen (< 0.01 mg/L), suggests that these diazotrophs are a potential nitrogen source supporting the community. Analysis of biosynthetic gene clusters (BGCs) revealed one microcystin BGC with no other cyanotoxin BGCs recovered in the MAGs. However, several uncharacterized BGCs and BGCs belonging to various cyanopeptides were identified. Untargeted HPLC-MS/MS was applied to identify the secondary compounds that were produced during this bloom and their potential producers. These data provide insights into the structure and function of late stage (i.e., diazotrophic-dominated phase) cyanoHAB, highlighting the shifts in bloom phenology and their implications.

Keywords: metagenomics, biosynthetic gene clusters, *Microcystis*



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Trade-off between interpretability and predictivity for forecasting algal bloom using machine learning and in situ monitoring data

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Harmful algal blooms (HABs) are increasingly impacting the ecosystem of lakes, reservoirs and estuaries globally. The integration of real-time monitoring and machine learning (ML) technology has opened up new horizons for early warnings of HABs. However, the "black-box" nature of ML techniques often lacks ecological meaning. Our study is based on seven years of daily *in situ* monitored data algal community and physiochemical factors to model population dynamics of algal blooms in the Laohutan Reservoir (China) by applying three alternative machine learning (ML) techniques: HEA, XGBoost and LSTM. The hybrid evolutionary algorithm HEA is integrating genetic programming with differential evolution to develop predictive models represented by logic IF-THEN-ELSE-rules. XGBoost is an advanced version of random forests and models complex data by large ensembles of decision trees enabling collective predictions. Long short-term memory neural networks (LSTM) are recurrent NN consisting of multiple LSTM layers. After training by long-term time-series data, they perform short-term predictions. The three ML techniques are applied to the same training and testing data, and assessed regarding their predictive accuracy and interpretability. Our results suggested that different machine learning models, although employing different strategies to produce model interpretability, reveal similar findings behind inherent. It offers novel perspectives for managing HABs through online monitoring and artificial intelligence in aquatic ecosystems.



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Pole-to-Pole cyanobacterial diversity under climate warming: Evaluation of long-read sequencing for understanding community composition at species-level

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Cyanobacteria dominate polar microbial mat communities and play critical roles in carbon and nitrogen fixation, toxin production, and as structural habitats for diverse microorganisms. Despite vast geographic separation, Antarctic and Arctic cyanobacterial communities share remarkably high species overlap, suggesting long-distance dispersal, potentially facilitated by birds or aerosols. Understanding how these mat communities respond to climate-driven changes is crucial, yet species-level assessments remain challenging due to methodological limitations in standard Illumina-based 16S rRNA sequencing. Illumina sequencing, typically targeting short (~300 bp) variable regions (V3-V4), excels in precision but struggles to provide the taxonomic resolution needed for phylogenetically closely related cyanobacterial species.

We aimed to overcome these limitations by comparing Illumina MiSeq short-read sequencing with Oxford Nanopore Technologies (ONT) long-read sequencing using full-length 16S rRNA genes. Antarctic cyanobacterial mats were incubated under laboratory-controlled conditions simulating current (3°C) and predicted warming scenarios (6°C and 10°C) over 84 days. Samples were sequenced at three time points (0, 42, and 84 days) to assess temporal shifts in community composition on the species-level. Bioinformatic workflows were optimized by combining ONT sequencing with quality filtering tools (Porechop, fastQC, PycoQC) and error correction using the Natrix2 pipeline and the EMU taxonomic assignment tool, which leverages an expectation-maximization algorithm for improved accuracy.

Our results demonstrate that long-read sequencing significantly enhanced species-level resolution compared to short-read approaches, enabling precise tracking of temperature-induced species shifts within cyanobacterial communities. We conclude that methodological choices, including sequencing platform, read length, bioinformatic pipeline, and reference database selection, fundamentally influence interpretations of microbial community dynamics under climate stress scenarios.



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Identification and Monitoring of benthic cyanotoxin-producers in Austrian bathing water

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Studies focusing on monitoring programs of planktonic cyanobacteria have been increased in recent years, however, knowledge about the distribution and formation of benthic toxic cyanobacteria proliferation is still limited. One hindrance is the higher effort required to collect and process benthic samples, compared to the relatively straightforward process of filtering planktonic samples. Nevertheless, benthic biofilms can contain fatal concentrations of the hepatotoxic microcystin (MC), the neurotoxic anatoxin-a (ATX) and homoanatoxin (HATX), as well as the cytotoxic cylindrospermopsin (CYR). In general, benthic toxic biofilms occur sporadic under oligotrophic and clear water conditions and are often overlooked unless cases of animal poisonings, especially dogs, are reported. Those animals suffered from signs of paralysis and even death after contact and uptake of algae from biofilms containing toxin-producing cyanobacteria. The aim of this presentation is to systematically record the distribution of benthic toxic cyanobacteria in Austrian bathing waters in relation to standard abiotic and biotic factors (e.g. daily variation in water temperature, Secchi depth, nutrients and substrate characteristics). During the summer seasons of 2022 and 2023, in total 84 benthic samples have been collected from 20 bathing sites. The DNA of the biofilm was extracted and sequenced with the use of universal 16S primers. A customized reference database was compiled, containing 150 toxic benthic strains which have been used for the taxonomic assignments of the ASVs. Furthermore, PCR was used to check for the presence of gene fragments indicative of toxin-producing genes, i.e. *anaC* indicative of (H)ATX production, *cyrJ* indicative of CYR production and *mcyE* indicative of MC production. Additionally, the cyanotoxin concentration of the biofilms was measured via HPLC-MS. The first results indicate that the overall abundance of toxigenic taxa in the studied bathing waters was low, wherein *Microcoleus autumnale* and *Tychonema bourellyi* occurred most frequently

Keywords: EU Bathing-Water Directive, Metabarcoding, genetic basis of toxin production, PCR product sequencing, HPLC-MS

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Planktonic and benthic cyanobacteria under participatory monitoring

Project owner: **Public Territorial Establishment of the Sèvre Nantaise catchment area (France)**

Partners and experts associated and/or consulted for this project:

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Since 2019, the public institution of the Sèvre nantaise catchment area (France) has implemented a participatory approach to visual observations following major blooms of cyanobacteria in rivers in 2018.

Canoe/kayak, fishing and wild swimming are the main activities on these rivers, likely to be impacted by these blooms. Volunteer observers are public service employees, users (fishermen, etc.), managers of drinking water supply or residents previously trained in the visual recognition of cyanobacteria. An online tool allows them to submit their observations directly in the field (data and photo) and ensures the instantaneous updating of an online map. The use of a phycocyanin probe by employees of public institution helps to validate the observations in case of doubt. This action therefore allows the population to be informed every summer from June to October of the presence of planktonic and benthic cyanobacteria at stations located on rivers and/or lakes (via the mapping consulted or by an SMS or email alert tool). Today this action involves the participation of 28 people and concerns 79 stations. This information collected improves knowledge of the state of the aquatic environment and promotes the prevention of health risks. This participatory approach is also a way of raising awareness of cyanobacteria-related issues.

Keywords: cyanobacteria, rivers, participatory approach, phycocyanin



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Control of planktonic cyanobacteria in preventive treatment with hydrogen peroxide to maintain recreational activities in freshwater lakes. 9 years of experimentation in France. Implementation of an application method

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Curative methods for controlling cyanobacteria are very uncertain and since Matthijs' work in 2012, the application of hydrogen peroxide appears to be an effective limiter of cyanobacteria biomass if it is carried out on a low biomass generating a low concentration of H₂O₂. This experiment was carried out in France on 3 lakes. Only one has been the subject of a recurrent action of application of H₂O₂ for 5 years to maintain nautical activity following a ban on activities in the presence of a bloom on the entire body of water and microcystins (> to 50 µg/L) by the genus *Microcystis*. This urban lake has a volume of 70 000 m³ and an average depth of 1.80 m. The method involves weekly monitoring from April to October of pigments in the water column (phycocyanin and chlorophyll) using specific sensors (Trios, Trilux, Ysi), and phytoplankton analyses to characterize the dynamics of cyanobacteria and species present and possibly microcystins.

At the threshold of 10 µg/L phycocyanin, an application for a total concentration of 5 mg/L of H₂O₂ is made over the entire water body. Closed to all activities for 24 hours, their resumption is only done if H₂O₂ is < to 0.5 mg/L and an absence of microcystins, analysed in the field by strip tests. In each case, the cyanobacteria abatement is 99%. A trial at 2.5 mg/L of H₂O₂ only allowed a reduction of 47%, prompting rapid reapplication. The results of the 5 years are: no cessation of nautical activities, no blooms, no problem with odors such as Geosmin or MIB, no fish mortality, recurrence of the different species of phytoplankton apart from the different species of *Microcystis*, which makes it a major result because of their ability to produce microcystins. Another essential point in this experiment was to highlight the natural production of H₂O₂ around 1 mg/L by cyanobacteria.

Keywords: Hydrogen peroxide, *Microcystis*, freshwater lake, cyanobacteria, phycocyanin

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Lake Põrmujärv – a heavily polluted water body forced local active residents to seek for scientific solutions

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We present a case study on Lake Põrmujärv, a central part of the small settlement near the city of Võru (Estonia). The lake's ecological status is currently very poor, characterized by high nutrient concentrations, low oxygen levels, and significant cyanobacteria biomass. Cyanobacterial biomass forms 80-98% of the phytoplankton biomass, ranging from 39 to 195 g WW/m³. The dominant cyanobacterium, *Planktothrix agardhii*, constitutes over 90% of the biomass in all seasons. Locals of the village have reported heavy, unknown pollution leading to unpleasant odours and extensive eutrophication during hot and dry periods. This pollution has resulted in the lake losing all ecosystem services, decreasing the attractiveness of the local area, and negatively impacting the well-being of residents. Consequently, the economic value of properties in the vicinity has significantly decreased, further affecting the local economy.

Lake Põrmujärv holds high public interest and is surrounded by active residents whose enthusiasm motivates experts to seek solutions. A research and restoration project BioFloat aims to design and test floating islands in three lakes in Estonia and Latvia. These human-made structures, with dense aquatic vegetation, serve multiple functions, including water purification, habitat provision, and landscape enhancement. Our objective is to improve the health of urban water ecosystems and enhance biodiversity richness. In Põrmujärv, we also aimed to investigate the bloom-forming and potentially toxic *Planktothrix agardhii* interaction with the aquatic food web. We collected zooplankton and fish samples to assess the contamination with microcystin-producing cells within variable consumers.

The BioFloat project will directly address the EU Water Framework Directive's goal of achieving good ecological status for all lake water bodies by 2027.

Keywords: Eutrophication, Cyanobacterial blooms, Ecosystem services, Floating island

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In Vivo Potential Androgenic Effects Induced by Pure Cyanotoxins (Microcystin-LR, Cylindrospermopsin)

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Potential androgenic-disrupting properties of cyanotoxins, such as cylindrospermopsin (CYN) and microcystin-LR (MC-LR) are of concern due to their increasing occurrence, the scarcity of reports on the topic (particularly for CYN) and the impact on human's health at different levels. Thus, this work explore the (anti)androgenic properties of CYN and MC-LR for the first time applying the OECD test guideline 441. For this purpose, the Hershberger assay was performed. In this trial, 2 groups of castrated rats for the agonist assay were established and treated by gavage with 75 and 300 µg/kg of CYN or MC-LR. For the antagonist assay, 3 groups treated with 75, 150 and 300 µg/kg of CYN or MC-LR and 0.4 mg/kg of testosterone propionate were established. The parameters measured were the body weight, water and food consumption, and the weight of 5 androgen-dependent tissues (ventral prostate, seminal vesicle, levator ani-bulbocavernosus muscle, paired Cowper's glands and the glans penis). In addition, the levels of different steroid hormones (testosterone, estradiol, follicle-stimulant hormone, luteinizing hormone and progesterone) and thyroid hormones (T3, T4 and free-T4) were measured. Results revealed a few significant changes in some of the weights of the different tissues after the exposure to the toxins but the acceptance criteria (significant changes in the weight of two of five tissues) were not reached. The most relevant hormonal changes were found in T, LH and FSH levels. Respect to the levels of thyroid hormones, only one significant change in comparison to the negative control was found in the 75 µg/kg CYN group (agonist assay). Taken together, these results point out that both toxins are not androgenic compounds at the conditions tested. However, more studies are necessary to determine their androgenic properties

Keywords: Cylindrospermopsin; Microcystin-LR; Androgen; Hershberger

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In Vitro Evaluation of the Androgen Receptor Transactivation by Arsenic, Cadmium and their Binary Combinations with Cylindrospermopsin

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Cylindrospermopsin (CYN) is a cyanotoxin produced by cyanobacteria that is of increasing global concern due to its bioaccumulative capacity and toxic effects. Previous studies suggest that there is an interaction between this toxin and other chemical contaminants found simultaneously in nature, and it is important to determine the effect of combinations of these compounds. The aim of this study was to evaluate the effects of arsenic (As), cadmium (Cd) and their binary combinations with CYN on the androgen receptor (AR) transactivation using the AR-EcoScreen cell line (OECD protocol 458). Firstly, a cytotoxicity test (MTS) was performed. Viability results showed a significant reduction in cell viability after exposure to As and Cd for 24 h, with EC₂₀ values of 19.89 ± 3.23 and 1.98 ± 0.20 μ M, respectively. For the transactivation assay, non-cytotoxic concentrations of each compound were selected. With regard to the agonist activity, for both compounds (As and Cd) and for their combinations with CYN, the levels of response induced were less than 10% of the response induced by the positive control (5 α -dihydrotestosterone). In the antagonist assay, both As and Cd showed antagonist activity by inhibiting AR activation at the highest concentration (1.9 and 0.19 μ M) by more than 30% compared to the positive control (hydroxyflutamide). In the case of combinations with CYN, both of them showed to inhibit the AR more than 30% in a similar way of compounds alone did. These results suggest that neither As nor Cd has agonist activity on the AR, while both compounds and their binary mixtures with CYN have antagonist effects under the conditions tested. However, further studies are needed to determine the endocrine properties of the binary combinations.

Keywords: Cylindrospermopsin; Arsenic; Cadmium; Androgen Receptor

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Employing Ozonation as a Pre-oxidation Method for Managing Toxic Cyanobacteria and their Cyanotoxins

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Cyanobacteria, commonly known as blue-green algae, remain a significant concern due to their persistence in aquatic environments and their ability to produce toxic metabolites (cyanotoxins). Nutrient enrichment from human activities, such as agricultural and sewage runoffs, drives the proliferation of these cyanobacterial blooms, which are increasing globally and pose significant risks to water quality and safety, ecosystem balance, and public health [1]. Cyanotoxins, such as microcystins and anatoxins, are associated with severe health impacts, including liver damage, gastrointestinal distress, and neurological effects [2]. These toxins also disrupt aquatic ecosystems and limit the use of water resources for recreation and fisheries [3]. This study investigates the effectiveness of ozonation as a pre-oxidation method for managing toxic cyanobacteria in drinking water treatment plants. Ozone not only disrupts cyanobacterial cell structures but also degrades cyanotoxins, offering a dual-action approach to controlling blooms [4]. Herein, we evaluated the response of three cyanobacteria species (*Microcystis* sp., *Aphanizomenon* sp., and *Cylindrospermopsis* sp.) and one green alga (*Scenedesmus obliquus*) to ozone exposure under controlled conditions (0.5–4 mg/L ozone, 20°C). Cyanobacteria exhibited greater sensitivity to ozone compared to green algae. A 4 mg/L ozone dose effectively disrupted cyanobacterial biomass without recovery, whereas lower doses allowed recovery within three days. Moreover, the age of cyanobacterial cultures significantly influenced their response to ozone treatment. These findings highlight the potential of ozonation as an efficient and scalable method for managing toxic cyanobacteria in water treatment systems. By targeting both cyanobacterial cells and their toxins, ozonation can be crucial in safeguarding water quality and addressing the global challenge of harmful algal blooms.

Keywords: cyanobacteria, cyanotoxins, ozonation, water treatment

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Toxic benthic cyanobacteria in Southern German lakes

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Benthic cyanobacteria have gained increasing attention in recent years, particularly due to toxic blooms, which led to deaths of mammals. For instance, in 2019, three dogs died during a massive bloom of the benthic cyanobacterium *Tychonema* in reservoir Mandichosee (River Lech, Germany)¹.

To improve the understanding of the distribution and toxic potential of benthic cyanobacteria, particularly within the genus *Tychonema*, aquatic biofilms were collected over several years from reservoir Mandichosee and from various lakes in Southern Germany. The cyanobacterial communities in these benthic samples were characterized through a combination of microscopy and 16S rRNA gene sequencing. Furthermore, the presence of specific genes coding for the biosynthesis of anatoxin, cylindrospermopsin, microcystin and saxitoxin was assessed using PCR with gene-specific primers. Toxin concentrations in selected samples were quantified by LC-MS/MS comparing the results with concentrations examined by ELISA.

Our results demonstrate that benthic cyanobacteria are widespread in many water bodies across Southern Germany and are beyond the point of being single occurrences. In several lakes, both toxin genes and their corresponding toxins were detected, highlighting the significant toxic potential of this group next to pelagic cyanobacteria. Toxin-producing strains of *Tychonema* were found not only in reservoir Mandichosee, but also in additional lakes, thereby expanding our understanding of their distribution range. Anatoxins were regularly detected in reservoir Mandichosee, with concentrations up to 68,000 µg/L in samples with high biomass. Anatoxins produced by the genus *Tychonema* consisted of up to 99 % of the variant dihydroanatoxin-a, which is not detectable by standard toxin ELISA tests.

Our findings underline the need for increased scientific attention towards benthic cyanobacteria, particularly in relation to their role in toxin production. Furthermore, they emphasize the importance of ongoing monitoring and research into the ecological and public health risks posed by these organisms.

Keywords: cyanobacteria, benthic, anatoxins, lakes, *Tychonema*

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P60 - 94

Empowering natural resource professionals to combat harmful algal blooms in privately-owned recreational and agricultural ponds

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Harmful algal blooms (HABs), particularly cyanobacteria that produce toxins, are increasingly affecting small ponds and lakes, posing significant risks to humans, pets, livestock, and wildlife. Rapid identification and response are critical, as detrimental health impacts can occur within hours of exposure. However, natural resource professionals—often the primary resource for rural residents and agricultural communities—have historically lacked training in HAB identification, risks, and mitigation strategies. To address this gap, Cooperative Extension staff from Clemson and South Carolina State Universities collaborated to develop an innovative train-the-trainer program to empower natural resource professionals to provide timely, informed responses to HAB events. This program combines in-person and on-demand online training courses with practical resources, including a quick-reference HAB manual and a waterproof field guide. These tools provide clear, actionable information for rapid HAB identification, response, and mitigation. The program also introduces a novel approach to accelerate HAB confirmation. By equipping Extension Agents with training and field-ready diagnostic tools, the initiative enhances collaboration between local professionals and the Clemson University Plant and Pest Diagnostic Clinic, shortening confirmation times and enabling swift, evidence-based responses. Since implementation, the program has improved HAB awareness, response capacity, and confidence among participants. Natural resource professionals now play a pivotal role in identifying blooms, mitigating risks, and educating residents who rely on small ponds for agricultural and recreational purposes. This project underscores the importance of equipping frontline professionals with targeted training and accessible resources to address the growing HAB threat effectively. The program's scalable model holds promise for other regions grappling with similar challenges, fostering proactive responses to safeguard water quality and public health in vulnerable communities.

Keywords: Harmful algal bloom response, public education, stakeholder engagement, train-the-trainer, privately-owned ponds

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Ultrasound as a tool for cyanobacterial control: Evidence of changes in the phytoplankton community

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In the last 20 years, ultrasound technology has emerged as an effective solution to control harmful algal blooms. Ultrasonic waves control these algae's growth by matching the algal cells' resonance frequency, which affects their cell structure. In cyanobacteria, this causes the rupture of gas vesicles and sedimentation of cells, disrupts cell division, and negatively affects photosynthesis, inhibiting their growth and modifying cell size and morphology.

By reducing or eliminating the dominant cyanobacteria in the phytoplankton community, habitat and resources are freed up that can be for other non-harmful algae to utilize, generating a change in the ecosystem conditions.

This paper presents ultrasound results for algae control in various aquatic ecosystems across different geographical regions and latitudes. The case studies include water bodies with different uses, such as deep and shallow recreational lakes, shrimp culture ponds, irrigation ponds, and water treatment ponds. Physicochemical parameters of the water column and microalgae composition were monitored periodically. After exposure to ultrasound waves, changes were observed in the taxonomic composition of the microalgae and their relative contribution within the community, with increases in species diversity and richness. In addition, improvements in water quality parameters such as transparency and pH were identified.

Ultrasound acts as an external disturbance that induces changes in the phytoplankton community, proving to be an effective tool to improve water quality by controlling harmful algae and cyanobacteria.

Keywords: Harmful algae and cyanobacteria control, ultrasound technology, lake restoration

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P62 - 85

Photocatalytic removal of *Raphidiopsis raciborskii* cells, saxitoxin and neo-saxitoxin using g-C₃N₄ coated beads and low-cost LED irradiation

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Access to safe drinking water has been an increasingly environmental and public health issue. Cyanobacterial blooms can produce saxitoxins, which are potent neurotoxins capable of causing severe illness and occasionally death [1]. There is an increasing effort to develop water treatment alternatives to efficiently remove cyanobacteria and its toxins from drinking water reservoirs. Conventional water treatment might be inefficient for the removal of cyanobacteria and cyanotoxins, therefore, it is necessary to evaluate complementary technologies that can be applied at source. Photocatalysis is a technology that can be used for the removal of cyanobacteria and cyanotoxins, however, the recovery of the powdered catalyst from water and the high costs associated with the implementation of UV illumination represent challenges for the application of photocatalysis at source.

To overcome the issue involving the recovery of powdered catalyst, porous beads made of recycled glass were previously used as photocatalyst immobilization matrix in a successful mesocosms-study in a drinking water reservoir for the photocatalytic removal of cyanobacteria [2]. In the current study, the same type of beads was coated with graphitic carbon nitride (g-C₃N₄) and activated by low-cost LEDs (£0.075/LED) to remove *Raphidiopsis raciborskii* (ITEP-A1) cells and toxins, saxitoxin (STX) and neo-saxitoxin (NEO), for 72h. A one-month-old culture of cell density of 40x10⁶ cells/mL (intracellular STX 17.6 µg/L, NEO 14.2 µg/L and extracellular STX 23.7 µg/L, NEO 6.3 µg/L) was used. The photocatalysis of *R. raciborskii* was demonstrated to be effective with photosynthetic activity significantly decreasing by 60% within 3h of exposure and no photosynthetic activity after 24h treatment until the end of the experiment at 72h. Within 24h of photocatalysis, *R. raciborskii* cells were completely removed and no STX and NEO were detected in the treatment samples. The results demonstrated rapid destruction of cyanobacterial cells and toxins providing a promising alternative water treatment technology.

Keywords: Photocatalysis, Cyanobacteria, Drinking water, Cyanotoxins, Saxitoxin

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Simple and Efficient Risk Assessment of Microcystin Variants in Fish Using a Single-Domain Antibody Preconcentration and MALDI-ToF detection

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Microcystins (MCs) can occur in high levels in fish and shellfish, posing a significant threat to consumers. However, the complexity, cost, and accessibility of the reference LC-MS/MS methods limit their routine use for risk assessment. Therefore, it is essential to develop simpler methods to identify and quantify individual MC- variants and their less toxic metabolites. Matrix-Assisted Laser Desorption/Ionization (MALDI-ToF) enables rapid, high-throughput analysis with minimal sample preparation, making it ideal for large-scale monitoring at low operational costs. To enhance selectivity and sensitivity, we developed a MALDI-ToF method that integrates an efficient purification and pre-concentration step using an immobilized high-affinity single-domain antibody for MCs, immobilized on magnetic beads (1). The MCs present in the fish extracts adsorbed to the beads with an immobilized internal standard can be rapidly identified and quantified by MALDI-ToF (2). The Method Reporting Limit for MC-LR, RR, YR, and WR was 0.3 ng/g ww. Since it corresponds to 1.3% the WHO Tolerable Daily Intake for a 60 kg adult (TDI = 24 ng/g for 100 g daily fish consumption), it is highly sensitivity for risk assessment. Fish muscle samples were collected over three years from the Negro River Basin, an eutrophic watercourse in Uruguay, where intermittent cyanobacterial blooms in summer and autumn can reach maximum MCs concentrations of 20,000–40,000 µg/L. The predominant MCs were MC-LR in the first and third campaign and [D-Leu1] MC-LR in the second. The median of results for the sum of variants was 3.8 ng MCs/g fish ww (p25% 1.0- p75% 27.6 ng MCs/g, n=83), evidencing the relevance of a routine monitoring for risk assessment. The reliability of the method was shown by the excellent correlation observed for the intercomparison with LC-QToF MS/MS (n=29): $r^2 = 0,995$ for MC-LR, 0,914 for [D-Leu1] MC-LR, 0,941 for MC-RR and 0,877 for the sum of variants.

Keywords: Microcystins, Fish, MALDI-ToF, Risk assessment

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Bacterial communities as important biological elements for nutrient-cycling in sequential-sedimentation biofiltration systems – a nature-based solution preventing accelerated eutrophication

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Nutrients (N), including nitrogen and phosphorus, are important elements promoting accelerated eutrophication of downstream reservoirs, consequently leading to the appearance of toxic cyanobacterial harmful blooms (chBs). The aforementioned problem results from different anthropogenic pressures surrounding lowland reservoirs. Furthermore, global climate change is expected to exacerbate the problem, and therefore, there is an urgent need for effective solutions. Sequential-sedimentation biofiltration systems (SSBSs) have been implemented to treat stormwater runoff from agricultural and urban areas. These multizone biofilters include sedimentation, macrophyte biofiltration, biogeochemical barriers (dolomite, limestone or a ceramic-calcite polymer "bioker") and denitrification barriers (brown coal) enhancing biological processes to transform/adsorb nutrients from polluted water (nature-based solution). Efficiency of SSBSs have been proved with average reduction of ammonium, nitrates and phosphates (up to 59.8%, 91.3% and 35%, respectively)^{1,2,3}. Microbial communities, especially bacteria, were noted to play an important role in N-cycling in SSBSs. Therefore, the aim of this study was to reveal favourable conditions promoting higher abundances of their communities in biofilms from SSBSs. Collective knowledge, using PCR analyses targeting functional genes involved in N-transformation, revealed that biogeochemical barriers containing limestone promoted higher abundances of nitrifiers (*amoA* gene) and phosphorus accumulating bacteria (*ppk1* gene)^{1,2}, while denitrifying barriers containing brown coal promoted denitrifying bacteria (*nosZ* gene)^{1,2}. Amplicon sequencing analysis (16S rRNA) suggested that *Crenothrix* and *Nitrospira* were associated to nitrification^{2,3}, and *Rhodoferrax*, *Hydrogenophaga*, *Flavobacterium* and Burkholderaceae to denitrification^{2,3}. In contrast, *Gemmatimonas* and Rhodocyclaceae were linked to phosphorus accumulation^{2,3}. Furthermore, higher porosity of *bioker* in geochemical barriers was attributed as an important factor influencing biofilm development with higher abundances of N-transforming bacteria³. This knowledge is nowadays considered for the construction of systems, to increase their nutrient removal efficiency. Nutrients are the most important anthropogenic factor stimulating chB development, so the use of SSBS is a promising tool to control their development in downstream reservoirs.

Keywords: nitrifying bacteria, denitrifying bacteria, phosphorus accumulating bacteria, 16S rRNA, cyanobacterial harmful blooms

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Hydroponic culture of Strawberry (*Fragaria vulgaris*) using Microcystins-contaminated water: Effects on plant physiology, antioxidant defense, microcystins transfer and health risk assessment

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The occurrence of cyanotoxin-producing cyanobacteria and the adverse effects concerning those secondary metabolites have gained more scientific attention. Irrigation water contamination, phytotoxic effects, and plant bioaccumulation are all consequences of cyanotoxins' persistence in irrigation water.

The use of cyanotoxins-contaminated water for hydroponic-based culture involves direct exposure of plants to cyanotoxins. For that, Strawberry (*Fragaria vulgaris* L.) grown hydroponically was used as a model to assess the phytotoxic effects, bioaccumulation, transfer, and human health risks of cyanotoxins (type microcystins (MCs)).

The exposure of *F. vulgaris* L. seedling to MCs concentrations (5-20 µg/L) lasted 60 days and induced several adverse effects. Besides plant growth parameters decreases, and photosynthetic disturbances, the oxidative stress indicators (Catalase, Peroxidase, and Superoxide dismutase) showed significant elevation at 10 and 20 µg/L of MCs.

The MCs bioaccumulation, transfer, and health risks were evaluated using strawberry plants and *Meriones shawi* animals (set as an example of food chain model). The bioaccumulation of MCs was found to be highest in the roots, followed by leaves, fruits, liver, stomach, and fecal matter. The bioaccumulation factor (BAF) was highest in roots (5.01), followed by leaves (1.55), stomach (0.87), and fecal matter (1.18). The transfer of MCs to animal organs was low, and the daily toxin intake of adult consumers of strawberry fruit receiving MCs (5- 20 µg/L) did not exceed the WHO recommended limit (0.04 µg MC-LR/Kg of bw/day). However, strawberry fruits (10 and 20 µg/L) and *Meriones*' consumption of plant-contaminated leaves may pose a moderate health risk.

The obtained results suggest that legislation regulating the quality of irrigation water in terms of MCs concentrations is necessary to prevent detrimental consequences to crops and human exposure, and to develop effective management strategies for mitigating the risks associated with MCs contamination.

Keywords: Microcystins, Phytotoxic effects, Bioaccumulation, Transfer, Health risks

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Breaking Down the Bottles: Do Marketed Bacterial Consortia Live Up to Their Cyanobacteria Harmful Algal Blooms Control Claims?

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Cyanobacteria harmful algal blooms (cHABs) pose a significant risk to water quality by generating toxins that threaten ecosystems, human health, and drinking water resources [1, 2]. Chemical algicides are widely used to control cHABs. However, they have disadvantages, such as secondary contamination, detrimental impacts on non-target species, and the risk of developing resistance development in cyanobacteria [3]. These issues have driven the search for sustainable alternatives, with bacterial-based algicides emerging as a promising solution.

Commercially marketed bacterial agents assert success in controlling cHABs; however, their mechanisms of action and long-term efficacy remain poorly understood. Therefore, this study evaluated the efficiency of four widely used commercial bacterial consortia in reducing toxin-producing *Microcystis aeruginosa* (*M. aeruginosa*). The bacteria in these products were classified into two groups: algicidal bacteria, which directly target cyanobacteria, and nutrient-consuming bacteria, which indirectly suppress cyanobacteria by depleting critical nutrients [4]. Water quality analysis, such as total nitrogen, total phosphate, turbidity, pH, total/dissolved toxins, and chlorophyll-a, were monitored along with microbial community responses. The results indicated that only one product, a *Bacillus*-based consortium, achieved significant cHABs management, reducing cyanobacteria levels by 97.9%. In contrast, the other products showed minimal short-term efficiency, with cyanobacteria reduction of only up to 30% compared to untreated samples. After 7 days of application, these products appeared to even promote cyanobacterial growth, most likely due to the release of intracellular organic materials and nutrients. These findings highlight the crucial need to understand the mechanisms of bacterial-based algicides—not only to validate their claims but also to optimize their application for long-term, successful cHABs control. The 16S/18S rRNA sequencing demonstrated strong microbial interactions between the product's bacterial consortia, *M. aeruginosa*, and co-existing bacteria/Eukarya in the bloom water. Metagenomic/metabolomic analyses were also performed to elucidate the mechanisms of cHABs inhibition. The outcomes of this study highlight the potential bacterial consortia as long-term solutions for cHABs control.

Keywords: Harmful Algal Bloom, Bacterial Consortium, Commercial Products, Biological Treatment

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P68 - 130

Searching for appropriate measures to avoid problematic cyanobacterial blooms in the Province of East Flanders (Belgium)

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The last few years Flanders (Belgium), which is a densely populated area with intensive agriculture, has to deal with an increase in problematic blooms of cyanobacteria promoted by changing environmental conditions. Following these frequent blooms, the Province's recreational areas with surface waters are more and more confronted with recreational bans. Due to the urgent need for a good water quality several monitoring campaigns have been set up to examine the current nutrient status of the systems (different seasons, surface/deep) and which nutrient fluxes are at play to see which management options might be appropriate in the specific context of each system. The main challenge is to find a balance between exploitation of the recreational area, the investments and the environmental restoration. In this study, we highlight some results and challenges from studies in different ecosystems spread throughout the Province of East Flanders. Two of these ecosystems (Blaarmeersen, De Ster) are rather isolated mesotrophic lakes with few fluxes of nutrients left that can be tackled to evolve to a good status. The addition of a P binder (Phoslock) is for most of the actors at De Ster an acceptable option, considering the severity and extent of cyanobacterial blooms the last couple of years. Nevertheless some environmental concerns remain. The same concerns make it for the actors in De Blaarmeersen only an option to use this P binder when the environmental quality further deteriorates. At De Gavers (mesotrophic deep lake), first the nutrient fluxes need to be tackled before other measures can be considered. Same for the Boerekreek (hypertrophic shallow lake) but because of intensive farming in the catchment and nutrient input through domestic discharge, less obvious. At Damslootmeer (eutrophic deep lake) where still several discharges of wastewater take place, a nutrient-loaden stream entering the lake hampers the restoration at this moment.

Keywords: cyanobacteria, recreation, monitoring, measures



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Differential resistance pattern of cyanobacteria species against hydrogen peroxide

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Hydrogen peroxide (H_2O_2) is an eco-friendly control of cyanobacterial blooms, yet species-specific susceptibilities remain underexplored. This study compared the response of *Microcystis aeruginosa*, *Raphidiopsis raciborskii*, and *Sphaerospermopsis torques-reginae* to 10 mg/L H_2O_2 under laboratory conditions. Additionally, two *M. aeruginosa* strains, differing in biovolume and toxin production (LETC-MC-02: $\sim 40 \mu m^3$ cell volume, highly microcystin producer and LETC-MC-32: $\sim 180 \mu m^3$ cell volume and slightly microcystin producer), were compared. Cell density, biovolume, chlorophyll-a concentration, and photosynthetic efficiency (yield) were monitored over 7 days of exposure to 10 mg/L H_2O_2 . *R. raciborskii* was the most sensitive species, with significant reductions in biovolume, chlorophyll-a and yield at the end of the experiment. *S. torques-reginae* displayed moderate resistance for these parameters compared to *R. raciborskii* following a potential recovery in photosynthetic activity over 7 days. Meanwhile, *M. aeruginosa* showed greater resilience, without significant changes in chlorophyll concentration although biovolume and yield presented a slight decrease in 24 and 72h, respectively. Microscopic analysis revealed severe damage in *R. raciborskii* and *S. torques-reginae*, while *M. aeruginosa* maintained structural integrity. Comparison between *Microcystis* strains was performed in different conditions, varying the moment of 10 mg/L H_2O_2 application according to the light cycle (either 6am or 6pm), and following H_2O_2 concentrations (20, 40 and 80 mg/L) in different initial cell densities (10^5 and 10^6), being monitored throughout 28 days. The timing of H_2O_2 application relative to light cycles did not significantly affect outcomes for both strains. LETC-MC-02 exhibited greater resistance than LETC-MC-32, even at higher H_2O_2 concentrations (40-80 mg/L), although being sensitive at lower cell density, following different H_2O_2 decomposition kinetics. This suggests intraspecific variability in oxidative stress defense. Ongoing gene expression analysis related to oxidative stress and photosynthetic activity will further elucidate defense mechanisms, supporting tailored bloom management strategies which require careful dosing to mitigate ecological impacts.

Keywords: Blue-green Algae; Mitigation; Water Management; Oxidative Stress; Resistance.

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Emergent Macrophytes as Sustainable Solutions for Mitigating the Eutrophication and Cyanobacterial bloom: A Comparative Study in an Economic Circular Perspective

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Eutrophication and cyanobacterial blooms are critical challenges for global water quality management. Constructed Floating Wetlands (CFWs) using emergent macrophytes offer a sustainable, nature-based solution (NBS) for mitigating these issues. This study investigated the potential of five tropical emergent macrophytes—*Alternanthera philoxeroides*, *Ludwigia leptocarpa*, *Polygonum ferrugineum*, *Typha domingensis*, and *Urochloa mutica*—to control eutrophication and cyanobacterial blooms, comparing their performance to the widely studied floating macrophyte *Pontederia crassipes*.

Phytoremediation experiments conducted in mesocosms (40-50 L) with high soluble reactive phosphorus (SRP) levels (400 µg L⁻¹) revealed that emergent macrophytes achieved >94% SRP reduction within 14 days, outperforming *P. crassipes* (86%), except for *T. domingensis* (64%). *L. leptocarpa*, particularly when paired with dried biomass of *P. crassipes* (DB Pc), demonstrated promising results in nutrient removal and cyanobacterial suppression, with phenolic compounds from DB Pc contributing allelopathic effects. Root exudates of *A. philoxeroides*, *L. leptocarpa*, and *P. ferrugineum* effectively inhibited the cyanobacterium *Microcystis aeruginosa*, eliminating chlorophyll-a within 7 days. Including DB Pc as a substrate increased total phenols but led to nutrient release, indicating a need for long-term monitoring. Metagenomic analyses of *L. leptocarpa* rhizosphere revealed high bacterial diversity, enhancing its phytoremediation potential.

These findings highlight the efficacy of tropical emergent macrophytes in CFW systems as cost-effective and sustainable tools for nutrient removal and cyanobacterial bloom control. The integration of local macrophyte species and innovative substrates, like DB Pc, represents a circular economic approach by reusing macrophyte biomass while addressing eutrophication. However, challenges such as long-term nutrient dynamics require further research to optimize these NBS for water management applications.

This study underscores the importance of tailored, eco-friendly strategies in mitigating eutrophication and cyanobacterial blooms in tropical aquatic systems.

Keywords: Phytoremediation, nutrients, emergent macrophytes, cyanobacteria, eutrophication, nature-based solutions.

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Pilot-Scale CWPO Process for Effective Removal of Toxic Cyanobacteria and Cyanotoxins: A Sustainable Approach

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The increasing occurrence of toxic cyanobacteria blooms in drinking water sources poses a global health concern [1], highlighting the urgent need for innovative, cost-effective, and sustainable treatments. Catalytic Wet Peroxide Oxidation (CWPO), using natural magnetite (Fe_3O_4) as catalyst, has shown great potential for the degradation of cyanobacteria and their associated cyanotoxins [2], yet scaling the process for continuous, large-scale applications remains a critical challenge. This study evaluated the CWPO process at pilot scale using a 3 L continuous stirred tank slurry reactor coupled with continuous magnetite recovery to degrade microcystin-LR (MC-LR), one of the most prevalent cyanotoxins worldwide, under ambient conditions. Operational parameters, including Fe_3O_4 concentration, initial H_2O_2 dose, and pH_0 , were optimized. Under optimal conditions ($[\text{Fe}_3\text{O}_4] = 8 \text{ g L}^{-1}$; $[\text{H}_2\text{O}_2]_0 = 12.6 \text{ mg L}^{-1}$; flow rate = 50 mL min^{-1} ; $\text{pH}_0 = 3.5$), up to 90% of MC-LR degradation was achieved. Furthermore, in the presence of *Microcystis aeruginosa* cells ($50 \mu\text{g L}^{-1}$ chlorophyll-*a*), the process achieved >99.9% cyanobacteria removal and 85% cyanotoxin reduction. Process intensification ($[\text{Fe}_3\text{O}_4] = 10 \text{ g L}^{-1}$; $[\text{H}_2\text{O}_2]_0 = 15 \text{ mg L}^{-1}$) further increased cyanotoxin degradation to 95%, producing a fully detoxified effluent. Continuous magnetite separation enabled catalyst reuse over multiple cycles, maintaining stable degradation rates with minimal iron leaching ($<0.1\% \text{ w/w}$), demonstrating the durability of magnetite as a catalyst. Finally, a design was proposed for an industrial-scale Drinking Water Treatment Plant (DWTP) serving 10,000 inhabitants. Economic optimization of the process resulted in a treatment cost of 0.19 € per cubic meter. This approach offers a safe, sustainable, and economical solution for water treatment, advancing innovative technologies to address global water quality challenges.

Keywords: CWPO, cyanobacteria, cyanotoxin, water treatment, pilot plant.

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Toxicity studies of terrestrial cyanobacteria isolated from a copper mine tailing site and loess environments intended for restoration and bioremediation

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Anthropogenically degraded and polluted land areas are a growing problem. Various forms of restoration, recultivation and bioremediation are being used to revitalise the damaged areas and to prevent the spread of damage to adjacent sites and sediment layers. Some restoration methods rely on the introduction and implementation of organisms which develop biological crusts. We have been isolating cyanobacterial strains from extreme environments and studying their potential to be used for the restoration process. There is an ecological rationale to use freshly isolated autochthonous (local) strains for inoculation. Such strains further need to be safe (non-toxic) and biotechnically fit for the purpose. We are currently studying cyanobacteria present in the former copper mine area Orijärvi (SW Finland) and loess environments (Serbia).

In Orijärvi, metal concentrations (e.g., Cu, Pb, Zn) are highly elevated in the sediment. Currently, oxidation is occurring in the uppermost 10 cm of the sediment layer, making it extremely acidic (pH <3) and causing metals to dissolve into a toxic form. The pH in the underlying undisturbed sediment remains near neutral (pH 4.6-5.9).

ELISA immunoassay was used to evaluate the presence of various toxins in strains isolated from the biocrusts of the Orijärvi mine tailings sediment. No detectable concentrations of anabaenopeptins, cylindrospermopsins, saxitoxins or microcystins were found. However, anatoxin-a (ATX-a) was detected in seven strains. Strains that produced higher ATX-a concentrations (50–100 µg/L) were sampled from medium containing the original mine tailings sediment and identified microscopically as *Fischerella* sp. and *Scytonema* sp. The strain that produced approximately 10 µg/L ATX-a was cultured in BG11+ medium without the mine tailings sediment and identified as *Fischerella* sp. Analyses by HPLC-DAD and LC-MS showed no signs of ATX-a in the same seven strains.

Our previous studies indicated that cyanobacterial strains from loess are non-toxic (Dulić et al., 2022). Further *Artemia salina* biotests confirmed the non-toxic nature of cyanobacterial isolates from loess environments.

Keywords: terrestrial cyanobacteria, toxicity, restoration, mining site, loess environment

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Enhanced mechanical properties of living and regenerative building materials by filamentous *Leptolyngbya boryana*

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Cement-free and cyanobacteria-based living building materials (LBMs) can be manufactured using microbially induced calcium carbonate (CaCO₃) precipitation (MICP) technology, which is regarded as eco-friendly because of the absence of CO₂ gas emissions during the manufacturing process. Here, we report that photosynthetic and filamentous cyanobacterium *Leptolyngbya boryana* GGD can precipitate substantial amounts of CaCO₃ with biofilm formation in our optimized medium. Compared to coccoid cells, filamentous cells have an extensive surface area that can efficiently agglomerate the formation of granular materials and fill the void spaces by forming bridging microstructures along with precipitated CaCO₃ in LBMs, which can enhance the mechanical properties of LBMs. Regenerative LBMs can possibly be reconstructed using old materials from parent LBMs without the addition of GGD strain cells. The physicochemical properties of the filamentous GGD strain hold promise as valuable components for maintaining the structural integrity of LBMs.

Keywords: biocomposite; cement-free materials; cyanobacteria; calcium carbonate; biomineralization

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From Blooms to Bioactives: Investigating the chemical diversity and the toxic potential of *Microcystis* (Cyanobacteria)

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Rising global temperatures and shifts in climate patterns, including more frequent droughts and intense rainfall, are expected to intensify toxic cyanobacterial blooms. These blooms pose significant risks to human health, water resources, fisheries, and ecosystems. Among cyanobacteria, *Microcystis* is particularly concerning due to its production of various harmful bioactive compounds, many of which remain poorly appreciated in terms of their toxicological impacts. Indeed, the current understanding of the cyanobacterial metabolome remains incomplete, with many compounds unidentified due to gaps in database annotations. Evidence suggests that toxic effects extend beyond microcystins (MCs), highlighting the need for more comprehensive chemical characterization and toxicity assessment of other potentially toxic compounds.

This study focuses on six highly toxigenic strains of *Microcystis*, selected among the most toxic of the Paris Muséum Collection (PMC) and Pasteur Cultures of Cyanobacteria (PCC) collection, according to preliminary tests on whole methanolic extracts. Initially, to investigate these strains, three advanced methods were used: genome mining, molecular networking, and (eco) toxicological risk assessment. The strains are notable for their embryo toxicity and teratogenic effects on Medaka fish and diverse fish cell lines, suggesting that novel metabolites may contribute to their toxicity, linked to specific geno-/chemotypes of the strains. Then, the metabolite characterization was first optimized on two strains presenting the highest toxicity, PMC 830.12 and PMC 727.11, using a dichloromethane/methanol mixture at room temperature. C18 Solid-phase extraction yielded five fractions of decreasing polarity. Each fraction was analyzed by ultra-high-performance liquid chromatography – tandem high-resolution mass spectrometry (UHPLC-HRMS/MS) and the resulted data were pre-processed using MZmine3 and finally, feature-based molecular networks were generated on the online platform GNPS, allowing to identify various chemical families, including the expected non-ribosomal peptides. The most promising hydromethanolic fractions from PMC 830.12 and PCC 727.11 strains were further fractionated by reversed-phase HPLC, thus permitting to isolate metabolites for toxicity evaluation and extensive structure characterization by NMR spectroscopy and mass spectrometry.

Keywords: *Microcystis*, chemical screening, toxicological evaluation, molecular networking, genome mining, structural analysis

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Inducing cyanobactericidal responses in the microbiome of *Myriophyllum spicatum* against *Microcystis aeruginosa* and associated microbial community dynamics

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Various methods have been developed to mitigate cyanobacterial harmful algal blooms (cyanoHABs). Among these, submerged macrophyte *Myriophyllum spicatum* (Eurasian watermilfoil) is documented for its allelopathic properties, particularly its inhibitory effects on bloom-forming cyanobacteria such as *Microcystis aeruginosa*. Previous studies have focused on identifying the allelochemical compounds produced by *M. spicatum* that suppress the growth of *M. aeruginosa*. In this study, we demonstrated that *M. aeruginosa* KW triggers cyanobactericidal responses in the microbiome of *M. spicatum*, and further examined the associated shifts in microbial community composition. The watermilfoil plants used in this study were collected from the Yudeung Stream in Daejeon, South Korea. After washing, 5-cm shoots were used for the experiments. Pre-culture conditions were compared across three groups: (1) *M. spicatum* (Ms) pre-cultured in BG11 medium, (2) Ms pre-cultured in *M. aeruginosa* KW culture (8.7×10^5 cells/ml), and (3) Ms pre-cultured in the filtrate of *M. aeruginosa* KW culture solution (filtered through 0.8- μ m membrane filter, BG11 10%). After 7 days of pre-culture under the three different conditions, *M. spicatum* shoots were inoculated into fresh *M. aeruginosa* KW culture (7.3×10^5 cells/ml). Group (2) exhibited a rapid inhibitory response against *Microcystis*, achieving 84% inhibition within the first day, while Group (1) showed slower responses, reaching 89% inhibition by day 7. In contrast, Group (3) maintained inhibition levels below 50% throughout the experimental period. A total of 28 bacterial taxa closely associated with the inhibitory response were identified, including *Bosea massiliensis*, *Pseudoxanthobacter* sp., *Pirellula* sp., *Ferrovibrio* sp., *Mesorhizobium* sp., *Devosia insulae*, *Candidatus Megaira* sp., and *Bdellovibrio bacteriovorus*. Our findings suggest that *M. aeruginosa* induces cyanobactericidal responses in the microbiome of *M. spicatum*. This response is accompanied by significant changes in the microbial community composition in the samples where the inhibitory reaction occurred.

Keywords: *Microcystis aeruginosa*, Bloom Control, *Myriophyllum spicatum*, Microbiome, Cyanobactericidal Induction

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Understanding the growth inhibition of *Microcystis* by *Cyanobium*: A transcriptome-based interaction study

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The occurrence and decline of cyanobacterial blooms, particularly those driven by *Microcystis*, are influenced by interactions with various microorganisms. These interactions are crucial for understanding bloom dynamics and the health of aquatic ecosystems. Recent research indicates that *Microcystis* is affected not only by heterotrophic bacteria but also by picocyanobacteria, which play a significant role in nutrient cycling and competition in aquatic environments. This study aimed to explore how and under what conditions the picocyanobacterium *Cyanobium* affects the growth of *Microcystis*, proposing potential mechanisms for these interactions based on transcriptome analysis. The strain *Cyanobium gracile* A950 demonstrated a more pronounced inhibitory effect on the growth of *Microcystis aeruginosa* KW at elevated temperatures. In co-culture experiments, *M. aeruginosa* KW exhibited downregulation of genes associated with photosynthesis, such as *psaC* and *psaE*, which hindered its energy production and light-harvesting capabilities. Additionally, the reduced expression of phosphate uptake genes, including *phoU* and *pstA*, indicates that *M. aeruginosa* KW faced disadvantages in nutrient acquisition compared to *C. gracile* A950. Conversely, *C. gracile* A950 quickly upregulated genes related to photosynthesis and ATP synthase within just 2 hours of co-culturing, enabling it to secure an early competitive edge over *M. aeruginosa* KW. These rapid transcriptional changes highlight the adaptability of *C. gracile* A950 in response to competitive pressures. These results suggest that the inhibitory effect of *C. gracile* A950 on *M. aeruginosa* KW primarily stems from the imbalanced expression changes in photosynthesis-related genes between the two species. Understanding these interactions is essential for predicting the dynamics of cyanobacterial blooms and developing strategies for managing water quality in affected ecosystems. Further research is needed to elucidate the broader ecological implications of these findings and to explore potential applications in bioremediation and bloom management.

Keywords: *Microcystis*, Picocyanobacteria, *Cyanobium*, Transcriptomics, Interaction

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Exploring interactions of MC-producing and non-producing *M. aeruginosa* with members of its phycosphere

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Rising occurrence of blooms of the photosynthetic bacterium *Microcystis* is a known global phenomenon. Among *Microcystis*-specific characteristics like the plasticity in carbon uptake, resilience towards changes in temperature, or regulation of buoyancy with gas vesicles, the success of this unicellular bacterium has been increasingly attributed to *Microcystis*' ability to form single colonies with specialized microbiomes [1]. Here it was shown that some environmental stressors (e.g. oxidative stress) are alleviated as a joint effort [2]. Interestingly, no core microbiome could be identified so far, suggesting that *Microcystis* microbiomes are highly specialized. Furthermore, *Microcystis* is known for the production of a miscellany of secondary metabolites including microcystins (MC), microviridins, aeruginosins and cyanopeptolins. *Microcystis* single colonies consist of unique genotypes, indicating the presence of unique combinations of small molecules in these colony microbiomes, which in turn could play a role for bacterial interactions. For example, while it is known that MC is toxic to mammalian life, it was shown for the laboratory strain *M. aeruginosa* PCC 7806 and its MC-deficient mutant $\Delta mcyB$ that the loss of the metabolite MC leads to alterations in the light adaption and cell surface characteristics as well as reshaped carbon metabolism [2]. This could result in an altered specificity of phototroph-heterotroph interactions.

In longitudinal experiments with synthetic communities consisting of a MC-producing WT (*M. aeruginosa* PCC 7806) and its MC-deficient mutant together with 21 heterotrophic isolates, we identified two bacterial isolates that showed reciprocal abundance. Additionally, we observed interesting dynamics in the bacterial growth as an intermittent stationary phase occurred after which a second growth period was visible [3].

Using the two candidate isolates we aim to further explore this growth dynamic in tri-partite experiments, especially under carbon limitation. For this, we aim to employ metatranscriptomic analysis of synthetic consortia together with a metabolic modelling approach.

Keywords: Microcystis, phycosphere, phototroph-heterotroph interactions, metabolic modelling

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Toxicity assessment of aquatic and terrestrial *Nostoc* strains using bioinformatics and zebrafish embryo biotests

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In this study, the toxicological potential of aquatic and terrestrial *Nostoc* strains was evaluated using the biotest with zebrafish embryos (*Danio rerio*) and bioinformatics analyses of cyanobacterial genomes. Zebrafish embryos exposed to extracts from the aquatic strain *Nostoc* Z1 exhibited dose-dependent developmental abnormalities, including skeletal deformities and yolk sac edema. Skeletal malformations affected up to 25% of embryos, while yolk sac edema was observed in fewer than 10%. These effects became evident after 72 hours of exposure, with no further changes in malformation rates observed thereafter. An EC₁₀ value of 204.9 µg mL⁻¹ was calculated for skeletal deformities. Exposure to extracts from the terrestrial strain *Nostoc* 2S3B similarly induced skeletal deformities, particularly at higher concentrations (160–250 µg mL⁻¹), with the highest incidence observed after 72 hours and remaining stable until the end of the experiment. Pericardial edema and underdeveloped eyes were also noted at higher concentrations, but malformation rates did not exceed 8.33%. An EC₁₀ value of 169.55 µg mL⁻¹ was determined for skeletal deformities, while no significant deviations in hatching rates were observed in any experimental group. Genome analysis of the aquatic strain *Nostoc* Z1 revealed the presence of 15 biosynthetic gene clusters, including one linked to the biosynthesis of the potentially toxic metabolite microviridin J. The identified genes showed complete sequence identity with those from other known toxic strains. The terrestrial strain *Nostoc* 2S3B contained 29 clusters, including those encoding peptides microviridin N9 and scytocyclamide A. The microviridin N9 cluster exhibited complete sequence similarity with the cluster found in *Nostoc punctiforme* PCC 73102, while partial homology (46%) was noted with the scytocyclamide A cluster of *Scytonema hofmannii* PCC 7110. These findings highlight the ecological and toxicological diversity of aquatic and terrestrial cyanobacteria, emphasizing the value of zebrafish biotests and genomic tools for assessing their toxin synthesis potential.

Keywords: Cyanobacteria, Toxicological Potential, Zebrafish (*Danio rerio*), Bioinformatics, Secondary Metabolites

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Metabolomic Profile Analysis after CRISPR-Cas9-Mediated Disruption of Microcystin Synthesis in *Microcystis flos-aquae* TAU-MAC 1510

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Microcystins are cyclic hepatotoxic heptapeptides, listed among the most commonly toxic secondary metabolites produced by cyanobacteria and synthesized nonribosomally by large enzyme complexes encoded by the *mcy* gene cluster. Although they are well characterised toxins, up to date, their possibly multifunctional biological role is not clearly determined. This study aims to interrupt the synthesis of microcystins in *Microcystis flos-aquae* TAU-MAC 1510, a microcystin-producing strain, to analyse the metabolomic profile and detect drifts in the mutant strain, which can contribute both to the understanding of the role of microcystins in cyanobacteria and to examining the involvement of the genes encoding them in multiple pathways. Two CRISPR-Cas9 genetic engineering platforms were designed, each inducing specific knockout mutations. The first one incorporated a cassette of two gRNAs that target the *mcyA* gene and inhibit microcystin production, aiming to analyse the metabolites produced in the absence of microcystins' synthesis. The second one also incorporated two gRNAs targeting the *mcy* gene cluster's bidirectional promoter with the goal of understanding if any of the ten *mcy* genes (A-J) is involved in the production of other metabolites based on the metabolomic profile changes when the promoter is knocked out, resulting in the inactivation of the whole *mcy* operon. Ongoing experiments aim to validate the knockouts through sequencing and metabolomic analyses. LC-HRMS and computational data analysis are expected to confirm the reduction of microcystin levels and provide detailed insights into the metabolic changes resulting from the genetic modifications.



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Possibilities and limitations of environmental cyanobacterial biomass application in biotechnology

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The potential of cyanobacteria in the bioindustry has been widely recognized due to valuable compounds they can produce. Cyanobacteria are considered a promising feedstock for biofuels and bioplastics as they accumulate lipids and synthesize polyhydroxyalkanoates. The latter is a biodegradable polymer that is already being used as an alternative to conventional plastics. Moreover, cyanobacteria have the potential to produce a wide range of high-value compounds, including pigments, vitamins, fatty acids, etc.

Despite their potential, scaling up cyanobacteria cultivation to an industrial level is challenging due to high infrastructure investments, operational costs and technical limitations. An alternative, harvesting bloom-forming cyanobacteria from nature, offers the dual benefit of controlling harmful algal blooms and improving water quality by removing nutrients and cyanotoxins. The harvested biomass could serve as a low-cost renewable resource for bioproducts and improve the economic sustainability of cyanobacteria applications.

However, ensuring the safety of bioproducts from harvested biomass remains a critical challenge. Many cyanobacteria species can produce harmful compounds, making their use difficult and leading to strict regulations, especially in the European Union. Another limitation is the variability in cyanobacterial bloom composition, which depends on environmental conditions and bloom phase, affecting the yield and quality of desired products.

At the same time, harvested cyanobacterial biomass can contribute to sustainability by improving soil fertility and structure, reducing reliance on chemical fertilizers or serving as a catalyst to increase biogas yields. They also produce bioactive compounds that act as biostimulants promoting plant growth and resistance to environmental stressors and pathogens.

The current situation underlines the need for a broader discussion on the potential use of environmental cyanobacterial biomass and the regulatory challenges involved. Key findings of EU-LIFE project "AlgaeService for LIFE" will be presented, in which a circular economy model was applied to explore the establishment of a value chain for cyanobacterial bioproducts.

Keywords: harmful blooms, bioproducts, cyanobacteria harvesting

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Evaluation of *Microcystis aeruginosa* biomass extracts against plant pathogens

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Cyanobacteria are gaining attention as a natural alternative to conventional chemical pesticides, as they can produce secondary metabolites with antiviral, antibacterial and antifungal properties. These bioactive compounds – including alkaloids, lipopolysaccharides, non-protein amino acids, non-ribosomal peptides, polyketides and terpenoids – contribute to their biocidal activity and make cyanobacteria a viable alternative to synthetic pesticides for the control of plant pathogenic bacteria and fungal diseases. Environmental biomass collected from cyanobacterial blooms in freshwater systems could serve as a valuable resource for the development of environmentally friendly, biodegradable biopesticides. Unlike many synthetic pesticides, cyanobacteria-based pesticides pose minimal risk to human health and non-target organisms while avoiding soil and water contamination.

However, the efficacy of cyanobacterial extracts can vary depending on environmental conditions, the predominant cyanobacterial species, the target pathogen group or species, and the application methods used. To address these challenges, the "HABpest" project (2023–2027) focuses on the evaluation of extracts from harvested in nature bloom-forming cyanobacteria against a range of plant pathogens, including bacteria and fungi, to assess their potential as natural biopesticides. The composition of cyanobacterial biomass, which is determined by a complex of species dominance, timing of bloom collection and environmental conditions, results in extracts with different secondary metabolite profiles.

In the initial phase of the project, cyanobacterial biomass was collected during the bloom of *Microcystis aeruginosa* in the Kaunas reservoir in September 2024. The aqueous and methanolic extracts obtained from this biomass are tested against various pathogenic organisms and preliminary results will be presented.

Keywords: biopesticides, bioproducts, environmental biomass, harmful blooms

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Baltic cyanobacteria – source of anticancer compounds

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Cyanobacteria produce a wide variety of bioactive metabolites. Most commonly these compounds are characterised by cytotoxic and enzyme inhibitory activities. For years, microorganisms from tropical regions were considered to be the richest source of bioactive metabolites. Here we present that temperate regions such as the Baltic Sea host cyanobacteria with promising pharmaceutical potential.

Our screening studies, with various Baltic cyanobacteria, revealed that *Pseudanabaena galeata* CCNP1313, *Nostoc edaphicum* CCNP1411 and *Spirulina subsalsa* CCNP1310 strains were characterised by the most potent anticancer activities.

The assays were performed against a wide spectrum of cancer cells: cervical (HeLa, SiHa, C33-A, CaSki), lung (A549), prostate (PC3), breast (T47D), and human dermal fibroblasts (HDFa).

In the second step, bioassays of samples obtained by fractionation of the extract lead us to determine the composition of the bioactive samples.

Based on LC-MS/MS analyses, we identified compounds from both, well-known classes of cyanopeptides as well as compounds that were not deposited in the CyanoMetDB.

The compounds varied with their effects on the proliferation of different cell lines. The most promising compounds were those that had no effect on HDFa cells and induced apoptotic changes in the cells.

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Potential of Cyanobacterial Blooms for Sustainable Biodiesel Production

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Cyanobacterial blooms have a negative impact on aquatic ecosystems, reaching high population densities under favorable environmental conditions and forming visible scum on the water surface (Akcaalan et al., 2014). However, this substantial biomass offers great potential for the production of economically viable products such as biodiesel and bioactive natural compounds (Madusanka and Manage; 2018; Duman et al., 2019). Thus, the exploitation of cyanobacterial blooms could provide both environmental and economic benefits. To explore this potential, water samples were collected from two artificial rainwater harvesting ponds in Gümüşyaka and Çakıl (Istanbul, Türkiye) between June and October 2024. Phytoplankton counts were performed to identify the dominant species, and biodiesel yield and quality were analyzed, focusing on the fatty acid methyl ester (FAME) content. The results showed that cyanobacteria represented more than 80% of the total algal abundance in both ponds. In Çakıl, *Microcystis* sp. was identified as the dominant species, while *Aphanizomenon* sp. and *Planktothrix agardhii* were dominant in Gümüşyaka. FAME content analysis showed that biodiesel yields ranged between 45% and 60% in Çakıl and between 40% and 50 % by weight in Gümüşyaka. In addition, the fatty acid composition met biodiesel quality standards (EN 14214 and ASTM D6751). These results showed higher FAME content compared to laboratory-scale cultures, suggesting that the natural stress conditions in these environments increased FAME production. The study highlights the significant potential of biomass derived from cyanobacterial blooms for sustainable and economically viable biodiesel production. This approach not only supports the bioenergy sector, but also helps mitigate the environmental impacts of harmful algal blooms.

Keywords: Cyanobacteria Bloom, Biodiesel, FAME, Rainwater Harvesting Ponds

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Exploring Phycobiliprotein Diversity and Extraction Potential from Wild Freshwater Cyanobacterial Biomass

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Cyanobacteria are unique organisms due to their ability to produce a wide range of bioactive pigments such as chlorophylls, carotenoids and phycobiliproteins, which have high potential for applications in the food, pharmaceutical, cosmetics and other industries. Among these, phycobiliproteins are the most prominent pigments due to their antioxidant, anti-inflammatory, anti-cancer and other properties. These water-soluble, fluorescent pigment-protein complexes, which play a key role in capturing light energy for photosynthesis, are widely used in the food and cosmetics industries as natural colourant and food supplements, as well as in biomedical research as fluorescent markers. Research on phycobiliproteins has mainly focused on cultured cyanobacteria, while environmental biomass from natural waters is an underexplored resource for the diversity and extraction of phycobiliprotein. Therefore, the study focused on the composition of phycobiliproteins in wild cyanobacterial biomass harvested from various freshwater aquatic ecosystems. It also aimed to assess the feasibility of using this biomass as a sustainable source for the extraction and purification of phycocyanin, the most promising phycobiliprotein in biotechnology due to its abundance, versatility and high market demand for organic products. The results revealed that the harvested cyanobacterial biomass from freshwaters was rich in phycobiliproteins, particularly phycocyanin and allophycocyanin. The non-toxic cyanobacterial biomass dominated by *Aphanizomenon flos-aquae* proved to be a valuable source for phycocyanin extraction. By applying different extraction and purification techniques, phycocyanin of varying purity was obtained, with purity levels (measured by the absorption ratio A620/A280) ranging from 1.5 (suitable for food and cosmetics) to 4.1 (suitable for pharmaceutical applications). In addition, the extracted phycocyanin demonstrated significant antioxidant properties with an ABTS scavenging activity of up to 85%. This research highlights the potential of wild cyanobacteria biomass to contribute to the development of environmentally friendly processes for phycocyanin extraction and broadens the understanding of its applications in various industries.

Keywords: harmful algal bloom, phycocyanin, biotechnology

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Evaluating Wild Cyanobacteria Biomass as a Sustainable Feedstock for Eco-Friendly Biocompounds

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Cyanobacteria are highly adaptable organisms that have become increasingly prevalent and widespread in aquatic ecosystems, making them significant for biotechnological and ecological research. The present study aims to evaluate the biotechnological potential of wild *Aphanizomenon* and *Microcystis* cyanobacterial biomass harvested from the blooming waters of the Kaunas reservoir in Lithuania between 2019 and 2021. The predominant cyanobacteria genera were identified and various components in the collected biomass were quantified, including pigments (chlorophyll *a*, carotenoids and phycobiliproteins), phenolic compounds, toxins as well as antioxidant, antibacterial and antifungal activities of the biomass. Notably, freeze-dried biomass exhibited higher levels of chlorophyll *a* and carotenoids compared to dried biomass, with the highest concentrations of 10.82 mg/g and 3.26 mg/g found in freeze-dried *Aphanizomenon* biomass. Phycocyanin was identified as the dominant cyanobacterial phycobiliprotein, accounting for up to 87% of the total phycobiliproteins in *Aphanizomenon* biomass. The maximum concentration of total phenolic compounds, expressed as gallic acid equivalents (GAE), was determined to be 9.26 mg GAE/g biomass in the dried *Microcystis* biomass, measured by the Folin-Ciocalteu method. In terms of antioxidant activity, *Aphanizomenon* extracts demonstrated the strongest results in the DPPH assay, reaching 3.46 mg GAE/g biomass, while the ABTS assay indicated the highest antioxidant capacity in the *Microcystis* dominated biomass extract in 2021 measuring 8.09 mg Trolox equivalent (TE)/g biomass. All tested cyanobacterial biomasses showed antimicrobial activity against Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and the yeast *Candida albicans*, with minimum inhibitory concentrations ranging from 250 µg/ml to 62.5 µg/ml against *E. coli* and *C. albicans*, and from 250 µg/ml to 7.8 µg/ml against *S. aureus*. The composition and bioactivity of the wild cyanobacterial biomass varied depending on dominant cyanobacterial species associated with the blooms in the Kaunas reservoir and biomass disruption and the extraction methods employed.

Keywords: (cyanobacteria, wild biomass, bioproducts)

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Detection of Bacterial Communities in a Cyanobacteria Bloom and Culture Samples by Nanopore Sequencing Approach

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Cyanobacteria cell surface favorable for other organisms such as heterotrophic bacteria and nutrient exchanges occur between these microorganisms. Recent advances of next generation sequencing techniques enabled metagenomic analysis of microalgal-bacterial communities based on taxonomic profiling by amplicon sequencing. In this study, we analyzed the composition of the bacterial community in three samples by nanopore sequencing: a cyanobacterial bloom sample of *Planktothrix rubescens*, two non-axenic culture of cyanobacteria including one toxic *Microcystis aeruginosa* and one taste and odour producer non-toxic *Microcoleus* sp. Using nanopore sequencing approach, we revealed a clear distinction at the species level. Pseudomonadota was abundant phyla in all samples (relative abundance was 12% in bloom, 11% in *M. aeruginosa* and 6% *Microcoleus*) followed by and Bacteroidota (2% in bloom, 0.6% in *M. aeruginosa* and 1.2% *Microcoleus*). Taste and odour producer *Microcoleus* cyanosphere showed 2 additional phylum Bacillota (2%) and Planctomycetota (0.2%). At the species level, abundant bacteria (>1%) were different in all samples suggesting a specific association between microalgal-bacterial communities. In bloom sample, *Curvibacter* sp. AEP1-3 (2.3%), *Rhodoferrax sediminis* (2%), *R. saidenbachensis* (1.4%) and *Aquirufa nivalisilvae* (1.4%) were abundant (>1%). In *Microcystis aeruginosa* culture sample, *Hydrogenophaga* sp. NH-16 (4%), *Porphyrobacter* sp. LM 6 (2%) and *Serpentinimonas maccroryi* (1.7%) were abundant. In *Microcoleus* sp. culture, *Crinalium epipsammum* (17%), *Limnospira indica* (2.4%), *Sphingopyxis* sp. EG6 (1.7%), *Allocoleopsis franciscana* (1.4%) and *Clostridium estertheticum* (1.3%) were abundant. Cyanobacteria associated bacteria could have important functions such as promoting cyanobacterial growth (*Curvibacter*, *Rhodoferrax* etc.), colony formation of cyanobacteria (*Porphyrobacter*) and also cyanobacteria could have important functions for promoting bacterial growth producing substrates for them (*Hydrogenophaga*). Further studies are needed to understand the ecological functions of associated bacteria in toxic and taste-odour producer cyanobacteria cyanosphere. The present study has shown that the nanopore sequencing approach is able to assess the diversity of these associated bacteria.

Keywords: Nanopore Sequencing, Cyanobacteria, *Microcystis*, *Planktothrix*, *Microcoleus*

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Innovative strategies to cultivate and optimize Guanitoxin production in *Sphaerospermopsis torques-reginae* (Cyanobacteria, Nostocaceae)

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Guanitoxin (GNT) is a potent neurotoxin produced by freshwater cyanobacteria^{1,2}. *Sphaerospermopsis torques-reginae*³, a filamentous species isolated from the eutrophic Tapacurá reservoir (Pernambuco, Brazil)⁴, is the only strain (ITEP-24) worldwide currently maintained in a biobank known to produce GNT. This study evaluated cell growth, chlorophyll-a production, and GNT synthesis by ITEP-24 across six culture media with varying nitrogen (N) concentrations: BG-11, BG-11 + N5%, ASM-1, ASM-1 + molybdenum, ASM-1 + N5%, and Z85,6,7. Cultures were maintained under controlled conditions (pH 7.5, 24 ± 2 °C, 12h:12h light:dark photoperiod, 40 μmol photons m⁻² s⁻¹) for 52 days. Samples were collected at regular intervals for cell counts, chlorophyll-a concentration, and qualitative GNT analysis via LC-MS/MS^{8,9}. Optimized toxin extraction using 70% methanol with 0.3% acetic acid and ultrasonic cell lysis yielded the highest efficiency (p < 0.05), balancing extraction efficacy with sample integrity. Purification via normal-phase silica gel reduced toxin loss and removed non-polar compounds, resulting in 1.2 mg of GNT from 1.375 g of dry biomass. Chromatographic analysis identified the ZIC-HILIC column as the most effective for GNT detection using LC-HR-QTOF-MS. The Z8 medium showed the best overall performance, achieving a GNT peak at day 45, while BG-11 + N5% excelled during early growth phases. Drying/concentration tests after SPE extractions revealed that guanytoxin (GNT) hydrolyzed during nitrogen gas drying procedures at room temperature (~23 °C), resulting in significant losses. Qualitative GNT analysis after one (GNT II) and two (GNT III) drying cycles showed a sharp decrease in toxin concentration compared to the initial sample (GNT I) (p < 0.001). Chromatographic analysis (LC-MS/MS) identified degradation products, confirming a trade-off between GNT concentration and hydrolysis after drying tests. These findings contribute to the optimized cultivation of *S. torques-reginae* and improved GNT purification and detection, highlighting their potential for applications in biotechnology and environmental monitoring.

Keywords: cyanobacteria, anatoxins, cyanotoxins, natural organophosphate, neurotoxins.

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Unveiling Bioactive Siderophores: Leveraging Computational Metabolomics to Investigate Mediterranean Deep-Sea Actinobacteria

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Deep-sea environments host a diverse array of microorganisms, including cyanobacteria and actinobacteria. Extremophilic actinobacteria, in particular, are prolific producers of secondary metabolites, exhibiting therapeutic potential due to their antimicrobial or cytotoxic properties (1). Among these, siderophores, iron-chelating compounds, offer a unique opportunity for drug discovery and biomedical applications. Here, we present an isolation and identification pipeline accelerated by *in silico* mass spectrometry approaches. We isolated Mediterranean actinobacteria from the Spanish coast grown in liquid cultures under the EU project SECRETed. The cultures were centrifuged, and both the supernatant and the pellet were examined separately. Following a multi-step liquid-liquid extraction, the supernatant was separated into two phases, while the biomass was extracted to obtain progressively polarity extracts. The profile of the obtained extracts was subjected to LC-MS/MS analysis, which served two purposes: semi-automatic dereplication of siderophore-related features, and method development for purification and validation of the predicted features. For the computational aspect, data acquisition was followed by processing through MZmine and comparative metabolomic analysis to annotate the chemical drivers distinguishing active from non-active siderophore extracts. These features were then examined through library matching in public mass repositories such as GNPS and supported by SIRIUS *in silico* fragmentation studies. Then data propagation through molecular networks annotate potential analogs. These networks were enriched with bioactivity metadata and ferric ion identity, highlighting active clusters. This guided the purification process, which was monitored by TLC FeCl₃ assays, and led to isolates which were dereplicated by HRMS and NMR. Key dereplicated compounds included linear and cyclic hydroxamate siderophores from the "ferrioxamine family" and spore germination inhibitors from the "germicidin family". Additionally, carboxylic acid derivatives, dipeptides, and nucleosides were identified, all with notable application potential. This advanced analytical workflow, enhanced by state-of-the-art cheminformatics, streamlined compound identification and prioritized bioactive candidates for structural elucidation and application.

Keywords: siderophore, actinobacteria, computational metabolomics, molecular networks, isolation

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Phytotoxicity and Phytostimulant Effects of Secondary Metabolites from *Sphaerospermopsis torques-reginae* ITP-024 in Cucumber (*Cucumis sativus*)

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Cyanobacteria, crucial for aquatic ecosystems, produce secondary metabolites with biological activities and potential toxins. *Sphaerospermopsis torques-reginae* ITP-024 can produce toxic metabolites, such as guanitoxin, which pose risks to the environment and human health¹. However, it can also produce, among others, mycosporine-like compounds and cyanopeptides, which may have toxic effects on aquatic organisms or potential applications in biotechnology^{2,3}. Therefore, the objective of this study was to investigate, through LC-QTOF-MS, the presence of secondary metabolites in the aqueous and methanolic extracts of ITP-24 and six different fractions (10%, 20%, 40%, 60%, 80%, and 100% methanol) obtained by SPE from the methanolic extract, and evaluate the phytotoxic or phytostimulant potential of these metabolites in cucumber (*Cucumis sativus*). Different secondary metabolites were identified in each fraction analyzed by LC-QTOF-MS, based on polarity. Guanitoxin was found in the aqueous extract, while mycosporine-like amino acids appeared in fractions ranging from 10% to 40% methanol. Cyanopeptides, such as namalides and spumigins, were present in the 60% methanol fraction, and anabaenopeptins in the 80% methanol fraction. Previous studies have confirmed the presence of these metabolites in *S. torques-reginae* extracts⁴. In the phytotoxic/phytostimulant assays with cucumber, stem and root lengths were evaluated after seven days of seed exposure to the treatments⁵. Preliminary results indicate toxicity of the aqueous extract containing guanitoxin, where no seed germination occurred. However, the methanolic extract of the biomass stimulated stem growth by 63% compared to the control (1% DMSO). These results suggest that the secondary metabolites of *S. torques-reginae* have potential for both biotechnological applications and environmental risks, advancing the studies on the toxicology of these compounds.

Keywords: cyanobacteria, cyanopeptides, guanitoxin, metabolomics, phytobiological effects.

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Environmental Monitoring: Advancing Cyanobacterial Bloom Monitoring through Remote Sensing and In Situ Observations in Three Italian Lakes

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Remote sensing technology has transformed environmental monitoring, allowing not only the measurement but also the discovery and better understanding of ecological phenomena (Ferrara *et al.*, 2017). This paradigm shift is important for managing harmful algal blooms (HABs), such as cyanobacterial blooms caused by *Planktothrix rubescens*. Cyanobacteria behave as sensitive bioindicators of environmental impact, showing the cumulative consequences of human-induced changes in aquatic ecosystems. Beyond ordinary observation, understanding the origins, dynamics, and underlying environmental drivers of such phenomena is critical for advancing environmental science and management (Lega *et al.*, 2018). The present study demonstrates the power of remote sensing in illuminating the complex dynamics of cyanobacterial blooms. Sentinel-2 multispectral satellite imagery was used not only to detect spectral signatures related to bloom events but also to analyze spatial and temporal bloom patterns (Laneve *et al.*, 2022). These remote observations were confirmed by in-situ water sampling and biological analyses. By combining these approaches, the study shows how remote sensing can go beyond monitoring to identify the ecological processes and interdependencies that regulate bloom dynamics, while also highlighting the importance of cyanobacteria as indicators of ecosystem health. The approach was applied across three Italian lakes—Albano, Vico, and Avernus—offering diverse ecological contexts. Spectral signature analysis revealed common trends between Albano and Vico, indicating shared environmental drivers and similar subspecies of *P. rubescens*. In contrast, Avernus exhibited unique bloom characteristics and distinct subspecies. This difference was confirmed by in-situ sampling, which observed different *Planktothrix* sub-species in Albano and Vico compared to Avernus one. This study establishes a spectral signature database for *P. rubescens*, enabling precise and rapid identification of harmful blooms. It underscores remote sensing's dual role as a measurement tool and a method for understanding ecological phenomena, with cyanobacteria acting as key bioindicators of human environmental impacts.

Keywords: Cyanobacterial blooms; *Planktothrix rubescens*; remote sensing; in-situ observations; spectral signature analysis

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Hydrodynamic effects on the aggregation and fragmentation of *Microcystis* colonies

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Hydrodynamics stresses, such as those generated by wind or artificial mixing, can greatly influence the formation of cyanobacterial colonies. A general framework describing the relative importance of flow-induced fragmentation and aggregation on colony size is still in its infancy. In this study, we present experiments and a numerical model that capture the effects of fluid flow on the size of colonies of the cyanobacterium *Microcystis*. Colonies from laboratory cultures and field samples are subjected to a controlled shear flow combined with direct microscopic imaging, which captures the dynamic changes in the colony size distribution. We demonstrate that colonies formed by clonal expansion have a strong mechanical resistance against hydrodynamic stresses due to the embedding layer of extracellular polymeric substances (EPS). In contrast, the aggregation of single cells into colonies leads to weaker structures than division-formed colonies. The relevance of the EPS layer is further evidenced by the higher resistance of field samples of *Microcystis* spp. against fragmentation in comparison to the laboratory cultures. At high levels of hydrodynamic stresses (exceeding those observed for wind mixing), single cells are eroded from colonies, leading to a reduction in colony size. Furthermore, we provide a numerical model that describes well the measured dynamical changes in colony size distribution. Our findings indicate that cell division is the main mechanism for colony formation under natural conditions, although aggregation can be relevant in dense scum layers [1].

Keywords: Fragmentation | Hydrodynamics | *Microcystis* | Colony formation

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Warming Surface and Lake Heatwaves as Key Drivers to Harmful Algal Blooms: A Case Study of Lake Dianchi, China

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Extreme heat events such as marine and atmospheric heatwaves have caused catastrophic impacts on marine and terrestrial ecosystems. Recent studies have shown that the warming surface continuously increases the frequency, duration, and intensity of lake heatwaves (LHWs) globally. However, the effects of LHWs on lake ecosystems, especially cyanobacterial harmful blooms (cHABs), are still lacking. This study reconstructed the daily Lake surface water temperature (LSWT) time series in Lake Dianchi from 1951 to 2023 with random forest. Then identified the trend and characteristics of LSWT and LHWs, and further analyzed the critical driving effect of LSWT and LHWs on cHABs. Results show that: the LSWT in Lake Dianchi significantly increased with a rate of 0.29 °C/10a, and the abrupt changing point was in 1992, which coincided with the first report of severe cHABs in Lake Dianchi. From 1951 to 2020, a total of 151 LHWs were detected, and 95.4% of them happened after 1992. Among all the potential climatic and water quality driving factors on Chl a variation, LSWT is the most important one. The Chl a and the intensity of LHWs are significantly correlated. The complex and strong interactions between LHWs and cHABs make it possible to suppress cHABs only when both remained low. The case study in Lake Dianchi highlighted the critical driving effect of LSWT and LHWs on cHABs, suggesting that continuously warming surface and enhanced LHWs in the future will further challenge more specific and effective lake management stratagems to suppress cHABs.



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Temperature regulation of microcystin degradation

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Toxic cyanobacteria blooms are increasing in frequency and severity with rising surface-water temperatures (1). Warming generates a positive feedback on harmful algal bloom development by promoting toxic cyanobacteria over non-toxic strains, stimulating toxin synthesis, and subsequently triggering toxin release (2). The release of cyanotoxins into the water column poses a serious threat to water quality and human health (3). Yet, cyanotoxins rarely accumulate in the water column long-term, suggesting that biodegradation by heterotrophic bacteria may play a role in the removal process. Owing to increased metabolic rates at higher temperatures, microbial-mediated degradation of cyanotoxins may be influenced by warming. In this study, we evaluated the ability for heterotrophic bacteria to degrade microcystin produced by a common cyanobacterium, *Planktothrix agardhii*, across a natural temperature gradient (from 5 to 20° C) during a laboratory incubation experiment. We measured microcystin concentration and bacterial abundance at 0, 6, 12, 24, 48, 96, and 192 hours. We found that microcystin concentration declined over time in all temperature treatments and degradation increased with warming. Patterns of microcystin degradation corresponded with increases in bacterial cell density over time that were enhanced by warming. Bacterial cell density increased most rapidly in the 20°C treatment resulting in the greater microcystin degradation than all other treatments. Degradation of microcystin was lowest in the 5°C treatment where the increase in bacterial cell density was the slowest. Bacterial density and microcystin degradation were similar between 10°C and 15°C treatments throughout the experiment and were consistently between 20°C and 5°C treatments. These results show that cyanotoxins can be reduced by microbial-mediated degradation and the rate of cyanotoxin degradation increases with water temperature.

Keywords: bacteria, climate change, cyanobacteria, toxins, water quality

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Bubble plumes promote deeper and longer recruitment of *Microcystis*

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In deep, stratified lakes, bubble plumes are effective in controlling cyanobacterial blooms [3], particularly those dominated by *Microcystis* spp.. While vertical migration has been identified as a key factor in bloom dynamics, our research suggests that recruitment processes, specifically the mobilization of benthic *Microcystis* populations, are equally important.

We conducted a long-term study in a 22-meter-deep Dutch lake to investigate the impact of bubble plume aeration on *Microcystis* recruitment. By combining *in-situ* measurements and laboratory experiments, we observed that aeration:

1. Extends the recruitment period: Aeration can stimulate *Microcystis* recruitment into autumn, even under conditions that would typically lead to sedimentation.
2. Mobilizes cells from deeper depths: Aeration can mobilize *Microcystis* cells from greater depths, potentially increasing the biomass available for bloom formation.

To understand these observations, we developed a quantitative model that simulates *Microcystis* sedimentation, overwintering, and spring recruitment based on [4]. The model incorporates temperature-dependent glycogen metabolism and light-induced buoyancy regulation [5]. Our simulations suggest that aeration can enhance recruitment by increasing water temperature and light availability at the sediment-water interface, thereby promoting cell buoyancy and mobilization.

While our study highlights the importance of recruitment in *Microcystis* bloom dynamics, further research is needed to fully elucidate the underlying mechanisms. Factors such as bioturbation by invasive species may also contribute to increased recruitment [6].

Regardless of the primary driver, our findings emphasize the need to consider recruitment processes in the design and implementation of aeration strategies for cyanobacterial bloom control. By increasing the capacity and duration of aeration, it may be possible to mitigate the negative impacts of *Microcystis* blooms.

Keywords: Recruitment, Bubble Plumes, *Microcystis*, Glycogen

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Environmental Impact Assessment: an advanced approach to the remote identification of Harmful Algal Blooms using Spectral Signatures

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Harmful Algal Blooms (HABs), and particularly cyanobacteria blooms, knowingly affect freshwater ecosystems and water quality. As anthropogenic activities and climate change continue to increase, the management of these phenomena becomes more critical. In this context, remote sensing presents an innovative approach to observe algal blooms, utilizing robust data archives and enabling large-scale monitoring while reducing costs and workforce demands compared to traditional methods. Additionally, different observation platforms allow to investigate these phenomena across different spatio-temporal scales, enabling multi-layer analyses (Lega et al., 2018). This study proposes an innovative method based on spectral signatures to monitor blooms of *Planktothrix rubescens*. Spectral signatures, which represent the reflectance of materials at specific wavelengths, are unique to each material and provide a powerful tool for the distinction of similar phenomena across diverse environments. This research aims to establish a spectral signature database designed for modern sensing technologies, enhancing the precision of HABs identification and monitoring in aquatic ecosystems while supporting a more comprehensive ecosystem analysis. For instance, cyanobacteria often exhibit a characteristic green-blue coloration, whereas *P. rubescens* is distinguished by a strong red colour. Employing Sentinel-2 data, the study focuses on three volcanic lakes: Albano and Vico, located in Central Italy (Lazio), and Lake Avernus, situated in South Italy (Campania), all of which have experienced blooms of *P. rubescens*. Previous studies by Messineo et al. (2006) and Laneve et al. (2024) on the Lazio lakes, and by Lega et al. (2023) on Lake Avernus, have analyzed these events. By deriving and comparing spectral signatures across these lakes, this approach highlights the potential to develop reference signatures for detecting and analysing similar bloom events globally. This type of analysis highlights the value of employing spectral data to advance the monitoring and management of algal blooms, offering insights that could support future research and environmental decision-making.

Keywords: Spectral Signatures, Remote Sensing, Harmful Algal Blooms

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What explain the variability in cyanobacterial community composition and cyanotoxin production in nearby shallow lakes located in Moselle (France)?

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The spatio-temporal dynamics of cyanobacterial community composition and cyanotoxin production in more or less connected waterbodies have been investigated as part of the NoCyano research project, which aims to develop a collaborative and sustainable approach to managing shallow lakes with multiple uses in eastern France. Within this framework, an assessment of the hazards associated with cyanobacterial blooms was conducted by monitoring cyanobacterial communities and their toxins in both water and fish.

To achieve this goal, water samples were collected during the summers of 2022 and 2023 from 14 sampling points across four lakes, along with a remote sensing analysis covering the period from 2017 to 2024. A participatory approach was also employed to gather fish from these lakes.

Large variations, depending on the time and the sampling sites (within and between the lakes), were observed over the two monitoring summers in (i) phytoplankton biomasses, which ranged from 1 to 453.5 $\mu\text{g.L}^{-1}$, (ii) the composition of cyanobacterial communities, with four dominant genera (Aphanizomenon, Dolichospermum, Microcystis, Planktothrix), and (iii) cyanotoxin concentrations, with microcystin levels ranging from 0 to 762 $\mu\text{g.L}^{-1}$ and anatoxin levels ranging from 0 to 2.1 $\mu\text{g.L}^{-1}$. No toxins were detected in the tissues (gut, liver, and muscle) of the 248 roaches analyzed. Finally, a strong correlation was observed between the phytoplankton biomass estimates derived from spectrophotometric measurements (after chlorophyll-a pigment extraction) and those obtained from remote sensing targeting chlorophyll-a or phycocyanin pigments.

An ongoing analysis is being conducted to assess the relative impact of variations in meteorological variables and lake water levels on the biomass and composition of cyanobacterial communities and on the cyanotoxin concentrations.

Keywords: shallow lakes, cyanobacteria, cyanotoxins, monitoring

Acknowledgements and Funding

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Toxin-Producing Cyanobacterial Blooms in the Waters of Blue Infrastructure of Lublin City (Eastern Poland): Implications for Water Quality and Ecological Status

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Toxin-producing cyanobacterial blooms have detrimental effects on aquatic ecosystems and water quality. We examined the seasonal variation and spatial distribution of phytoplankton, including cyanobacteria, of the main components of Lublin's blue infrastructure, namely the Zemborzycki Reservoir and three upland rivers in Lublin (including the Bystrzyca River flowing through the reservoir). Sampling was conducted in spring, summer, and autumn of 2023. Phytoplankton was predominated by diatoms (Bacillariophyceae), with the lowest abundance recorded in summer and the highest in autumn. In summer, the greatest phytoplankton abundance was observed in the Wrotków and Marina bathing areas (in the reservoir) and Bystrzyca River below the reservoir. The lowest abundance was found in the Bystrzyca River upstream of the reservoir. Phytoplankton abundance in the Bystrzyca River below the reservoir was 2-3.5 times higher than upstream, exhibiting a composition similar to that of the Zemborzycki Reservoir. Notably, during summer cyanobacterial blooms, the cyanobacteria *Aphanizomenon flos-aquae*, *Planktolyngbya limnetica*, and *Microcystis* spp. were detected throughout the Bystrzyca River downstream of the reservoir. The study also revealed the presence of intracellular microcystins (MCs), primarily MC-RR, in both the reservoir and Bystrzyca River downstream of the reservoir, with the highest concentration of MC-RR in Wrotków (1.27 µg/L). Additionally, MC-LR and [DAsp³]MC-LR were detected. The cyanobacterial abundance and MC concentration in Bystrzyca waters decreased with the distance from the reservoir. In August, in the cyanobacterial scum created in the Wrotków bathing area, intracellular total MC concentration reached 102.1 µg/L. The diatom index indicated that the Zemborzycki Reservoir and Czechówka River had a moderate ecological status, while other waters maintained a good ecological status. The ecological status of the Bystrzyca River declined below the reservoir compared to the upstream river. These findings underscore the impact of Zemborzycki Reservoir and cyanobacterial blooms on the water quality within the urban blue infrastructure of Lublin.

Keywords: Microcystins, cyanobacteria, Zemborzycki Reservoir, Bystrzyca River, water quality

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Climate change vs. nutrient control? Changes in river hydrology trigger cyanobacterial blooms

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For decades, mitigation measures to decrease nutrients (i.e. phosphorus) have successfully reduced eutrophication in many European inland waters including larger rivers¹. Nevertheless, in recent years a number of events indicate a recurrence of eutrophication phenomena such as phytoplankton blooms and oxygen depletion. While each event has unique circumstances and needs to be assessed individually, there are some overarching patterns indicating that climate change is counteracting established eutrophication control strategies².

Cyanobacterial blooms of *Microcystis aeruginosa* have been occurring regularly since 2017 in the regulated Moselle, a large temperate river, suggesting climate-induced eutrophication³. Examination of a long-term dataset (20 years) of water quality parameters along with hydrological modelling identified hydrological (i.e. discharge, flow velocity and residence time) and physical (temperature, light) factors as main drivers of the blooms. While nutrient concentrations have been decreasing over the past 20 years, and were thus not the initial cause, concentrations remained above limiting conditions and supported high primary production. The years 2021 and 2022 provided suitable examples for wet, cyano-free (2021) and dry, cyano-dominated (2022) conditions. They were studied in more detail by longitudinal profiles and remote sensing covering spatial bloom dynamics. Average summer flow velocity was significantly lower in 2022 with on average 0.13 m/s compared to 0.39 m/s in 2021 and residence time was up to 28 days higher. Total nitrogen was significantly lower and phosphorus higher during low water phases, indicating a shift in nutrient ratios during drought periods.

In conclusion, drought or dry weather conditions can change riverine hydrology in favour of cyanobacterial or other harmful algae blooms. This necessitates the adaption of water quality mitigation strategies including an extension of the established nutrients control measures. The Moselle may serve as an example for the future of regulated rivers in temperate regions under the accelerating impact of climate change.

Keywords: *Microcystis aeruginosa*, harmful algae bloom, eutrophication, discharge, regulated rivers

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Anatoxin biosynthesis gene abundance predicts anatoxin concentration in benthic cyanobacteria mats

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Reliable tools are needed to evaluate the risks associated with toxic cyanobacteria, and approaches based on PCR that detect and quantify toxic cyanobacteria by targeting the genes responsible for toxicity are gaining traction as they offer selectivity, sensitivity and relative simplicity. We compared a new droplet digital PCR (ddPCR) approach that permits the absolute quantitation of toxin genes with an analytical chemical approach. Benthic cyanobacteria samples ($n = 171$) were collected bi-weekly at 8 sites in the Fredericton region of the Wolastoq, New Brunswick, Canada, from June to September 2019 (Beach et al., 2022). Concentrations of anatoxin-a (ATX), homoanatoxin-a (hATX) and dihydroanatoxin-a (H₂ATX) were measured using DART-HRMS/MS analysis (Beach et al. 2022) and screened for the presence of the *anaC* gene, which is involved in the first step of the anatoxin biosynthesis pathway. All *anaC*-positive samples were then quantified using ddPCR. Anatoxin concentration and *anaC* abundance were low in June, increased until late August when values of 1648 μg anatoxins g^{-1} mat and 5.5×10^9 *anaC* copies g^{-1} mat were observed, and declined thereafter. Both anatoxins and the *anaC* gene were measured in 111 samples and correlated with an R^2 of 0.63. Cellular toxin quotas were calculated for each pair of samples and reached 274 pg cell^{-1} . The median quota was low in June, peaked at 0.44 pg cell^{-1} in July, and decreased thereafter. The July peak coincided with exponential growth of toxigenic *Microcoleus* and a shift in dominance from non-toxigenic to toxigenic *Microcoleus* subspecies (Valadez-Cano et al., 2023). The agreement between DART-HRMS/MS and ddPCR methods suggests that, for routine cyanotoxin monitoring applications, ddPCR provides a relatively accessible and cost-effective alternative. However, by pairing ddPCR together with analytical chemical approaches, cellular toxin quotas can be calculated and provide valuable insight into the seasonality of toxin biosynthesis.

Keywords: Anatoxin-a, Droplet Digital PCR, Mass Spectrometry, Microcoleus, Benthic Cyanobacteria

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P101 - 118

Divergent Cyanobacterial Responses to Climate Change in Temperate and Tropical Lakes

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Climate change is a key driver of cyanobacterial blooms, with increased cyanobacteria abundance and toxin production documented in rapidly warming lakes globally. This study investigates whether temperate (Canada) and tropical (Uganda) lakes exhibit similar trends in response to recent climate shifts. Climate records were analyzed to identify long-term changes and paleolimnological techniques were used to reconstruct cyanobacteria and cyanotoxin histories in Ugandan lakes. Our findings reveal that both temperate and tropical lakes have become warmer since the 1990s. Sediment records starting in the 1950s showed similar patterns in phytoplankton trends. Both temperate and tropical lakes showed early signs of cultural eutrophication in highly impacted catchments, marked by elevated cyanobacteria. These trends intensified during the 1980s, coinciding with regional climate shifts and temperature increases of about 2°C above the pre-1990 mean. In contrast, lakes in minimally impacted catchments exhibited a single productivity spike during the same period, aligning with the onset of accelerated warming since the 1980s. However, sediment records revealed divergent patterns in cyanotoxin production potential. Using the microcystin (mcyE gene) as a marker of cyanotoxin, temperate lakes showed relatively low microcystin potential, which increased in tandem with an increase of 2°C, which raised the mean snow-free temperatures to 15°C. In tropical lakes, however, microcystin potential was initially high but declined with an increase of 2°C, which raised the mean temperatures to 25°C. These differing trends emphasize the complex response of cyanotoxin potential to rising temperatures. This cross-regional comparison underscores climate change's pervasive yet variable influence on lakes, providing critical insights into region-specific responses and potential risks associated with cyanobacterial blooms globally.

Keywords: global change, paleolimnology, temperate, tropical lakes, microcystin

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Exploring the dynamics of cyanobacteria growth in urban ponds of Brussels using the CYBLOOM model

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The development of cyanobacteria blooms and the impact of environmental conditions (nutrients, light, and water temperature) on their growth were explored with the mechanistic CYBLOOM model. The CYBLOOM model describes the dynamics of phytoplankton (with four groups, including an explicit representation of cyanobacteria), zooplankton, nutrients (ammonium, nitrate, phosphate, and silica) and organic matter remineralization in the water column. As a first step, the cyanobacteria growth was calibrated and validated in batch conditions and compared to existing laboratory studies on *Microcystis* and *Dolichospermum*. Results showed that the growth of both cyanobacteria was favored under high nutrient and high temperature conditions. However, *Microcystis* was more competitive than *Dolichospermum* under phosphorus-limited conditions and at high temperatures, consistent with current knowledge. The CYBLOOM model was then applied to study the seasonal development of cyanobacteria blooms in Brussels ponds and was compared to field data collected in 2022. Results demonstrated the model's ability to accurately reproduce the occurrence and magnitude of cyanobacteria in summer, as observed. Sensitivity analysis were conducted to further investigate environmental conditions that promote cyanobacteria development in eutrophied urban ponds and the potential impacts of expected future changes in temperature and nutrient loads. An earlier onset of cyanobacteria blooms and an increase in bloom magnitude were simulated in response to rising water temperature. A higher supply of nitrogen and phosphate resulted in longer and more intense cyanobacteria bloom.

Keywords: cyanobacteria bloom, mechanistic model, eutrophication, urban ponds

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Assessing the frequency of microcystin biodegrader and *mlrA* gene detection in Lake Zurich

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Global warming and anthropogenic-induced alterations in nutrient ratios favour harmful algal blooms (HABs) in freshwaters, posing a threat to human and animal health. In Lake Zurich, the filamentous cyanobacterium *Planktothrix rubescens* benefits from lake warming and has become the dominant microbial organism, forming massive seasonal blooms. Abiotic stressors such as intense UV radiation and turbulences disrupt *P. rubescens* filaments, releasing the bioactive secondary metabolite microcystin (MC) into the surrounding matrix. Although physico-chemical transformation of MC is slow, concentrations of MC remain undetectably low throughout all seasons. Thus, we hypothesized that MC biotransformation must be a common trait in Lake Zurich. We assessed the seasonal and spatial dynamic of bacterial MC degraders in the context of *P. rubescens* population dynamics and investigated the ecological role of the only described MC degradation pathway via the *mlr* gene cluster. We used a dilution to extinction strategy coupled with the most-probable number method to determine the frequency and seasonal succession of microcystin-degrading bacteria in three water layers. Lake water samples were inoculated with lysed axenic *P. rubescens* filaments and MC concentrations quantified by HPLC-MS. The presence of *mlrA* gene in selected enrichments was examined with semi-nested PCR. Highest abundances of degraders was found in the upper hypolimnion during summer stratification. By early September, the degrader maxima shifted upwards, persisting in the metalimnion until late autumn, when thermal mixing caused equal distribution throughout the water column and induced a pronounced increase in degrader abundance. This coincides with the population pattern of *P. rubescens*. Detection of the *mlrA* gene in the most abundant fraction of MC degraders remained unsuccessful. In conclusion, our study highlights the dynamic interplay between *P. rubescens* proliferation and the seasonal abundance of MC degrading bacteria.

Keywords: *Planktothrix rubescens*, microcystin (MC), MC biotransformation, degraders, *mlrA* gene



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Activation and bio-decontamination of water by cold atmospheric plasma, towards green fertilizer with lower environmental impact

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The reactive chemical species generated by cold atmospheric plasma of electrical discharges can be trapped in liquids, particularly water, to produce a chemically rich solution called plasma-activated water (PAW) [1]. PAW has demonstrated strong antimicrobial properties and has been extensively studied for its wide range of applications in food preservation, agriculture, and biomedicine and has a great potential for wastewater treatment, especially to inactivate toxic microorganisms [2]. So far the studies applying plasma discharges and PAW against cyanobacteria are rare [3].

By combining two distinct plasma sources - a transient spark, generating nitrogen oxides and nitrous/nitric acids, and an ozone generator based on dielectric barrier discharge - we were able to generate two distinct types of PAW. The first type, rich in NO_2^- (nitrite ions), exhibits strong antimicrobial properties and is suitable for the bio-decontamination of water. The biocidal effects of this PAW, generated by the transient spark, were verified on several microorganisms [4, 5]. Further research, including tests on cyanobacteria, is planned to assess its efficacy against a wider range of microbial contaminants. The second type of PAW, rich in NO_3^- (nitrate ions), shows promise as an alternative fertilizer for plants, presumably with a lower potential for causing harmful cyanobacteria blooms compared to traditional nitrogen-based fertilizers.

Keywords: nitrogen fixation, green agriculture, water bio-decontamination, cold plasma

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Disappearance of *Planktothrix rubescens* in a drinking water reservoir over the years (Sapanca Lake, Türkiye)

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Sapanca Lake is one of the major drinking water sources for the city of Kocaeli and Sakarya.. It has a deep monomictic character and is stratified between late April and mid-November. Since the 1990s, the lake has faced a constant threat due to the presence of *Planktothrix rubescens*, a potential cyanotoxin-producing species. The lake has been monitored for more than 30 years using different sampling depths and strategies. This extensive dataset indicates that in the 2020s, *P. rubescens* biomass decreased gradually.

In this study, we focused on three specific years: 2016, characterized by the highest *P. rubescens* biomass and total microcystin levels; 2021, which exhibited reduction on *P. rubescens* biomass; and 2024, when the *P. rubescens* population shifted entirely, and no microcystin was detected. As *P. rubescens* primarily resides in the metalimnion, water samples were collected from depths of 0 m, 10 m, 15 m, and 20 m were analyzed. According to the trophic status index, based on Total Phosphorus (TP), Chlorophyll-a and Secchi disk depth, Sapanca Lake transitioned from a mesotrophic status in 2016 to an oligo-mesotrophic status by 2024. The mean biomass of *P. rubescens* decreased by 78.1% between 2016 and 2021 and by 98.7% between 2021 and 2024. Its mean concentration decreased by 86.3% from 2016 to 2021 and by an additional 9% from 2021 to 2024. Among the measured parameters, Total Nitrogen showed the most significant variation over time. This decline led to disappearance of *P. rubescens* and the emergence of other species. Our findings suggest that the decrease in total nitrogen concentrations is a key factor influencing *P. rubescens* biomass. However, other factors, such as temperature-driven water column mixing and light availability, may also play important roles.

Keywords: Climate change, Nutrients, Long-term data, Microcystin



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Biotechnological potential of cyanobacterial blooms for sustainable and environmental solutions

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Cyanobacterial blooms, driven by eutrophication and climate change, represent a critical global challenge, threatening water quality, public health, and ecosystem balance^{1,2}. These blooms, marked by rapid cyanobacteria proliferation, disrupt aquatic ecosystems and release harmful cyanotoxins, posing significant challenges for water management and human safety^{2,3}. The increasing frequency and severity of blooms underscore the urgent need for innovative strategies to mitigate their impacts while harnessing their biotechnological potential⁴. This study investigates cyanobacterial biomass collected from eutrophic waters in Sabino, São Paulo, Brazil. Remote sensing tracked chlorophyll-a concentrations, revealing the spatiotemporal dynamics of blooms. Ethanol-assisted greenhouse drying proved practical and scalable, preserving biomass quality for diverse applications. The biomass exhibited high levels of essential nutrients, such as phosphorus, potassium, calcium, and magnesium, vital for soil fertility⁵. Combined with significant nitrogen levels, these elements establish the biomass as a valuable biofertilizer, enhancing agricultural productivity and reducing reliance on synthetic fertilizers. The presence of micronutrients like iron and zinc further supports its role as a soil amendment, fostering plant growth and resilience⁵. Additionally, the amino acid profile, rich in glutamic and aspartic acids, underscores its potential as a sustainable protein source for aquaculture and animal feed, aligning with circular bioeconomy principles⁶. The biomass also demonstrated significant metal bioaccumulation, such as iron and phosphorus, making it suitable for bioremediation and pollutant removal⁷. Elevated carbon content reinforces its role in carbon sequestration and climate change mitigation⁸. Although cyanotoxins like microcystins, saxitoxins, and cylindrospermopsin present ecological and health risks, controlled applications unlock opportunities for high-value products, including pharmaceuticals, bioplastics, and biofuels^{3,9}. This duality of risks and opportunities highlights the importance of innovative strategies to transform cyanobacterial blooms into valuable and sustainable resources, addressing environmental challenges while fostering economic and technological advancements.

Keywords: Eutrophication, biomass valorization, circular economy, cyanotoxins, biofertilizer.

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Engaging Citizen Scientists in Research on Toxic Cyanobacterial Harmful Algal blooms

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Cyanobacterial blooms pose considerable risks to water quality, aquatic ecosystems, and public health due to their potential for producing harmful toxins. Effective monitoring of these blooms requires the application of diverse and multi-level tools to understand and better assess the associated risks. This project emphasizes the role of citizen science in augmenting data collection efforts, enhancing research, and raising environmental awareness. The collected data will be promptly published online, offering a valuable public service by informing community members about specific times and locations to avoid water-related activities such as swimming. Additionally, the project aims to collect high-frequency samples to monitor spatial and temporal variations in the phytoplankton community, thereby improving our understanding of phytoplankton dynamics. Our results showed that higher frequency data contributed to more understanding of algae concentration dynamics in the lake, methods and impact can be further improved and assessed.

Keywords: Water quality monitoring, ecosystem service, high-frequency data, public engagement

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P108 - 280

Exploring the role of climate change in toxic benthic cyanobacterial proliferation in Lake Lugano (Switzerland and Italy)

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Free-floating toxic cyanobacterial mats were observed for the first time in Lake Lugano (Switzerland and Italy) during the past two summers. The underlying causes of the rapid proliferation of benthic cyanobacteria remain unclear, and the potential negative effects on the ecosystem and water quality pose a new challenge for water management. Climate change is promoting the dominance of cyanobacteria in aquatic ecosystems; however, the effects on benthic species are still poorly understood. To investigate the source of cyanobacterial mats and the drivers of their proliferation in Lake Lugano, we conducted a study on the community structure and distribution of benthic cyanobacteria, focusing particularly on toxic species. We collected samples of cyanobacterial mats from the watershed of Lake Lugano and assessed their taxonomic composition using microscopy and high-throughput sequencing of the 16S rRNA amplicon sequence variants (ASVs). Cyanotoxins were quantified using liquid chromatography-mass spectrometry (LC-MS). The results were compared with the community structure and cyanotoxin profiles of floating mats and physicochemical variables. Our findings revealed that potential toxin-producing genera were widespread in benthic mats (e.g., *Oscillatoria* sp., *Phormidium* sp., *Tychonema* sp., *Scytonema* sp.). We observed distinct spatial distributions of anatoxin-producing and microcystin-producing cyanobacteria, which appeared to be influenced by different environmental variables (e.g., temperature, phosphorus levels, light availability). These results suggest that ongoing environmental changes could drive the future increase of toxic benthic cyanobacterial blooms. Although identifying the source of floating mats is challenging, the study emphasizes the urgent need for extensive research to identify high-risk areas and develop a management strategy to assist relevant authorities in mitigating the impact of these harmful blooms.

Keywords: benthic cyanobacteria, anatoxin, microcystins, deep lakes, climate change

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P109 - 245

Portable device for electrochemical detection of saxitoxin based on covalent organic polymers

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Toxic harmful cyanobacterial and algal blooms have intensified in recent years due to climate change, eutrophication, and human activities, leading to global environmental concerns [1]. Their proliferation has become a global environmental issue, as toxins can be released into waters or accumulate in the food chain, particularly in seafood and fish, affecting water and seafood safety and aquaculture sector [2]. Saxitoxin (STX) is a highly toxic and fast-acting neurotoxin produced by cyanobacteria in freshwaters and dinoflagellates in marine waters. It induces symptoms as numbness, headache, muscle weakness, respiratory failure, and even death, being one of the main responsible of the paralytic shellfish poisoning [3]. Thus, rapid, sensitive, and selective detection of STX is crucial. Currently available methods (mouse bioassay, high-performance liquid chromatography–mass spectrometry and enzyme-linked immunosorbent assay) have limitations such as ethical concerns, low sensitivity, high cost, need of complex equipment, and poor stability of reagents [3]. This work presents a novel portable electrochemical sensor based on covalent organic polymers (COP) for STX detection. The carboxyl-functionalized COPs (TpPa-COOH COP) offer high adsorption rates for STX [4], excellent chemical stability, and reusability, making them ideal for portable electrochemical devices. A gold nanoparticle-coated COP composite was prepared by the in-situ growth of GNPs on this COP via a seed-assisted method and it was used to modify the surface of electrochemical sensors. The surface modification process was characterized by scanning electron microscopy, confocal Raman microscopy, electrochemical impedance spectroscopy (EIS) and voltammetry techniques. The STX detection was performed by EIS and results indicate that 10 μ M STX was able to induce a significant electrochemical signal compared to the control. These sensors are well-suited for integration into an automated and portable device, allowing for the real-time monitoring of STX in real water samples and providing an efficient and effective solution for continuous environmental monitoring.

Keywords: Harmful cyanobacterial and algal blooms, paralytic shellfish toxins, saxitoxin, electrochemical sensor, covalent organic polymers

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Biotic interactions shape the realised niche of toxic cyanobacteria

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Cyanobacterial blooms pose a significant threat to freshwater ecosystems, driven by eutrophication and climate change. While traditional models aiming at these occurrences primarily focus on abiotic factors, our study highlights the critical role of biotic interactions in shaping cyanobacterial growth dynamics over temperature and phosphorus levels. Using five years of high-frequency data from a eutrophic lake, we employed state-space reconstruction techniques to investigate the complex interplay between cyanobacterial daily net growth rates and co-occurring plankton taxa. Results show that biotic interactions can change the magnitude and shape of cyanobacterial growth response to temperature and phosphate levels and that both zooplankton and phytoplankton interactions can significantly alter the temperature and phosphorus requirements for a positive net growth in all studied cyanobacteria. This can have significant impacts on our understanding and prediction of cyanobacterial blooming. Our findings suggest that incorporating biotic factors into bloom mechanistic or forecasting models is crucial for accurate understanding, predictions and effective management strategies.

Keywords: biotic interactions, realised niche, empirical dynamic modelling

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Impact of *Microcystis aeruginosa* on Fish Appetite through Intestinal Inflammation

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Microcystis aeruginosa is a dominant and widely distributed cyanobacterium that plays a major role in the formation and recurrence of harmful algal blooms in lakes. While it is known to cause intestinal inflammation and appetite dysregulation in fish, the relationship between these effects and their underlying molecular mechanisms remains poorly understood. This study explored the impact of *M. aeruginosa*-induced intestinal inflammation on fish appetite, with a focus on the underlying molecular pathways, using *Pseudorasbora parva* exposed to *M. aeruginosa* for 96 hours. We found a significant increase in inflammatory cells in the intestinal tract, accompanied by notable changes in glycerophospholipid and tryptophan metabolism, pathways associated with inflammation and appetite regulation. Key inflammatory markers (IL-6, IL-8, IL-1 β , NF- κ B, and TNF- α) were significantly elevated, while appetite-stimulating factors (orexins, ghrelin, and acetylcholine) were markedly reduced. In contrast, appetite-suppressing factors (5-HT, GABA, POMC, GLP-1, and CCK) showed significant increases. Correlation analysis revealed that inflammatory markers were negatively correlated with appetite-stimulating factors and positively correlated with appetite-suppressing factors. These findings indicate that *M. aeruginosa*-induced intestinal inflammation is a potential mechanism underlying reduced appetite in fish. This study provides novel insights into the toxic effects of *M. aeruginosa* on fish and offers a new perspective on the mechanisms by which it suppresses fish appetite, contributing to the broader understanding of the ecological and physiological impacts of cyanobacterial blooms.

Keywords: *Microcystis aeruginosa*; *Pseudorasbora parva*; Inflammatory response; Appetite regulation; Intestinal metabolites

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Toxicity of *Raphidiopsis raciborskii* extracts

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Raphidiopsis raciborskii (Wołoszyńska) Aguilera & al. 2018 is a cosmopolitan cyanobacterial species known for its ability to produce numerous bioactive secondary metabolites and the diversity of its biological activity has sparked significant interest in exploring the harmful potential of strains from various geographical origins. Therefore, exploring the biological activity of *R. raciborskii* metabolites was the goal of our research.

The toxicity of extracts of *R. raciborskii* strains from different localities was assessed. Most examined strains were non-toxin producing (isolated in Poland and Israel), but there were also two cylindrospermopsin producers isolated in Australia (CS-505 and CS-506). Cyanobacterial biomass was harvested after five weeks of growth and extracted with 75% methanol. The extracts were air-dried and subsequently reconstituted in phosphate-buffered saline (PBS) for use in the assays. A range of biomass concentrations, spanning from 75 µg/mL to 3 mg/mL, was tested to evaluate the biological activity using several toxicological assays (acute toxicity assay, cytotoxicity assay and alkaline comet assay). Examined cyanobacterial extracts seem to contain potent bioactive compounds with harmful effects on other organisms, cellular metabolism and gap junctional intercellular communication (GJIC), and genetic stability. Toxic effects included mortality of invertebrates, disruption of cellular metabolism and GJIC, as well as DNA damage in human mononuclear cells. Even though only two strains contain known cyanotoxin, the described effects were found in other strains as well, suggesting a presence of some other biologically active compounds that could potentially explain the observed effects. Furthermore, due to diverse nature of the applied toxicological tests, a wide array of responses was noted for each strain depending on the test, indicating that strains produce unique compounds with varying effects. The described biological activity of *R. raciborskii* highlights potential ecological and health risks associated with this cyanobacterial species.

Keywords: *Raphidiopsis raciborskii*, toxicity, biological activity

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How insecticides affect the toxicity of natural organophosphate guanitoxin during Zebrafish embryo-larval development

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Guanitoxin (GNT) is a neurotoxin produced by cyanobacteria and the only natural organophosphate documented in the literature, exhibiting potential effects similar to synthetic insecticides. This study evaluates the toxicity of GNT, produced by *Sphaerospermopsis torques-reginae*, and its combined effects with synthetic organophosphate insecticides (malathion - MLT and trichlorfon - TCF) on the embryo-larval stage of Zebrafish (*Danio rerio*), aiming to understand how insecticides influence GNT toxicity. *D. rerio* embryos were exposed for 120 hours to MLT, TCF, and GNT extracts, both separately and in mixtures, including three binary combinations (MLT+GNT, MLT+TCF, TCF+GNT) and one tertiary combination (MLT+GNT+TCF). Various endpoints were evaluated, such as morphology, cardiology, and behavior. The results showed significant alterations in larvae exposed to the chemicals, with the most pronounced morphological damage including yolk and heart edema, uncontrolled movements, no reaction to triggers, malformed head, and altered axis structure. Measurements showed up- and down-regulation of biomarkers, including increased yolk and pericardial areas and decreased body length, eye size, swim bladder area, and swim bladder inflation. Animals exposed to higher concentrations of TCF and MLT individually exhibited reduced heart rates, as well as in the GNT+MLT and TCF+MLT combinations. Behavioral observations revealed a reduction in locomotor activity, typically seen in the dark (light-seeking behavior). For TCF and GNT extracts, reduced swimming activity was observed across all treatments during the dark phase, and MLT showed significant effects at higher concentrations. Most binary and tertiary combinations showed a reduction in movement distance. In conclusion, both individual compounds and mixtures caused morphological, cardiovascular, and behavioral alterations, with potentially synergistic or additive effects in GNT, MLT, and TCF combinations. This study contributes to addressing knowledge gaps regarding GNT's impact on aquatic organisms and emphasizes the need to explore the mechanisms of the observed toxic effects.

Keywords: behavior, cardiotoxicity, cyanotoxins, ecotoxicology, pesticides

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A Kinetic Analysis of the Inhibition of Protein Phosphatases by the Cyanobacterial Toxin Microcystin-LR

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Cyanobacteria produce the toxic peptides, Microcystins (MC). These toxins inhibit the enzyme protein phosphatase (PP) through both reversible and irreversible binding. The mode of action is often associated with the covalent binding of MCs to an essential cysteine within the enzyme binding site. Here we conduct a kinetic analysis of the ability of microcystin LR to inhibit the enzymes protein phosphatase 1 and 2. PP1 used in these experiments showed Michaelis Menten kinetics with a V_{max} of 2.5×10^{-8} M/s and K_m of 27.7 mM for para nitrophenyl phosphate (pNPP). Microcystin LR was a non-competitive inhibitor of PP1 with a K_i of 2.7×10^{-8} . This indicated MC-LR reversibly bound to the enzyme at a site separate from where pNPP binds in the active site. The V_{max} for this enzyme did not change when the enzyme was incubated with MC-LR for up to 6 hr, indicating that covalent linkage of MC-LR to the enzyme did not occur during this time. The reversible inhibition by microcystin of its potential target which occurs within minutes to hours, rather than the irreversible binding to the enzyme that could lead to enzyme destruction which occurs over days, may have important implications for the biological function of this toxin *in situ*.

Keywords: Microcystin enzyme kinetics noncompetitive inhibitor

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Toxicity analysis of *Oscillatoria* B1 strain: combined *in vitro* and *in vivo* testing approaches

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In this study, the toxic potential of the cyanobacterial strain *Oscillatoria* B1 was assessed using zebrafish embryos (*Danio rerio*), the crustacean *Daphnia magna*, and human neuroblastoma (SH-SY5Y) and hepatocellular carcinoma (HepG2) cell lines. Zebrafish embryos were exposed to varying concentrations of extracts, and several sublethal effects, including skeletal deformities such as spinal and tail curvature, as well as pericardial and yolk sac edemas, were observed. Skeletal malformations were first detected after 48 hours in groups exposed to 200 and 250 $\mu\text{g mL}^{-1}$, with their frequency increased significantly with prolonged exposure, indicating a time-dependent response. An EC_{50} value of 162.5 $\mu\text{g mL}^{-1}$ was determined for skeletal deformities at 120 hours post-fertilization. In contrast, no acute toxicity was observed in the *Daphnia magna* biotest. At the molecular level, alterations in the relative expression of certain genes and disruption of homeostasis in both organisms were noted. Significant changes were observed in pathways associated with estrogenicity (*vtg1*) in zebrafish and xenobiotic metabolism (*cyp314*) in *D. magna*. Cytotoxicity was assessed *in vitro* using the MTT assay, which showed a reduction in viability in both tested cell lines treated with *Oscillatoria* B1 extracts. The dose- and time-dependent toxicity was observed, with an IC_{50} value of 1723 $\mu\text{g mL}^{-1}$ for SH-SY5Y cells and 534 $\mu\text{g mL}^{-1}$ for HepG2 cells, after 72 hours of exposure. The sublethal effects observed in zebrafish embryos and influence at the molecular level suggest that the strain *Oscillatoria* B1 produces metabolites that disrupt normal developmental processes in vertebrates. Coupled with the evidence of cytotoxicity obtained through *in vitro* testing, these findings highlight the strain's significant toxicological impact. They also emphasize the importance of identifying the specific bioactive compounds responsible for these effects and understanding their mechanisms of action at different levels of biological organization.

Keywords: Biotests, Cyanobacteria, Cytotoxicity, *Daphnia magna*, Zebrafish (*Danio rerio*)

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Assessing direct and indirect effects of cyanotoxins on *Daphnia*: survival dynamics under different exposure scenarios

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Harmful cyanobacterial blooms are a significant concern in freshwater ecosystems, impacting water quality and food web dynamics. Zooplankton, such as *Daphnia*, are particularly vulnerable to cyanobacterial toxins, which can disrupt population dynamics and ecosystem functioning. In this study, we explored the direct and indirect effects of toxic and phylogenetically similar non-toxic cyanobacteria on two Cladocera species (*Daphnia magna* and *Daphnia galeata*), together with a cyanotoxin extract, through life-table response experiments.

We conducted both ingestion and environmental (diffusion-based) exposure tests. Our results demonstrated clear distinctions in survival and reproduction across species and exposure methods. Direct exposure to toxic cyanobacteria and toxin extracts caused rapid mortality in both daphniid populations, with *D. magna* showing slightly greater resilience. Non-toxic cyanobacteria, on the other hand, induced minimal mortality, confirming the importance of toxins rather than nutritional quality in these effects. Starvation-induced mortality occurred gradually, highlighting the toxicity-specific impacts of cyanobacteria and emphasizing the role of toxins over food deprivation.

Furthermore, indirect exposure experiments, designed to allow the transfer of toxins without access to whole cells, revealed a significant reduction in survival. *D. magna* exposed to toxic cyanobacteria and toxin extracts indirectly exhibited a delayed but ultimately sharp decline in survival compared to direct exposure, suggesting that toxins alone are sufficient to cause substantial harm. Non-toxic cyanobacteria elicited no significant mortality under indirect exposure conditions.

Overall, our findings highlight the multifaceted ecological impacts of cyanobacterial toxins, revealing their capacity to disrupt zooplankton populations through both direct ingestion and environmental toxin release.

Keywords: Cyanotoxins, *M. aeruginosa*, *Daphnia*, Direct and indirect exposure

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Cyanobacterial blooms: a newfound threat to the wild Iberian Lynx?

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Wild animals often encounter toxic cyanobacteria in their habitats, particularly in freshwater bodies where cyanobacterial blooms frequently occur. Despite the growing evidence linking cyanotoxins to wildlife morbidity and mortality¹, the true extent of cyanobacteria's impact on the health of wild animals remains an under-researched topic. Understanding how toxic cyanobacteria poison animals may contribute to the effective clinical diagnosis in instances of unexplained wildlife health crisis, which pose significant challenges for natural wildlife conservation areas. By monitoring freshwater bodies in these regions, we can better assess the risk of cyanotoxin exposure among wild animal populations and enhance management strategies for species conservation programs. Additionally, the issue of cyanobacterial blooms is increasingly recognized within the One Health framework, as these freshwater environments may also serve as bathing areas and irrigation sources, potentially impacting human health and agricultural productivity.

The Guadiana River Valley Natural Park, in southern Portugal, is a conservation area for the Iberian Lynx, one of the world's most endangered feline species. Until 2015, it was classified as Critically Endangered by the International Union for Conservation of Nature². However, conservation efforts have led to a positive shift, with the Iberian Lynx now globally listed as Vulnerable, and Endangered within Portugal. The region's Mediterranean climate, characterized by high temperatures and drought conditions, favors the proliferation of cyanobacterial blooms.

In this study, we focused on monitoring reservoirs and ponds in the Guadiana River Valley that serve as watering spots for the Iberian lynx, for the presence of cyanobacteria, cyanotoxins and *mcy* genes. Preliminary findings indicate the presence of *Microcystis* spp. blooms at lynx crossing points, underscoring a potential risk of exposure to microcystins. This information may be crucial in understanding the environmental challenges faced by the Iberian Lynx and highlights the need for ongoing monitoring and conservation efforts in these habitats.

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cyanobacteria, cyanotoxins, Iberian Lynx, freshwater, Guadiana Natural Park



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The impact of anthropopressure in toxic cyanobacteria and bacteria communities at the volcanic-origin lakes of Amatitlán and Laguna del Pino, Guatemala

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The global occurrence of cyanobacterial harmful blooms (cHBs) is increasing due to prolonged anthropogenic pressure from urban, rural, and agricultural catchments. Wastewater, industrial effluent, and agricultural runoff discharged to freshwater bodies increase nutrient concentrations leading to the appearance of cHBs. In Guatemala, Amatitlán Lake is particularly affected by a segregated catchment with intense urban and agricultural land use, whereas Lake Laguna del Pino is a National Park mainly surrounded by natural forest with small impact from rural areas. The present study is a novel and first comparison of phytoplankton communities (prokaryotic and eukaryotic) and toxigenicity of cHBs in mentioned lakes of volcanic origin experimenting different anthropogenic pressures. Water samples were collected from: 1) the hypereutrophic Amatitlán Lake, at two different points, one influenced by an urban catchment and the second by an agriculture catchment; and 2) the mesotrophic Laguna del Pino Lake with more natural catchment. Microscopic analysis and 16S rRNA amplicon sequencing was performed to characterize the phytoplankton and cyano-bacterioplankton communities, respectively. Toxigenicity of blooms was also addressed with the detection of cyanotoxin genes: microcystins (*mcyE*), cylindrospermopsins (*cyrJ*), anatoxins (*anaC*) and saxitoxins (*sxtA*). The phytoplankton communities of the urban and agricultural catchment in Amatitlán were dominated by *Microcystis*, while eukaryotic taxa of *Cyclotella* (diatom) dominated in Laguna del Pino. Amatitlán showed toxigenic potential for microcystins (+ *mcyE*) and anatoxins (+ *anaC*), and Laguna del Pino showed toxigenic potential for microcystins (+ *mcyE*) and saxitoxins (+ *sxtA*). These results suggest that different land uses could influence the composition of phytoplankton and bacterioplankton communities. Although cyanobacteria were not highly detected for Laguna del Pino, its toxigenic potential suggests that it is on risk for deterioration, and therefore the local authorities must be informed in order to continue to effectively protect the lake from the phenomenon of increasing eutrophication.

Keywords: land use, toxigenic genotypes, phytoplankton communities, 16s rRNA

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P120 - 160

Role of cyanotoxins in horizontal gene transfer of antibiotic resistance genes

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In 2015, the World Health Organization (WHO) assessed antimicrobial resistance (AMR) as a major public health threat. It promoted a Global Plan of Action to fight AMR with a One Health approach, emphasizing the close link between human, animal, and environmental health ¹. In the past decade, it has been acknowledged that the environment influences the spread and transmission of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) through various mechanisms. These include horizontal gene transfer (HGT) which can involve the transfer of genetic information between bacteria of the same or different species, contributing to the rapid spread of ARG in the environment ².

Cyanobacteria, photosynthetic prokaryotic organisms, occur naturally in many freshwater habitats, and many species produce potent cyanotoxins ³.

Some recent studies suggest a role for cyanobacteria in the spread of ARBs in aquatic environments by increasing the populations of ARBs possibly present during their blooms and cyanotoxins may affect the rate of HGT between bacteria ⁴. Our work aimed to test whether, under controlled laboratory conditions, cyanotoxins can increase the conjugation frequency of the *incHI2* plasmid (> 200kb), a natural plasmid with low conjugation frequency. For this purpose, we tested the ability of two variants of microcystins MC-LR and [D-Asp3] MC-RR (the major cyanotoxins produced by *Microcystis aeruginosa* and *Planktothrix rubescens* respectively, two of the most widespread species in Italy) in promoting HGT of this plasmid carrying an antibiotic resistance gene between strains of *Escherichia coli*. We have observed that both microcystins, across a wide concentration range covering those normally measured during the development and maturity phases of blooms, increased the transfer rate between bacteria. This result highlights a possible mechanism for the increase in antibiotic-resistant bacteria during cyanobacterial blooms.

Keywords: Toxic Cyanobacteria, Horizontal gene transfer (HGT), Antibiotic resistance genes (ARGs)

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Occurrence of methanogenic archaea in freshwater bodies experiencing cyanobacterial blooms

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Methane is the second most abundant greenhouse gas, for this reason it is significant to define the sources of its origin and cycling in the environment. Microbial methane production in lakes occurs mainly in anoxic sediments; however, recently methanogens were observed in *Microcystis* aggregates (1). Anoxic microniches occur in *Microcystis* aggregates and scums and are important for anaerobes such as methanogenic archaea (2). In an era of increasing anthropopressure and eutrophication, understanding the correlation between cyanobacterial blooms and methanogenic archaea could be crucial for the environment due to its potential to contribute to increased methane emissions into the atmosphere.

This study is one of the first to compare methanogenic archaea occurrence to cyanobacterial occurrence in freshwater bodies across continents. In 2023, water samples were collected from four water bodies (1-4), in three countries: Poland (POL 1 and POL 2), United States of America (USA 3) and Singapore (SIN 4). 16S rRNA amplicon sequencing was used to determine dominant groups of cyanobacteria. Shotgun metagenomic analysis allowed us to extract the composition of archaea. The qPCR of *mcrA* (a methane reductase gene) was used to determine total abundance of methanogenic archaea.

The highest amount of methanogenic archaea was found in the USA 3. This site showed the highest relative abundances of methanogens including members belonging to *Methanothrix*, *Methanobacterium*, *Methanoregula* and an unclassified taxon of Methanoregulaceae. The POL 2 followed with high abundances of *Methanosarcina* and *Methanothrix*. In the remaining reservoirs (POL 1 and SIN 4), we did not record a significant amount of methanogenic archaea. Statistical analysis revealed that *mcrA* gene copy numbers were positively correlated to the relative abundance of *Microcystis* PCC7914, suggesting that coccoidal colonies of *Microcystis* provide another ecological niche for methanogenic archaea. The analyses will be extended for the subsequent year.

Keywords: methane, *mcrA*, 16S rRNA sequencing, *Microcystis*

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Assessment of lethal and sublethal effects of *Raphidiopsis raciborskii* extracts on zebrafish embryos using automated phenotyping method

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Extracts of six *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera et al. 2018 strains were evaluated for their potential lethal and sublethal effects on zebrafish embryos. The strains included two toxic (CS-505 and CS-506) and four nontoxic (UAM/DH-BiRr, UAM/DH-PnRr, UAM/DH-KmRr and KLL07). Cyanobacterial biomass was extracted with 75% methanol and air dried. Dry extracts were reconstituted in sterile PBS at 10 mg/mL. Zebrafish embryos were exposed from 2 hours post-fertilization (hpf) to 120 hpf at concentrations of 200, 100, 50, 25, and 12.5 mg/L diluted in E3 medium. Mortality rates were recorded at the end of the exposure period, and surviving embryos were phenotyped using a novel automated imaging-based method for quantitative assessment of morphological changes.

The phenotyping method employed lateral images analyzed via FishInspector, a software leveraging deep-learning models to detect and annotate morphological features. Sensitivity ratios for phenotypic endpoints were determined by dividing EC50 values by the experimental LC50 value of each extract. LC50 values were only calculated for the three strains (UAM/DH-PnRr, UAM/DH-KmRr, and CS-505), that led to mortality of zebrafish, which were determined to be 20.3 mg/L, 42.2 mg/L, and 63.2 mg/L, respectively.

Prominent morphological effects included non-inflation of the swim bladder, body length reduction, head malformations, notochord curvature, and impaired yolk sac absorption. These findings provide insights into the sublethal impacts of cyanobacterial metabolites on early developmental stages of aquatic organisms.

This study highlights the potential environmental and human health risks posed by cyanobacterial metabolites, particularly those with unknown toxic effects. The use of zebrafish embryos as a model system, combined with advanced imaging techniques, offers a robust framework for assessing the toxicity of environmental contaminants and contributes to a better understanding of their ecological impacts.

Keywords: zebrafish, *Raphidiopsis raciborskii*, teratogenicity, FET test, cyanotoxins

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Exploring the Bioactive Metabolites of *Raphidiopsis raciborskii* Strains: Beyond Cylindrospermopsin

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Raphidiopsis (*Cylindrospermopsis*) *raciborskii* is a bloom-forming cyanobacterium originally described in tropical regions but now increasingly found in temperate areas, posing ecological and human health hazards. Strains from different regions vary in their ability to produce toxic secondary metabolites. Strains from Australia, New Zealand, and Asia produce cylindrospermopsin (CYN), while those from South America and Southeast Asia can produce saxitoxins. However, European and Mediterranean strains seem to lack the production of well-recognized cyanotoxins.

In this study, we compared the metabolomic profiles and toxicity towards mammalian cells of five distinct *R. raciborskii* strains: Australian CYN-producing strains CS-505 and CS-506, and non-CYN-producing strains KLL07 (Lake Kinneret, Israel) and two Polish isolates from lakes Pniewskie (UAM/DH-PnRr) and Kierskie Małe (UAM/DH-KmRr).

Regardless of CYN production, aqueous methanolic extracts (75%) from the biomass of Australian and Polish strains exhibited comparable *in vitro* cytotoxic activity in rat liver cells WB-F344, while KLL07 was relatively less potent. Additionally, non-cytotoxic concentrations of the extracts inhibited gap junctional intercellular communication (GJIC), a process crucial for maintaining homeostasis in mammalian tissues. Disruption of GJIC via nongenotoxic mechanisms has been linked to tumor promotion.

The effects on GJIC varied among the extracts. Kierskie Małe, Pniewskie, and CS-506 induced rapid inhibition of GJIC within 30 minutes. Kierskie Małe's effects on GJIC were transient and not observed after 24 hours, while its cytotoxicity progressed. In contrast, the GJIC-inhibiting effects of the other extracts increased with exposure time, with CS-505, CS-506, and Pniewskie being 3–10 times more potent than the KLL07 extract.

Moreover, LC-HRMS analysis revealed distinct profiles of secondary metabolites among the five strains. This study suggests that even non-CYN-producing *R. raciborskii* strains can produce highly bioactive metabolites with cytotoxic effects comparable to CYN-producing strains, and also metabolites capable of affecting intercellular communication and disrupting homeostasis in mammalian tissues.

Keywords: *Raphidiopsis*; gap junctional intercellular communication; cytotoxicity; metabolome; nongenotoxic carcinogens

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Anatoxin-a survey in environmental samples. Electrophysiological profile of anatoxin-a and dihydroanatoxin-a on human $\alpha 7$ nicotinic acetylcholine receptor

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Worldwide, cyanobacterial blooms in marine and continental waters are of public concern regarding the capacity of certain cyanobacterial strains to produce hepatotoxins and neurotoxins among other compounds that can affect public health, human activities and wild and stock animals. Cyanobacterial neurotoxins described to date target cholinergic synapses (anatoxin-a, homoanatoxin-a, dihydroanatoxin-a: nicotinic acetylcholine receptors; guanitoxin: acetylcholinesterase) and voltage-gated sodium channels (saxitoxin; antillatoxin; jamaicamide; kalkitoxin). With the exception of saxitoxin and microcystin most of the cyanotoxins are not internationally regulated. Further monitoring of anatoxin-a is needed to improve evidence gathering of the occurrence in environmental waters in Europe and specially in France where mortality of dogs is yearly reported due to anatoxin-a- producing benthic cyanobacteria. We have sampled the Tarn River (Tarn-et-Garonne, France), the Brielse Meer (Netherlands), and purchased two environmental edible *Nostoc* sp. (*Ilullucha*) samples from Ancash and Puno, Peru. Cyanobacterial isolates were cultured in BG11 medium. Water soluble extracts were obtained and analyzed by UPLC-MS/MS using microcystin LR, dihydroanatoxin-a, homoanatoxin-a, anatoxin-a and cylindrospermopsin certified standards. Microcystin, homoanatoxin-a and dihydroanatoxin-a were detected in minute amounts in few isolates. Cylindrospermopsin and anatoxin-a were detected in most of the isolates tested. Tarn River may be a hotspot for benthic cyanobacteria producing anatoxin-a and cylindrospermopsin. Further, anatoxin-a is an agonist of nicotinic acetylcholine receptors with IC₅₀ values in the micromolar range¹. We have evaluated the mode of action of dihydroanatoxin-a compared to anatoxin-a against the human neuronal $\alpha 7$ nicotinic acetylcholine receptor by using two-electrodes voltage clamp on *Xenopus laevis* oocytes expressing the human receptor.

Keywords: Cyanotoxins; anatoxin-a; nicotinic acetylcholine receptor; two-electrodes voltage clamp

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Temporal dynamics of cyanobacteria and cyanotoxins in the reservoirs of the Pedrógão subsystem within Alqueva, South Portugal

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Freshwater resources are being compromised by anthropogenic activities and climate change. Eutrophication, which is particularly prevalent in lentic ecosystems, promotes the proliferation of cyanobacteria, photosynthetic bacteria that can produce cyanotoxins threatening human, animal and environmental health (One Health). The Alqueva Multi-Purpose Project (EFMA), located in southern Portugal ensures water for various purposes through hydraulic infrastructures, like reservoirs. This study focuses on Pedrógão subsystem, which comprises the Pedrógão, São Pedro and Magra reservoirs, aiming to assess the temporal dynamics (2014–2024) of phytoplankton communities, especially cyanobacteria and cyanotoxins. This assessment was conducted using the company's historical monitoring data. Among the various phytoplankton groups, Cyanobacteria were dominant. However, their concentrations declined over this period. The most prominent blooms were dominated by species such as *Aphanizomenon flos-aquae*, *Raphidiopsis raciborskii*, *Dolichospermum smithii* and *Pseudanabaena limnetica*. Cyanobacterial biovolumes in Pedrógão and São Pedro were consistently elevated during the warmer months, with average values of 2.66 mm³/L and 3.57 mm³/L, respectively, and peak levels reaching 22.42 mm³/L and 28.84 mm³/L. These values are within the WHO moderate risk category (< 4 mm³/L), but occasionally exceed the high- risk threshold (> 4 mm³/L), presenting potential risks for water use. In the case of the Magra reservoir, which supplies water for human consumption, the average biovolume was 2.59 mm³/L, with a peak value of 21.37 mm³/L, exceeding the high-risk threshold. Additionally, the monitored cyanotoxin levels revealed peak microcystin concentrations of 1.72 µg/L in Pedrógão and 1.62 µg/L in São Pedro. These concentrations did not directly coincide with the highest biomass peaks, suggesting that environmental factors, such as temperature and nutrient availability, can influence cyanotoxin production. These findings highlight the significance of integrated water resource management and the need for further research into the factors contributing to eutrophication and subsequent toxin production, particularly considering the impacts of climate change.

Keywords: Cyanobacteria; cyanotoxins; climate change, Alqueva; One health.

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EYDAP ensures drinking water quality.

EYDAP supplies Athens with high quality water, among the best in Europe. The main water sources and the reservoirs used are located in pure areas, free from agricultural and industrial activity, so that Athens is supplied with high quality water, while the water is transported naturally by gravity, thus with low energy consumption. With a sense of responsibility towards consumers, and with continuous investment in technology, EYDAP ensures that drinking water is one of the best in Europe.

The European Benchmarking Co-operation, during its international evaluation of water supply companies, rated EYDAP's water with 99.6% for its quality, a higher value than the average rating among water supply companies in Western Europe. EYDAP carries out daily quality checks on both raw and drinking water, having established an appropriate framework of control to ensure that the water supplied to the consuming public is safe.

In order to monitor water quality, about 1.000 representative points have been selected throughout EYDAP's water supply system:

- in the reservoirs from which it derives,
- in the four Water Treatment Plants, and
- at representative points in the distribution network

Every day, 365 days a year, samples are taken and analyses are carried out, according to a defined complex program, in the Company's ISO 17025 accredited chemical and microbiological laboratories, where the most modern analytical methods are applied with state-of-the-art laboratory equipment.

In fact, the samples checked, particularly on drinking water, exceed the required by the legal requirement. Indicatively, the number of drinking water samples tested for microbiological parameters exceeds 9,000 per year, compared to the 3,300 required by the legislation.

Monitoring is also carried out in real time, with the online monitoring of critical quality parameters in both raw and drinking water, with the help of sophisticated systems that send real-time telemetry results and, in case of extreme values, timely alerts.

In the water supply network, there are about 1000 drinking water monitoring points (700 for chlorine measurements and 300 for combined chlorine and other parameters) always according to the requirements of the legislation.

Most recently, 150 new drinking water quality control points were added to enrich the sampling points and to cover the new networks received by EYDAP.

Safety is one of the main pillars of the Company's strategy and is not limited only to the analysis of qualitative factors, but is active in a wide range of activities such as:

- investment in infrastructure, technology and staff training
- protecting reservoirs and aqueducts by complying with strict legislation
- prevention and readiness to deal with risks
- the specification of strict sanitary requirements for procedures and materials for the abstraction, treatment and distribution of drinking
- the systematic monitoring of international trends

In addition, the Company participates in research projects that offer multiple benefits for better water management and the application of innovative technologies and techniques for monitoring water quality and biosecurity, and systematically keeps up with international trends.

At EYDAP we operate with responsibility towards society, with the aim of ensuring the safety of both people and the environment. Our ultimate goal, through continuous investment in infrastructure projects and innovative applications, is to ensure the quality of drinking water and to continue to produce superior quality water, accessible to all, using science and technology.

