NaToxAq

CONFERENCE
ABSTRACT BOOK
AND PROCEEDINGS

Natural Toxins

Environmental Fate and Safe Water Supply

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MUNI | RECETOX

UNIVERSITY OF COPENHAGEN

“Progress is impossible without change, and those who cannot change their minds cannot change anything.”

George Bernard Shaw

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Proceedings of the online conference
Abstracts of presented contributions

Natural Toxins: Environmental Fate and Safe Water Supply

24th–25th September 2020

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Hans Christian Bruun Hansen (Head of NaToxAq, University of Copenhagen)
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NaToxAq MUNI | RECETOX UNIVERSITY OF COPENHAGEN

01

Introduction

Introduction

Nature is a big book to look into to understand pollutant fate and properties. Since the mid-20th century environmental chemists, biologists, toxicologist and managers have strongly improved in analysis, monitoring and understanding the distribution, fate and ecosystem and human health effects of a wide diversity of man-made pollutants ranging from polyhalogenated substances, heavy metals and PAHs to pesticides, pharmaceuticals and food ingredients.

We are far from done and new pollutant classes emerge such as nanoparticles, plastics, and perfluorinated compounds. Broad screening programs tell us that we should pay more attention to polar, persistent and toxic compounds. Despite intensive environmental research actions over the last 80 years we are not yet in position to predict the toxicological profile of a given water sample based on measurement of known pollutants in the water. The topic for our final NaToxAq conference is yet another lesson. This is about how Nature itself produces harmful substances. During millions of years of evolution, plants, microorganisms and other organisms have developed sophisticated ways to cooperate, to compete and to defend themselves. A huge diversity of specialized natural compounds are being synthesized, covering chemical structures that will leave the organic chemist jealous. These bioactive compounds may serve as chemical weapons for the producing organism, e.g. for a plant to avoid attack by an insect. Other metabolites may serve as signal compounds, for instance for a plant to shape the biome in the rhizosphere. Many natural compounds serve as inspiration for the agrochemical and pharmaceutical industry to develop new pesticides and medicine. We do not know exactly how many of these bioactive natural compounds we have got, but numbers probably exceed 100,000. A larger fraction of the compounds are toxic to humans - and we will here term them as natural toxins. The environmental fate and toxicology of natural toxins is sparsely studied except for a few dozens of compounds. What is the load of natural toxins to the environment, what are their chemical-physical properties, how do they transfer from the organism to soils and water, how do they degrade and at which speed, do they leach to drinking water reservoirs and how do we get exposed? These and many other questions are those we will address at the conference. We are grateful for the many interesting contributions submitted to the conference to help grow this emerging field of natural toxin chemistry and toxicology. We were very much looking forward to host you at the originally planned conference in Brno, Czech Republic, in June 2020. Unfortunately, the COVID-19 pandemic has made the conference a special challenge. Great efforts are taken to transfer the conference into a successful online event. Special thanks go first of all to the RECETOX organizing team in Brno, the NaToxAq management, and the organizing committee. Your continuous enthusiasm and creativity has been a fantastic platform for developing the conference. Lean back, browse the nice book of abstracts and prepare yourself for the conference, the 24th and 25th of September.

On behalf of the organizing committee and the NaToxAq consortium,

Hans Christian Bruun Hansen
Head of NaToxAq

Overview of the NaToxAq project

Clean drinking water is crucial to human health and wellbeing. The ambition of the NaToxAq ETN network is to expand the research basis for EU's leading role in securing high quality drinking waters for its citizens. Focus is on natural toxins – a large group of emerging contaminants with unknown impact on drinking water resources. Both known toxins, like cyanotoxins, cyanogenic glucosides and terpenes and not yet explored toxins will be investigated.

Twenty leading universities, research institutions, and water enterprises will pioneer the field through joint training of 15 ESRs investigating natural toxin emission via water reservoirs to water works and consumers. The natural toxin challenge is addressed by the concerted work of the ESRs within 4 scientific work packages comprising origin, distribution, fate and remediation.

Priority toxins are selected using in silico approaches accompanied by novel non-targeted and targeted analyses to map natural toxins along vegetation and climatic gradients in Europe. Invasion of alien species, toxin emission, leaching and dissipation will be under strong influence of climate change. Data collected for toxin emission, properties and fate will be used to model effects of climate, land use, and design of remediation actions. Special attention will be paid to toxin removal at water works including development of new technologies tailored to remove natural toxins. The results will contribute to strengthening of European policies and regulation of drinking water, while new business opportunities within the fields of water supply and treatment, chemical monitoring and sensing, and the consulting sector will arise from academia-industry collaborations. The urgency of the challenge, its eminent knowledge gaps, its multifaceted and multidisciplinary nature, and the need for scientific and public awareness to be communicated by ESRs in a balanced way makes the topic ideal for a European mobility and training network.

Hans Christian Bruun Hansen
Head of NaToxAq

Words from ESRs

Since 2017 we are all on our common, yet individual journey within NaToxAq which is largely coming to an end with this conference. For all of us NaToxAq was opening a new chapter, since we were required to relocate to new places and plunged head-first into the ambitious visions of our supervisors, who came up with the innovative idea to investigate natural toxins in waters on a large scale and all different levels: new sources, new toxins, how do they travel in the environment?

How much raw water can I safely drink? How about treated water? For us students, however, the first questions often were: what is bracken? A fern and toxic?! What does "toxic" mean? I should solve all this in just three years?!

The ducklings plunged into the water (Fig. 1) started to swim – it went surprisingly well! We stumbled, were stuck, were desperate and had to "prioritize" – yet here we are: a group of 16 – mature, proud ducks. Over the past years, we met on many occasions and we were very much looking forward to have this final get-together of our "NaToxAq family" (Fig. 2) in Brno this summer, right before parting our ways and waddle into new adventures – be it in science or other fields. The past years were certainly also filled with lots of fun excursions, laughter, new friends, numerous valuable experiences, and a lot of exciting science! We think we can speak for all of us in thanking the whole NaToxAq family for this special time, but also our close friends and families for supporting our choices and ambitions. Further, we want to thank you, the participants of this conference, for allowing us to share the outcomes of the past years with you and learn more about your natural toxin research in exchange. It is a privilege to be able to do so, even in these rather difficult times and the still rather uncommon online format. We hope you will enjoy the meeting and gain new insights into the fascinating field of natural toxin research!

Barbara Kubíčková and Carina Schönsee

ESR representatives

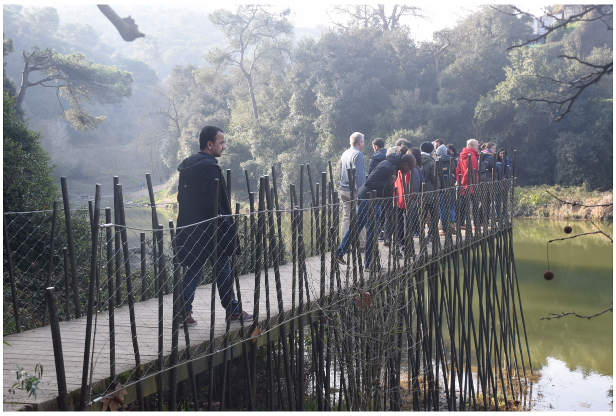

FIGURE 1

Ducklings ready for the jump.

Photo by K. Kirkensgaard, 2019.

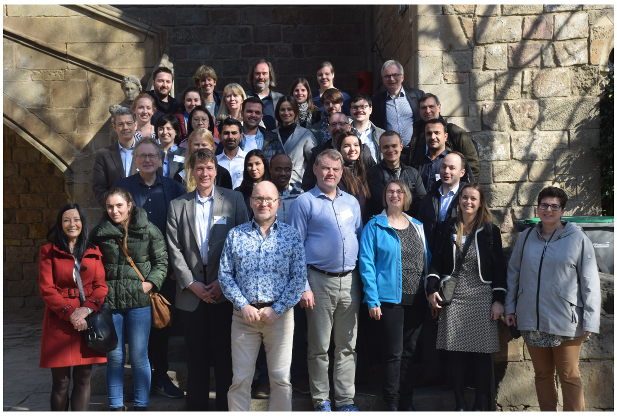

FIGURE 2

The NaToxAq consortium.

Photo by K. Kirkensgaard, 2019.

01

Program

Thursday
24th September 2020

Online venue

SESSION

Emerging compounds and sources of natural toxins

9:00–9:20	OPENING SESSION
9:20–10:00	KEYNOTE SPEAKER **Ingrid Chorus** (UBA) Cyanobacterial contamination of surface waters
10:00–10:20	**Barbara Günthardt** (Agroscope) Suspect screening for phytotoxins – new insight into the occurrence of natural toxins in surface waters
10:20–10:40	**Ellie Stone** (Fera Science Ltd) Expansion of the Toxic Plants-Phytotoxins Database for the European Union
10:40–11:00	**Mulatu Nanusha** (Helmholtz- Centre for Environmental Research) Occurrence of (toxic) Secondary Plant Metabolite Fingerprints in River Waters
11:00–11:20	COFFEE BREAK
11:20–11:40	**Xiaomeng Liang** (University of Copenhagen) LC-MS Screening of Natural Toxins Leaching from Lupinus angustifolius L.
11:40–12:00	**Vaidotas Kisielius** (University College Copenhagen) Invasive plant contaminates stream and seepage water saturating groundwater wells with hepatotoxic pyrrolizidine alkaloids
12:00–12:40	LUNCH
12:40–13:00	**Bettina Gro Soerensen** (Helmholtz–Centre for Environmental Research) Toxicity Assessment of Identified plant secondary metabolites in water environment
13:00–13:20	**Luděk Sehnal** (RECETOX) Bioprospecting of Antarctic microbial communities dominated by cyanobacteria
13:20–14:20	**Poster Session** Toxicity to aquatic/terrestrial organisms and humans + Emerging compounds and sources of natural toxins
14:00–14:20	COFFEE BREAK
14:20–14:40	**Iwona Jasser** (University of Warsaw) Limited toxin production by canobacteria from microbial mats in cold deserts
14:40–15:00	**Raegyn B. Taylor** (Baylor University) Identifying toxins associated with Prymnesium parvum elicited acute toxicity to fish
15:00–15:20	**Dana W. Kolpin** (USGS) Are Food Processing Plants an Environmental Source of Phytoestrogens and Mycotoxins?
15:20–16:00	KEYNOTE SPEAKER **Alan T. Stone** (Johns Hopkins University) Aqueous chemistry of natural compounds
16:00–16:20	EVALUATION
16:20–16:30	OPEN DISCUSSION

SESSION

Analysis of natural toxins, sampling, and monitoring to determine exposure

9:00–9:20	OPENING DISCUSSION
9:20–10:00	KEYNOTE SPEAKER **Ingrid Chorus** (UBA) Cyanobacterial contamination of surface waters
10:00–10:20	**Elisabeth Janssen** (EAWAG) Improved quantification of cyanopeptides in field samples by online SPE-LC-HRMS/MS and suspect screening
10:20–10:40	**Daria Filatova** (CSIC) SPE-UHPLC-HRMS method for the determination of multi-class cyanotoxins in freshwater
10:40–11:00	**Massimo Picardo** (CSIC) Suspect screening of natural toxins in surface water; A case study for Ter River (Catalonia, Spain).
11:00–11:20	COFFEE BREAK

SESSION

Toxicity to aquatic/terrestrial organisms and humans

11:20–11:40	**Barbara Kubíčková** (RECETOX) Cyanobacterial retinoids affect the differentiation pattern of human neural stem cells in vitro
11:40–12:00	**Lenka Šindlerová** (IBP CAS) Lipopolysaccharides from cyanobacteria and cyanobacterial water blooms activate various human cell types
12:00–12:40	LUNCH
12:40–13:00	**Ondřej Vašíček** (IBP CAS) Newly recognized cyanobacterial toxins induce disruptive and pro-inflammatory prosesses in Caco-2 human intestinal barrier model
13:00–13:20	**Marek Pípal** (RECETOX) In vitro and in vivo bioactivity of retinoid compounds detected in the environment in waters with developed cyanobacterial bloom
13:20–14:20	**Poster Session**: Toxicity to aquatic/terrestrial organisms and humans + Emerging compounds and sources of natural toxins
14:00–14:20	COFFEE BREAK
14:20–14:40	**Lea Lovin** (Baylor University) Behavioral effects of anatoxin-a with parallel gene and protein expression responses in larval Pimephales promelas and Danio rerio
14:40–15:00	**Emily Eagles** (University of Plymouth) Assessing the toxicity of two mycotoxins, deoxynivalenol and zearalenone, to freshwater organisms.
15:00–15:20	**Bryan Brooks** (Baylor University) Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat? Perspectives from Fifteen Years with Prymnesium parvum
15:20–16:00	KEYNOTE SPEAKER **Alan T. Stone** (Johns Hopkins University) Aqueous chemistry of natural compounds
16:00–16:20	EVALUATION
16:20–16:30	OPEN DISCUSSION

Friday
25th September 2020

Online venue

SESSION

Analys is of natural toxins, sampling, and monitoring to determine exposure

9:00–9:20	OPENING SESSION
9:20–10:00	KEYNOTE SPEAKER **John Fawell** (Cranfield University) Importance of sound science underpinning regulation
10:00–10:20	**Gloria N. D. Addico** (Council for Scientific and Industrial Research) A Review of Cyanobacteria and Cyanotoxins in Drinking Water Reservoirs in Ghana
10:20–10:40	**Petra Labohá** (RECETOX) Waterborne cyanobacteria in the air: exposure and effects
10:40–11:00	COFFEE BREAK
11:00–11:20	**Theodoti Papadimitriou** (University of Thessaly) Hepatotoxins in groundwater wells and their accumulation in edible plants through irrigation in an intensive agricultural region of Greece
11:20–11:40	**Eleni Keliri** (Cyprus University of Technology) Monitoring and treatment of St. George lake to mitigate Cyanobacterial Harmful Algal Blooms (cyano-HABs)
11:40–12:40	**Poster Session** Analysis of natural toxins, sampling, and monitoring to determine exposure + Physicochemical properties, environmental distribution, and fate + Risk assessment, removal, and management of natural toxins in (drinking) water
12:20–13:00	LUNCH

SESSION

Physicochemical properties, environmental distribution, and fate

13:00–13:20	**Inés Rodríguez Leal** (Stockholm University) Evaluating in silico tools for estimating physico chemical properties of natural toxins relevant to water quality
13:20–13:40	**Bilal Tariq** (Fera Science Ltd) Simulation of Phytochemicals in water with the PEARL model
13:40–14:00	**Daniel Garcia Jorgensen** (University of Copenhagen) A naturally occurring carcinogen in Pteridium aquilinum: I) modelling the fate of ptaquiloside in soils.
14:00–14:20	COFFEE BREAK
14:20–14:40	**Carina Daria Schoensee** (Agroscope) Varietas Placuerit - Variability in Natural Toxin Environmental Mobility
14:40–15:00	**Jawameer Hama** (University of Copenhagen) Fate of Quinolizidine Alkaloids from blue lupin (Lupinus angustifolius L.)–an Agricultural Field Test
15:00–15:20	**Regiane Natumi** (EAWAG) Photochemical transformation of emerging cyanobacterial peptides in surface waters
15:20–16:00	WRAP-UP + PRESENTATION/POSTER AWARDS
16:00–16:20	FRIDAY CHEERS

SESSION

Risk assessment, removal, and management of natural toxins in (drinking) water

9:00–9:20	OPENING SESSION
9:20–10:00	KEYNOTE SPEAKER **John Fawell** (Cranfield University) Importance of sound science underpinning regulation
10:00–10:20	**Tomoaki Itayama** (Nagasaki University) Microcystin removal from lake water for potable water using Bio-fence around Nyanza Gulf of Lake Victoria
10:20–10:40	**Marcel Schneider** (RECETOX) Degradation and detoxification of cylindrospermopsin by hydroxyl and sulfate radical-based advanced oxidation processes
10:40–11:00	COFFEE BREAK
11:00–11:20	**Natasa Skrbic** (University of Copenhagen) Removal of natural toxins in biological sand filters for drinking water treatment
11:20–11:40	**Elzbieta Wilk-Wozniak** (Polish Academy of Sciences) Tools to manage cyanobacteria agglomerations in freshwater ecosystems
11:40–12:40	**Poster Session** Analysis of natural toxins, sampling, and monitoring to determine exposure + Physicochemical properties, environmental distribution, and fate + Risk assessment, removal, and management of natural toxins in (drinking) water
12:20–13:00	LUNCH
13:00–13:20	**Myrto-Foteini Touloupi** (National Center for Scientific Research Demokritos") Degradation of cyanotoxin cylindrospermopsin in water using ultrasonication: Role of Reactive Radical Species
13:20–13:40	**Erika Berenice Martinez Ruiz** (Technische Universität Berlin) Biotransformation of cylindrospermopsin by manganese-oxidizing bacteria
13:40–14:00	COFFEE BREAK
15:20–16:00	WRAP-UP + PRESENTATION/POSTER AWARDS
16:00–16:20	FRIDAY CHEERS

03

Poster session program

Thursday
24th September 2020

13:20–13:40	**Toxicity to aquatic/terrestrial organisms and humans** 01
	Strobel B.W. Toxicity of Plant Alkaloids in Daphnia magna
	Davidović P. Effects of the cyanobacterial strain Nostoc zobnatica on gene expression in Daphnia magna
	Virmani I. DESTROY - lipiD droplEts aS a TaRget fOr cYanotoxins Application of systematic review framework to validate this association
	Emerging compounds and sources of natural toxins 01
	Kotačka T. Cyanobacterial water blooms dominated by species from different orders produce teratogenic retinoid-like compounds into surface waters
	Martinez Yerena J.A. First insight into the aetokthonotoxin's biosynthesis
	Zervou S.-K. Characterization of new anabaenopeptins from cyanobacteria of Greek lakes
	Peteva Z. Detection of marine biotoxins in Bulgarian costal waters in 2018-2019
	Jasser I. Toxin production by cyanobacteria in altitude gradient in small water bodies of Pamir mountain desert
13:40–14:00	**Toxicity to aquatic/terrestrial organisms and humans** 02
	Skočková V. Cyanobacterial LPS induces inflammatory response in epithelial cells in vitro
	Adamovský O. Elucidating mechanisms of cyanotoxin induced immunotoxicity
	Adamovský O. Does host microbiome play role in adverse outcomes of cyanotoxins?
	Kubickova B. Cyanobacterial toxins and the intestines - What are we missing?

	Toxicity to aquatic/terrestrial organisms and humans 03
13:40–14:00	**Spasic S.** Cyanobacterial neurotoxin 2,4-diaminobutyric acid produces three-fold higher depolarization of leech Retzius neurons than other excitatory amino acids implicated in neurodegeneration
	Martinková Š. Effect of cyanobacterial retinoids on nuclear receptor activation and neural differentiation in vitro
	Roy Chowdhury R. Dynamic bioreactor 3D hepatospheroid cultures to study cyanotoxin-induced chronic hepatotoxicity and liver disease
	Felipe Grossi M. Human 3D hepatic spheroids for in vitro assessment of hepatotoxic and steatogenic cell events in response to cylindrospermopsin and microcystin-LR.
	Toxicity to aquatic/terrestrial organisms and humans 04
14:00–14:20	**Sychrova E.** The cyanobacterial community compositions and bacteria-bacteria interactions related to their potential impact on human health
	Brózman O. Effects of the cyanotoxin microcystin-LR in human bronchial epithelial cells in vitro
	Hildebrandt J.-P. Cylindrospermopsin exposure causes attenuation of cell proliferation and elongates the metaphase in mitotic human airway epithelial cells
	Kubickova B. Effects of the cyanobacterial toxin cylindrospermosin on human airway epithelial cells in vitro

Friday
25th September 2020

Analysis of natural toxins, sampling, and monitoring to determine exposure

01

Janssen E.
Open access knowledge of cyanobacterial metabolites for suspect screening by LC-HRMS/MS

Maršálková E.
Early warning system for on-line monitoring of water resources

Van Hassel W.H.R.
The influence of pH in water samples on the recovery of microcystin congeners by MS/MS

Truc Ly L. H.
Influence of environmental factors on Microcystins degradation bacteria and toxigenic cyanobacteria bloom a Bayesian approach

11:40–12:00

Risk assessment, removal, and management of natural toxins in (drinking) water

01

Li J.
Stressing cyanobacterial risk in drinking water supply by using eutrophic freshwater as source

Simonazzi M.
Degradation of cyanotoxins produced by the cyanobacterium Microcystis aeruginosa using chlorine-based compounds implication for drinking water management

Schneider M.
Degradation of a cyanobacterial toxin by non-thermal plasmas

Skrbic N.
Monitoring of the natural toxin ptaquiloside in groundwater

12:00–12:20

Analysis of natural toxins, sampling, and monitoring to determine exposure

02

Garcia Jorgensen D.
A naturally occurring carcinogen in Pteridium aquilinum: II) A field monitoring study

Picardo M.
Occurrence of natural toxins. An LC-HRMS/MS approach for their tentative identification in surface water.

Keliri E.
Automated in-situ cyanotoxin assessment toolbox for real-time surface water monitoring

Wilk-Wozniak E.
Cyanobacterial diversity, biomass and cyanotoxins across the latitude in European freshwaters

12:00–12:20	**Physicochemical properties, environmental distribution, and fate** 01
	Schoensee C.D. Fighting over Alkaloids – Clay versus Organic Carbon
	Drejer M. Occurrence and stability of the Illudane glycosides ptesculentoside, caudatoside and ptaquiloside from Bracken ferns (genus Pteridium) in surface waters
	Rasmussen L. H. Stability of the fern carcinogen ptaquiloside in water resources
12:20–12:40	**Physicochemical properties, environmental distribution, and fate** 02
	Hama J. Leaching of Pyrrolizidine Alkaloids from Ragwort (Senecio jacobaea L.) Plant to Corresponding Soil and Water
	Hama J. Are Natural Alkaloids Transported from Soybeans to Streams?
	Rodríguez Leal I. / Schoensee C.D. Modeling K_{OC} of natural toxins
	Bošković N. Commercial coated stir bar usage for microcystin bioavailability and bioaccessibility assays

04

Keynote Abstracts

K01

Cyanobacterial contamination of surface waters

Speaker

Ingrid Chorus
German Environment Agency (UBA)

About keynote speaker

After her Ph.D in the context of ecological interactions in a lake ecosystem Ingrid Chorus worked as research scientist in two fields: lake restoration and toxic cyanobacteria, initiating and leading a number of research consortia to address cyanotoxin occurrence, toxicity, control measures and the elucidation of further bioactive metabolites. From 2007 until 2018 she led the Department for Drinking Water and Swimming Pool Hygiene of the German Federal Environment Agency. In this function she was actively involved in the development of regulations, including the EU Drinking Water Directive and the German Drinking Water Ordinance with the aim of introducing a comprehensive approach to planning, managing and monitoring water use systems. As co-editor of three WHO Guidebooks ("Protecting Groundwater for Health", "Protecting Surface Waters for Health" and "Toxic Cyanobacteria in Water") she continues to work for the World Health Organisation in the area of management and control of water resources.

Cyanobacterial toxins are probably the toxins that occur most widely surface waterbodies that humans use: they seem to occur in concentrations potentially relevant to human health more often than other natural and anthropogenic toxins. Does that make them very dangerous" - as expressed in the introduction of many scientific publications? How can we assess human health risks from cyanotoxins, and what is our current state of knowledge to assess this? The presentation aims to trigger discussion on these issues. This includes an overview of the current status of WHO guideline development as well as the research gaps that remain important for more comprehensive and balanced risk assessment. The presentation further addresses patterns of cyanotoxin occurrence and their key drivers, and it briefly introduces the levels at which we can control occurrence and thus human exposure: from catchment and waterbody management over optimising site selection for drinking-water offtake and recreation to the removal through water treatment. Approaches to situation and risk assessment need to be comprehensive, but specific to the respective waterbody, and the concept of developing site-specific Water Safety Plans offers a good platform to bring together the expertise necessary for risk assessment and effective control.

K02

Aqueous chemistry of natural compounds

Speaker

Stone Alan T.
Johns Hopkins University, USA

About keynote speaker

Alan Stone completed his bachelor's degree in Chemistry from the University of Maryland and his master's and Ph.D. degrees in Environmental Engineering Science at Caltech. Since then, he has been a member of the faculty of the Whiting School of Engineering, Johns Hopkins University. His research group uses comparison experiments, employing suites of organic compounds where small, systematic changes to functional groups and structure have been made, to explore pathways and rates of metal-organic and mineral/water interface reactions. Subjects addressed include nutrient acquisition by plants and soil microbes, reactive sinks for natural products, the chemistry of biofluids, and cell growth media design.

Every report of a new natural product comes with a description of its atoms, functional groups, and molecular arrangements. To place each natural product in its aqueous context, properties need to be evaluated, e.g. Kow, pKas, logKs for metal ion coordination, ability to adsorb at mineral/water interfaces, and tendency to partition into natural organic matter. As far as chemical reactivity is concerned, all aqueous media support hydrolysis, but pathways and rates depend upon pH and identities and concentrations of potential catalysts. Evaluating oxidations, reductions, and nucleophile-electrophile reactions requires careful inventory of all possible reactants and catalysts within the medium of interest. In surface waters, soils, and sediments, key reactants are often not ones that a synthetic organic chemist would select, e.g. manganese(III,IV) oxyhydroxides for oxidation, Fe(II) for reduction, polysulfides as nucleophiles. Not surprisingly, complex suites of intermediates and products can arise. Within organisms and in microenvironments under close biological control (e.g. rhizospheres, biofilms) peptides, proteins, and biomolecules are relevant reactants too. Ambient oxidants, reductants, nucleophiles, and electrophiles that survive shipment back to the lab for analysis are not necessarily the most reactive ones present in aqueous media. If a reactant is potent enough, it can be important even if it is ephemeral. Sink reactions don't matter as long as there are sources. Ascorbic acid and uric acid in blood, respiratory tract fluids, and tears are beneficial antioxidants. When oxidized, however, electrophilic reactivities of dehydroascorbate and alloxan oxidation products are so high that hydration (nucleophilic attack by water) and adduct formation with proteins, nucleotides, and other nearby biochemicals occurs almost immediately. Natural organic matter within soils has the ability to covalently sequester nucleophiles, but what are the electrophilic groups involved? Benzoquinones generated through the oxidation of dihydroxybenzenes within lignin source materials, and alpha-ketocarboxylates generated through the oxidation of alpha-hydroxycarboxylates (e.g. malic acid, cutins, suberins) are among the possibilities. Of course, natural products themselves can be pro-toxins, chemically activated within environmental media or within organisms by cytochromes or other enzymes. Natural products and the biogeochemical media within which they reside are subject to evolutionary forces. Changes to neighboring functional groups and molecular structures can lead to exquisite molecular fine-tuning, evident in such molecules as folate, curcumin, and aflatoxin B-1. As a consequence, appraising both desirable and problematic traits can be a formidable challenge.

K03

The importance of sound science underpinning regulation

Speaker

John Fawell
WSI, Cranfield University, UK

About keynote speaker

John Fawell has worked in the water field for 40 years, first at Water Research Centre and as an independent consultant. He has been involved in research and risk assessment of a wide range of drinking water contaminants, including cyanobacterial toxins. He has been a member of the team working on the WHO Guidelines for drinking water quality since 1986. He is a member of the WHO expert committee on the Guidelines. He works closely with WHO regional offices, particularly European Office through which he was one of the main authors of the advisory document on the revision of the drinking water directive. He is a visiting professor in Cranfield University Institute for Water Science.

Regulations for both anthropogenic and natural toxins require a balance between health protection and the achievability of any regulatory value. It is therefore vital that the science surrounding the determination of what might be a safe level of exposure, data on occurrence and data on means of mitigation are robust and can withstand scrutiny. In particular, it is important to understand and communicate the uncertainty around any values that are calculated. This is needed for risk managers and politicians to recognise the possible consequences of their decisions and understand what room for manoeuvre is available to them. One important point to recognise is that a relatively small difference in a standard may not be significant in terms of toxicity but may make a huge difference in achievability. In addition we know that repeating a study may well lead to different results so we try to achieve a balance of evidence in developing regulations. There is also a need for some stability in regulations that necessitate investment to achieve what is required because changes can be very difficult to implement. It is, therefore, incumbent on scientists to be aware of the requirements for good regulations and make sure that the science that we want to underpin those regulations is the best that we can achieve.

05

Platform Abstracts

S01

Suspect screening for phytotoxins – new insight into the occurrence of natural toxins in surface waters

authors

Günthardt Barbara F. [1, 2, 3]
Hollender Juliane [2, 3]
Singer Heinz [2]
Scheringer Martin [3, 4]
Bucheli Thomas D. [1]

Phytotoxins are natural toxins produced by plants with widely varying structures and toxic effects. In contrast to anthropogenic contaminants, phytotoxins have only received little attention as micropollutants, although, they can occur in high concentrations in vegetation. The aim of this study was to perform a suspect screening investigating the phytotoxin contamination in Swiss surface waters. A previously performed assessment identified the potentially most relevant phytotoxins based on plant occurrence, persistence, mobility, and toxicity resulting in 520 suspects. Additionally, for the pyrrolizidine alkaloids (PAs), a critical phytotoxin class, a retrospective quantitative data analysis was carried out using a comprehensive time-continuous data set. Water samples were taken monthly (May – November 2019) over three days at seven sites in the Swiss Plateau with different land uses. The water samples were enriched with solid phase extraction (SPE) and analyzed using liquid chromatography coupled with electrospray ionization to high-resolution mass spectrometry (LC-HRMS). The suspect screening was evaluated using 80 targets available with reference standards. For the retrospective data analysis, an HRMS data set was used from a time-continuous monitoring (March-August) of five more sites in 2015. The target evaluation showed that most phytotoxins were quantifiable in the low ng/L range, but some classes were less well ionizable, e.g. steroids or glycosylated compounds. In the screening workflow the distribution of the plants and the co-occurrence of different toxins were valuable additional filtering steps. The screening results confirmed the occurrence of over 15 phytotoxins of different classes. Isoflavones were detected at several sites in proximity to grasslands, confirming the occurrence of these estrogenic phytotoxins in surface waters reported in the literature. Furthermore, we found for the first time gramine, an indole alkaloid, and seven PAs with total concentrations of over 100 ng/L. At some sites, that received runoff from highways PA occurrence could be rationalized by the worrying presence of the PA-producing, invasive Senecio inaequidens. Therefore, we performed a retrospective data analysis of the PAs and confirmed their occurrence at all five sites. The highest concentrations were observed after rain events, indicating a wash out effect. The varying toxin patterns suggest that the toxins originated from different PA-producing plant species.

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S02

Improved quantification of cyanopeptides in field samples by online SPE-LC-HRMS/MS and suspect screening

authors

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Cyanobacterial bloom events intensify regarding their duration and geographical occurrence and with that the risk of exposure to toxic metabolites. Microcystins are known liver toxins and have been studied intensively and the wealth of evidence regarding exposure concentrations and toxicity led to the inclusion of one variant (MC-LR) in risk management frameworks for water quality since the 1990s.
Over the past decades, a legion of cyanobacterial metabolites has been identified. However, we lack essential information about concentrations in surface waters and environmental behavior of most compounds. The question arises, whether these compounds are of human and ecological concern regarding their abundance, persistence and toxicity. The key challenge presents their large structural diversity, lack of reference standards, and thus the high analytical requirements for identification and quantification. Liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) is the state-of-the-art analytical method to detect cyanopeptides. We validated structural information of known compounds to performed suspect screening of more than 300 microcystins and more than 1000 other cyanopeptides by LC-MS. To allow reproducible and sensitive analysis of the water samples, we validated an online enrichment by solid phase extraction coupled to LC-HRMS/MS for cyanopeptides. This method was used to identify production dynamics across the season and between affected lakes in Switzerland.

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S03

Expansion of the Toxic Plants-Phytotoxins Database for the European Union

authors

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Phytotoxins are compounds produced by plants for purposes other than survival, such as defence or species interaction. These secondary metabolites vary hugely in characteristics, with many comparable to anthropogenic micropollutants. However, to date, phytotoxins are rarely considered by regulators in environmental risk assessments, including those for drinking water. The Swiss Toxic Plant - Phytotoxin (TPPT) Database has collated information on toxic plants found in Switzerland, identified the associated phytotoxins and estimated some physical and chemical properties of the phytotoxins using QSAR modelling. Using the data, an aquatic micropollutant analysis was conducted to identify which phytotoxins were persistent, mobile or toxic (PMT) in an aquatic environment. For the TPPT database to be applicable to the whole EU, a new version was developed, the Phytochemical Plant Database. It expands on Swiss toxic plants to include species grown all over the EU, inclusive of agricultural, invasive and water plants, as well as adding additional information such as habitat and medicinal use. QSAR modelling of phys-chem properties and the aquatic micropollutant analysis will be conducted on the new phytotoxins, following the TPPT method, to complete the database and identify new PMT phytotoxins. Using the database and toxin transport model, a preliminary risk assessment of phytotoxin in drinking water will be conducted. This will aim to identify the degree of risk the phytotoxins could pose to drinking water, mitigation measures to minimise the risk, as well as examining what policy measures could influence the mitigation measures.

Fera Science Ltd

S04

SPE-UHPLC-HRMS Method for the Determination of Multi-Class Cyanotoxins in Freshwater

authors

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Cyanobacteria are the components of regular microbial succession in periphyton formation, and they are spread globally in different aquatic environments. Some cyanobacteria produce toxins, which vary in structure and harmful properties. The most described and diverse cyanotoxins are microcystins (MCs). During the past decades, there has been a noticeable increase in cyanobacterial blooms, dominating in many freshwater bodies worldwide. Human hazard may arise from drinking water supply, recreational water use, and consumption of food in which toxins may have bioaccumulated (De La Cruz et al., 2013). Thus, the World Health Organization appointed a provisional guideline value for total MC-LR in drinking water of 1 µg/L (WHO, 2011). To monitor cyanotoxins' levels and prevent both human poisoning and wildlife damage, suitable analytical methods need to be developed. In this work, a sensitive, fast and robust method for the determination of 10 cyanotoxins in freshwaters was developed, validated and applied. The method is based on ultra-high performance liquid chromatography coupled to high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). For the sample pretreatment, dual solid-phase extraction using 2 cartridges has been validated and employed. The chromatographic separation was achieved using a C18 analytical column (150x2.1 mm, 2µm) with acidified acetonitrile and water as a mobile phase. The total chromatographic run was 10 min. The chromatographic separation was coupled to a Q-Exactive Orbitrap instrument (Thermo Fisher Scientific), and the MS data were acquired in both full scan and data-dependent modes. This method provides targeted determination of cyanotoxins of different chemical classes (cyclic peptides: MCs, nodularin; a bicyclic secondary amine anatoxin-a, and an alkaloid cylindospermopsin) with method limits of detection (MLODs) ranging from 2-150 pg/L, much lower than the limits established by legislation. To our knowledge, these are the lowest reported MLODs for the determination of multi-class cyanotoxins. Method recoveries were evaluated by analyzing artificial freshwater samples in triplicate spiked with 10 cyanotoxins at 3 concentration levels (2, 10, and 20 ng/L). Recoveries at the highest tested level were in the range of 66.6 – 87.3 %, except for MC – LW (47.8 %). HRMS provides an assessment of the potential presence of transformation products and other non-targeted toxins in the samples. The developed method was applied for the study and characterization of cyanotoxins concentrations in Spanish, Swiss, and British freshwater samples.

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S05

Occurrence of (toxic) Secondary Plant Metabolite Fingerprints in River Waters

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It is known that, in their life time, plants are capable of producing and synthesizing a large number and variety of secondary metabolites (SMs) and release them to the environment. The SMs are critical components for plant survival and some of them might also play a role in supporting human health. On the other hand, from the environment (e.g. surface water), many of the secondary metabolites can have short and long term effect on humans and animal health. However, it is not yet intensively investigated to what extent these metabolites are present in surface waters in order to cause these effects. Therefore, the focus of this study is to investigate the occurrence of SMs in river waters that are not yet considered as environmental contaminants. Thus, to achieve our goals, we analysed aqueous plant extracts and river water from adjacent locations using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). We could detect several thousand features occurring in both, plant extracts and waters based on MS1 data, showing the contribution of vegetation to the chemical mixtures in river waters. Henceforth, the MS/MS fragmentation record for the common features revealed several common peaks representing the same chemical structure (Current work focuses on confirmation and quantification of these peaks using reference standards.) Similarly, a target screening of river waters employing a pre-selected set of metabolites revealed the presence of about 16 toxic plant metabolites, which belong to different classes. Coumarins (bergapten, fraxidin and psoralen), flavonoids (Formononetin and alpinetin) and alkaloids (lycorine and narciclasine) are among the identified toxic plant metabolites. The metabolites are distributed among the analysed river waters in various concentration ranges. Thus, our study demonstrated a brief overview of the impact of vegetation on the quality of river waters. Therefore, owing to their adverse effect, consideration should be given for the prevention and monitoring of SMs.

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S06

Suspect screening of natural toxins in surface water; A case study for Ter River (Catalonia, Spain)

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Freshwater scarcity, to meet the current drinking water demand, is an increasing global problem. In addition, freshwater contamination and eutrophication together with global warming lead to increase episodes of harmful algal blooms (HAB) and the proliferation of organisms producing natural toxins. These toxins can spread in water bodies reducing its availability to be used as a drinking water supply [1]. During the last decades, have been proposed robust and sensitive analytical methods able to identify their occurrence in the environment [2]. In this work, a tentative identification approach based on target and suspect screening using liquid chromatography-high resolution mass spectrometry (LC-HRMS) in full scan and data-dependent acquisition mode (FS-ddMS2) was applied to assess the presence of natural toxins in surface water. For the tentative identification, different on-line databases were used including ChemSpider, MzCloud and, an in-house database including 2480 natural toxins. Also, thanks to the FS-ddMS2 data, tentative identification confidence to level 2 was achieved for most compounds [3]. However, confirmation was done using certified standards. Analytical parameters were also validated, and the quantification was possible by external calibration. This approach was applied to study natural toxins along the Ter River in Catalonia. In this preliminary study, 53 natural toxins were tentatively identified and 6 of them confirmed. A study regarding their occurrence in different seasons was also carried out. Principal Component Analysis showed correlations with the dry and wet periods with differences in the content of plant and mycotoxins in differents spots.

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S07

LC-MS Screening of Natural Toxins Leaching from Lupinus angustifolius L.

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Natural toxins synthesized from plant secondary metabolism possess a wide array of chemical, physical and biological properties. Many of them are of great interest to pharmaceutical sciences, while some may result in negative impacts on the environment when they are leached to the vegetation field and accumulate to a threshold. In order to determine the environmentally relevant natural toxins as well as their transformation products, a state-of-art analytical strategy based on LC-ESI-HRMS(MS) is explored in the work. The established strategy is applied to a five-month field study of Lupinus angustifolius L. Samples of soil and drainage water are collected at different stages of plant growth, analyzed, and compared to the profiles obtained from the plant. Results indicate some classes of secondary metabolites, e.g. quinolizidine alkaloids, can be released from the plant to soil and migrate, and act as a potential source of contamination considering their abundance and toxicity.

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S08

Cyanobacterial retinoids affect the differentiation pattern of human neural stem cells in vitro

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Cyanobacterial blooms increase in frequency and intensity due to anthropogenic eutrophication of (fresh-)water ecosystems and climate change. They can produce a large variety of bioactive secondary metabolites, including hepatotoxins (microcystins, nodularins, cylindrospermopsin), neurotoxins (anatoxin-a, saxitoxin) and retinoid substances that may affect the quality of drinking water sources. Retinoid compounds have been shown to cause teratogenic effects in fish (Danio rerio) and frog (Xenopus laevis) embryos at environmental levels. Particularly all-trans retinoic acid (atRA), the bioactive metabolite of retinol (vitamin A), plays a pivotal role during vertebrate development. Through retinoic acid and retinoid X receptor (RAR and RXR) signaling pathways, anterior-posterior patterning of developing vertebrates is established. Retinoids are also involved in early organogenesis, including the development of the nervous system. The occurrence of these teratogenic substances in the environment poses the question to which extent retinoid compounds originating from cyanobacterial blooms could represent a threat to human development and health. To study the effects of cyanobacterial retinoid substances (atRA, 9-cis retinoic acid) on the development of the human nervous system we are using human neural stem cells (NSCs) that differentiate in vitro into a mixed culture of neurons and glial cells within 22 days. Samples were collected at three timepoints during differentiation. Endpoints assessed include network morphological patterning and the expression of biomarkers for neuroepithelial cells (nestin, SOX2), committed and mature neurons (β-III tubulin, TBR1, neurofilament 200), synaptogenesis (synaptophysin) and glial cells (GFAP). Upon exposure to 40-1000 nM retinoic acid (RA), we observe a dose-dependent decrease in network formation. Interestingly, the structural impairment of the neural network is not reflected by the abundance of committed neurons, as characterized by TBR1 biomarker detection with qPCR. Additionally, retinoic acid appears to decrease the number of cells committed to the glial lineage (GFAP expression) in favor of neural maturation in early differentiation stages. Taken together, we successfully established a state-of-the-art non-animal alternative for human developmental neurotoxicity testing, including endpoints relevant towards neurodevelopmental adverse outcome pathways. We also demonstrated the applicability of this advanced in vitro model to study the effects of cyanobacterial retinoids. Future studies will expand the applicability of this model to mixtures of retinoids and cyanobacterial toxins that can interfere with the nervous system (microcystins, anatoxin-a) and, for the first time, reveal mixture effects of cyanobacterial contamination to the developing human brain.

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S09

Invasive plant contaminates stream and seepage water saturating groundwater wells with hepatotoxic pyrrolizidine alkaloids

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Pyrrolizidine alkaloids (PAs) are persistent mutagenic and carcinogenic compounds produced by many common plant species. These compounds are regulated in food and medicinal products to ensure consumer health and safety. However, there is little awareness that PAs can contaminate water resources. Therefore, no regulations exist to limit PAs in drinking water. This study measured a PA base concentration of ~70 ng/l in stream water adjacent to an invasive PA-producing plant Petasites hybridus. After intense rain the PA concentration increased 10 fold. In addition, PAs measured up to 230 ng/l in seepage water from groundwater wells. The dominant PAs in both water types corresponded to the most abundant PA types in the plants. According to acceptable levels proposed by health authorities, drinking 2 l of the well water reported in this study would violate the daily PA ingestion limit. The study presents the first discovery of persistent plant toxins in well water and their associated risks.

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S10

Lipopolysaccharides from cyanobacteria and cyanobacterial water blooms activate various human cell types

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Harmful algae blooms (HAB) dominated by cyanobacteria are an important source of lipopolysaccharides (LPS) in the aquatic environment. HAB-LPS can originate both from cyanobacteria as well as from gram-negative heterotrophic bacteria associated with HAB. Bacterial LPS is known to be a potent pro-inflammatory agent but the cyanobacterial LPS is not studied well. To study its ability to induce pro-inflammatory responses in human cells, LPS extracts from HAB biomasses dominated by different cyanobacterial species as well as from axenic cultures of the same species were prepared. First, pyrogenicity of these LPS was tested using Pyrogene assay. There was a wide range of pyrogenic activity among all samples, from dozens to millions of EU/mg LPS. Since the epithelial cells are the first target after the exposure to the toxin, its effect on intestinal and skin cells in vitro was studied. It was shown that not only environmental mixtures but also pure cyanobacterial LPS is able to activate these cells to produce pro-inflammatory cytokines. Taking into account that inflamed epithelium can become permeable for toxins enabling them to reach blood stream, effect of LPS on human peripheral blood mononuclear cells was studied. Changes in surface markers expression and cytokines production, the markers of cell activation, were observed. To clarify the mechanism of cyanobacterial LPS action, murine macrophages in vitro were used. Surprisingly, the mechanism hypothesized, i.e. activation of Toll-like receptor 4 (TLR4), was not proven. Moreover, LPS with very low pyrogenic activity in the Pyrogene assay was strongly positive in different cell models showing pro-inflammatory effects comparable with LPS with high endotoxin activity and/or positive control, Escherichia coli LPS. In conclusion, LPS from HAB and also pure cyanobacteria is able to activate different types of human cells and induce production of pro-inflammatory signalling molecules by the cells. However, the activation does not require TLR4. What is really interesting and important is that testing of LPS samples only by Pyrogene assay could significantly underestimate their biological activity.

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S11

Toxicity assessment of identified plant secondary metabolites in the water environment

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More than 40 plant secondary metabolites (PSMs) have been identified in the water environment in relation to the NaToxAq project. However, for most of these compounds only very little is known about their potential toxic effects. The aim of the present study was to fill this gap by investigating the toxicity of the detected compounds, thus enabling a risk assessment of plant natural compounds as water contaminants. As model organism, we chose Daphnia magna; a highly used organism for the assessment of acute toxicity, responding specifically to neurotoxins such as acetylcholinesterase inhibitors and thereby hypothesized to sensitively respond to PSMs produced to defend against insects. 39 PSMs were screened in three concentrations expressed as factor to predicted baseline toxicity (48H exposure, 5 daphnids per concentration). Of these, three compounds had a toxic ratio (TR) >100. The toxic ratio expresses excess toxicity and indicates specific effects. Full dose-response relations were investigated for these compounds (48H exposure, 20 daphnids per concentration, 2-3 replicates in time) and the concentrations causing 50% immobilization (EC50) established. The EC50 of one pyrrolizidine alkaloid detected in high concentrations in the environment was additionally estimated, though only having a TR<10. By comparison to the reported environmental exposure concentrations, risk quotients could be calculated and compared to anthropogenic contaminants. Based on the results, the individual compounds are not expected to cause acute effects, but chronic effects cannot be excluded. Further, they may be considered additional stressors in the complex chemical mixture found in the environment.

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S12

Newly Recognized Cyanobacterial Toxins Induce Disruptive and Pro-Inflammatory Processes in CACO-2 Human Intestinal Barrier Model

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Water bloom cyanobacteria produce wide range of different toxic secondary metabolites (cyanotoxins). Some of them have been studied deeply in last years (e.g. microcystins) but mechanisms of toxicity of some of them are still unknown. Puwainaphycins (PUW) and minutissamides (MIN) are decapeptides containing a ß-amino acid which were firstly isolated from Anabaena sp. The first possible target of human exposure to these compounds is intestinal epithelium but effect of PUW/MIN on enterocytes has not been described yet. This study was focused on effects of PUW F and 3 natural modifications of MIN (A, C, D) on differentiated Caco2 monolayer as a model of intestinal epithelial barrier. Cells were exposed to different concentrations of PUW/MIN for 24 hours and their cytotoxicity as well as ability to induce interleukin 8 (IL8) production, changes in permeability and modulation of tight junction (TJ) were studied. All studied compounds were cytotoxic with PUW F being the most effective one. PUW F, MIN A and MIN C were also able to induce IL8 production in the non-cytotoxic concentrations. On the other hand, MIN D did not show any pro-inflammatory effect. Further, based on trans-well assay with FITC dextran detection, changes in permeability of the Caco2 monolayer were observed after exposure to PUW F, MIN A and MIN C. Surprisingly, pro-inflammatory activation of the Caco2 cells by PUW F and MIN C resulted in a significant increase in TJ protein claudin 4 expression determined by western blot analysis and confirmed by confocal microscopy. Furthermore, decrease in expression of zonula occludens 3, another TJ protein, was observed after the exposure to PUW F. Taken together, PUW F, MIN A and C are shown to be highly cytotoxic for human intestinal epithelial cells. Moreover, these cyanobacterial lipopeptides elicited effects in differentiated Caco2 cells which could lead to dysregulation of intestinal barrier function and inflammatory responses. Taking into account that these newly recognized toxins, or genetic elements for their biosynthesis, have been recently detected in a variety of different, environmentally prevalent cyanobacterial genera, PUW/MIN represent potential human health hazards. This study was supported by Czech Science Foundation (GA19-09980S).

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S13

Bioprospecting of Antarctic microbial communities dominated by cyanobacteria

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The extreme conditions of polar regions make the local dwellers attractive material for researches. Microorganisms have special importance in these ecosystems. The importance of the microorganisms inhabiting polar regions is not only in the species uniqueness but also in the diversity and unusual metabolic properties. However, our knowledge about microbial life in polar regions and their metabolic weapons such as cyanotoxins is still poor. To improve the state of knowledge, we have established AntArctic Microbial Biobank (AntAMB) for bioprospecting of polar microbes which constitutes a long-term, stable platform for both scientific and biotechnological use. To get the first insight, we have analyzed all samples in a biobank for the presence of cyanotoxins and confirmed the presence of microcystin, nodularin, cylindrospermopsin, saxitoxin, and various retinoids. This is the first report of saxitoxin and retinoids in the Antarctic. Furthermore, we have carried out 16S and 18S rRNA analysis of twelve Antarctic benthic mats. The analysis revealed significant diversity especially in static aquatic ecosystems such as lakes or ponds. Thus, observed diversity of both cyanotoxins and microbial species suggests a vast potential of not only cyanobacteria but all polar microbes to the production of diverse metabolites. To inspect this potential, we used PCR with degenerated primers to make screening for the presence of genes involved in the biosynthesis of interesting biologically active metabolites such as polyketides or nonribosomal peptides. We confirmed the presence of these genes in samples from both Arctic and Antarctic. Hence, we have constructed a fosmid library (eDNA from a benthic mat of the Antarctic lake serves as source material) and confirmed the successful transfer of these biosynthetic genes into a fosmid library. Moreover, created fosmid library constitutes a powerful tool for the mining of various novel natural products, not only polyketides or nonribosomal peptides. To conclude, we have established long-term platform for both genomic and chemical study of polar microbial communities, which has already helped us to expand our knowledge about microbial life in polar regions, diversity and potential to natural products biosynthesis.

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S14

In vitro and in vivo bioactivity of retinoid compounds detected in the environment in waters with developed cyanobacterial bloom

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Cyanobacterial water blooms and their production of diverse bioactive compounds represent a major source of natural toxins in the environment. Their metabolites have been linked with adverse effects on exposed organisms and potential health risks. Newly described products of cyanobacteria are retinoid-like compounds, but there is little information on their occurrence, potential adverse effects and associated risks. Detected compounds in the environment include all-trans retinoic acid (ATRA), 9/13-cis retinoic acid (RA), 4OH-RA, retinal (RAL) or 4keto-RAL as well as some novel metabolites, such as 5,6epoxy-RA or 4keto-ATRA. Some of these are known as strong teratogens and disruption of the retinoid signalling pathway can have fatal consequences as it regulates crucial processes during the early development of vertebrates such as the formation of the nervous system. Our studies employed four luciferase reporter cell lines for the characterization of the affinity to retinoid (p19/A15 with retinoic acid receptor- RAR and GeneBlazer UAS-bla HEK 293T with RAR and retinoid X receptor- RXR) and thyroid receptor (PZ-TR) and disrupting potencies of the pure compounds. In vitro potencies were compared with their ability to cause teratogenic effects in vivo using zebrafish (Danio rerio) embryotoxicity test (zFET). To provide further information on the mechanisms of action leading to the observed adverse outcomes the exposed zebrafish embryos were subjected to label-free proteomic analysis and qPCR analysis of selected genes expression. The results document the ability and potency of the compounds produced by cyanobacteria detected in the environment to interfere with retinoid signalling and provide a unique comparison between the responses in individual specific cell-lines and apical effects in vivo. Effective in vivo concentrations for single compounds are in nM range, which is lower than the levels of individual retinoids detected in the environment. Moreover, in nature, the compounds occur in complex mixtures within which they can pose an even greater risk. Relative potencies of the retinoids determined in the in vitro assays showed relatively good predictability towards their in vivo teratogenic potency and with the additional information from the proteomics and gene expression analysis it is clear that these compounds are able to disrupt the retinoid signalling pathway and early development at very low concentrations.

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S15

Limited Toxin Production by Cyanobacteria From Microbial Mats in Cold Deserts

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Cyanotoxin production is a characteristic of many cyanobacterial species. It has a genetic basis and toxin production is determined by gene expression processes which are influenced by numerous factors. Most of the data on cyanotoxin production comes from planktonic cyanobacteria, but there are also some records of toxigenic benthic, mat-forming cyanobacteria. However, the data concerning toxic cyanobacteria from Central Asia from both planktonic and benthic communities is very limited. Eastern Pamir, (Tajikistan) provides a unique, extreme environment for microorganisms because of its high insolation and UV radiation, as well as fluctuating temperatures, pH, salinity and nutrient availability in water reservoirs. We examined 52 cyanobacterial mats from small water bodies in a cold high-mountain desert in Eastern Pamir in terms of cyanobacterial diversity and communities' structure, presence of toxicity genes and occurrence of cyanotoxins. The microbial mats were examined using amplicon-based sequencing targeting the V3-V4 hypervariable region of 16S rDNA, PCR-based methods as well as ELISA, HPLC and LC-MS/MS methods. Identification of cyanobacterial sequences in QIIME2 based on SILVA database showed that potential toxin producers were present in most of metagenomes and often dominated them. The PCR revealed the presence of mcyE and ndaF genes in 11 samples and mcyD in six. In metagenomes with toxin-encoding genes, amplicon sequences were highly similar (>95%) to those available in databases and grouped with Nostoc PCC-73102, Nostoc PCC-7524, Scytonema UTEX 2349, Planktothrix NIVA-CYA and Tychonema CCAP 1459. Blasted mcyE sequences showed high sequence similarity to Nostoc, Planktothrix and Microcystis. In situ ELISA gave positive results for microcystins in seven samples. HPLC and LC-MS/MS analyses identified one microcystin congener (dmMC-LR) in sample E08 and two congeners ([ADMAdda] MC-RR and [d-Asp3,ADMAdda5] MC-LR) in sample E030, but they were not detected by ELISA. The results suggest that in this extreme environment, the cyanobacteria very seldom produce toxins, despite the high diversity and biomass and the widespread occurrence of potentially toxic taxa. The reason of such low cyanotoxicity in these extreme habitats will be discussed.

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S16

Behavioral effects of anatoxin-a with parallel gene and protein expression responses in larval Pimephales promelas and Danio rerio

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Anatoxin-a is a globally occurring neurotoxic cyanotoxin, which has been proposed to elicit toxicity through nicotinic acetylcholine receptor agonism, and has been shown to affect growth, reproduction, and survival of aquatic organisms. Studies characterizing its behavioral effects and altered gene and protein expression in fish models are limited. In order to fill this gap, the current study examined effects of anatoxin-a on behavioral response profiles and gene and protein expression using the fathead minnows (Pimephales promelas) and zebrafish (Danio rerio) models. Four replicates of both fathead minnow larvae (<48 hours post hatch) and zebrafish (4 - 4.5 hours post fertilization) were exposed to (±) anatoxin-a fumarate through water immersion at concentrations of 0.01, 0.1, 0.5, 1, and 1.5 mg/L for 96 h. Water concentrations were analytically verified using isotope dilution LC-MS/MS. Reconstituted hard water and caffeine were used as a negative and positive control, respectively. Multiple swimming behaviors for larvae of both species were analyzed following previously reported methods from our laboratory. Expression of genes related to neurotoxicity, oxidative stress, DNA damage, immune response, and hepatotoxicity were evaluated using qRT-PCR. In parallel with changes in gene expression, changes in protein expression was carried out using shotgun proteomics. We observed duration and total distance of fathead minnows bursting swimming speed (>20 mm/s) to be significantly lowered (ANOVA, $\alpha = 0.1$, $p<0.1$) by anatoxin-a at the 0.1, 1, and 1.5 mg/L treatment levels and caffeine (56.4 mg/L) under dark conditions, while the opposite occurred with zebrafish in which anatoxin-a stimulated an increased trend in bursting swimming speed from the negative control in both light and dark. Both species showed minor to no photomotor response changes from the negative control. Fathead minnow larvae behavioral responses to this toxin may be more sensitive than the zebrafish model. Target genes such as syn2a, tuba1, neurog1, gst, gpx1a, and cyp3a65 were all upregulated at the 3 highest anatoxin-a concentrations, but no statistical difference (ANOVA, $\alpha = 0.05$) was found between the control and fish exposed to anatoxin-a.

In analyzing proteome changes, PCA was used to classify toxicity relationship based on specific toxin treatment levels revealing a similar grouping of 0.1 mg/L anatoxin-a with caffeine (0.412 mg) for zebrafish. However, this trend was not observed in the fathead minnow with overlap of caffeine and anatoxin-a only seen at the highest concentration of 1.5 mg. Differentially expressed proteins also varied with species (~124-400) and concentration suggesting species specific differences in sensitivity. Ongoing work is exploring differences in response pathways using KEGG.

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S17

Identifying toxins associated with Prymnesium parvum elicited acute toxicity to fish

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Prymnesium parvum is a mixotrophic haptophyte that is notably euryhaline and eurythermal and has been detected in all continents except Antarctica. P. parvum causes harmful algal blooms (HAB) in both coastal and inland waters. These HAB events have been devastating to aquaculture ponds and aquatic ecosystems. Whereas several compound classes have been isolated from P. parvum and thought to be the cause of P. parvum related acute toxicity to gill-breathing organisms, the responsible toxin(s) have not been confirmed. Here we evaluated a top-down analytical approach using LC-HRMS that analyzed the entire chemical fingerprint and linked peak area differences with the magnitude of acute toxicity. A 2 × 2 factorial design with two nutrient levels (f/2 and f/8) and two salinities (5 ppt and 2.4 ppt) was employed to examine P. parvum growth, acute toxicity and toxins production. A positive relationship was found between higher acute toxicity to fish and elevated prymnesins concentration in experimental units, but non-targeted analysis and relative difference plots suggested other molecules may have contributed to observed toxicity. To further investigate the potentially responsible toxin(s), we then performed a sunlight study with samples from each experimental unit, because previous work indicated that acute toxicity to fish is ameliorated when P. parvum cultures are exposed to light. However, these previous studies did not incorporate analytical measurements of chemicals in toxic and nontoxic samples. We exposed each treatment combination to three light scenarios, ambient light in the field, dark conditions in the field and laboratory dark controls maintained at 4 °C. Following a 2-h light study period, juvenile Pimephales promelas were exposed to each treatment combination for 24-h to assess acute toxicity. Each treatment combination and light scenario was sampled prior to and following the 2-h light study period for LC-HRMS analysis. We observed acute toxicity to be completely ameliorated in light exposed samples but retained in field dark samples and laboratory dark controls. We further identified prymnesins in pre-exposed samples and a decrease in relative concentrations of these toxins in samples where toxicity disappeared. Thus, concentrations of prymnesins were relatively consistent between light scenarios except for the sunlight exposed conditions where acute toxicity and prymnesins concentration decreased. These results provide multiple lines of evidence supporting prymnesins as toxins responsible for P. parvum elicited acute toxicity to fish.

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S18

Assessing the toxicity of two mycotoxins, deoxynivalenol and zearalenone, to freshwater organisms

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Mycotoxins are natural toxins produced by various species of fungi. Mycotoxins are being considered as contaminants of emerging concern in freshwaters, with agricultural run-off highlighted as one of the main sources of input. Recent studies looking at mycotoxins in freshwater have confirmed their presence and, in some locations, relatively high spikes in concentrations during sampling periods. Climatic changes and farming practices are driving an increase in fungal infestations. Deoxynivalenol and zearalenone are two prominent mycotoxins which can both be produced by F. graminearum and F. culmorum. These species of Fusarium are widespread globally, known for being the main causative agents of fusarium head blight in cereal crops. Toxicity data for mycotoxins in freshwater organisms has formerly been centred around zebrafish. Here, we have investigated the toxicity of deoxynivalenol and zearalenone in multiple plant and invertebrate species to widen the understanding of the toxicity of two commonly detected mycotoxins. Test organisms included: Brachionus calyciflorus, Chironomus riparius, Daphnia magna, Hydra vulgaris, Lemna minor, Lymnaea stagnalis, Pseudokirchneriella Subcapitata, Thamnocephalus platyurus and Tetrahymena thermophila. Results of acute studies showed the most sensitive organism was dependant on the mycotoxin. Deoxynivalenol poses a particular threat to crustaceans, with both D. magna and T. platyurus showing a high sensitivity in this study. Whereas, zearalenone was most toxic to L. stagnalis embryos and H. vulgaris, at a comparable level to that reported by previous studies for zebrafish embryos. For both mycotoxins the variety in responses demonstrated the importance of considering a wide variety of species when assessing the potential toxicity of contaminants, particularly for deoxynivalenol which may have previously been considered of little concern based upon the absence of effects in zebrafish embryo exposures. This insight into the toxicity of two commonly detected mycotoxins aids towards understanding the potential risk of contaminated freshwaters when considered alongside available environmental data for mycotoxin presence. However, to sufficiently assess the risk of these mycotoxins on freshwater ecosystems more work into the temporal variation of mycotoxins is needed to understand the duration of exposure to peak levels of potentially hazardous concentrations.

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S19

Are Food Processing Plants an Environmental Source of Phytoestrogens and Mycotoxins?

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v Food, beverage, and feedstock processing plants transform raw agricultural materials into a variety of food ingredients and final products. Many of these plants are National Pollutant Discharge Elimination System (NPDES) permitted facilities discharging to the environment. Nevertheless, they are an under-investigated potential source of chemical and microbial contaminants to the environment. In 2018, effluent samples from 23 food processing plants (e.g. meat, seafood, fruit and vegetable, dairy, brewery/distillery operations, and ethanol production) were sampled from 17 states across the U.S. and analyzed for more than 530 target organic chemicals (e.g. natural toxins, pesticides, pharmaceuticals, hormones, and volatile organic chemicals). Effluent extracts were additionally screened for activity of approximately 70 biological endpoints using Attagene FactorialTM assays and endocrine pathway specific transcriptional activations assays. More than 170 chemicals were detected at least once in the 23 food processing plant effluent samples collected. At least one natural toxin was detected at 48% of the sites (11 sites: five meat, three beverage, one vegetable canning, one cheese, and one ethanol processing plant) with one to three natural toxins detected in these effluent samples. The most frequently detected natural toxin was genistein (22%, phytoestrogen) followed by daidzein (17%, phytoestrogen), zearalenone (13%, mycotoxin), equol (9%, phytoestrogen), formononetin (9%, phytoestrogen), beauvericin (4%, mycotoxin), biochanin A (4%, phytoestrogen), and α-zearalanol (4%, mycotoxin). Detected natural toxin concentrations, however, were generally low with 89% of the detections being between 1 and 10 ng/L. Initial results indicate food/feedstock production effluents represent a potential source of natural toxins to receiving surface waters. In addition, the measured natural toxins are included in the U.S. Environmental Protection Agency's Toxicity Forecaster (ToxCast) database of chemical-biological interactions. A comparison between concentration and bioassay results (e.g. total estrogenicity) will be conducted to determine if a statistical relation exits between natural toxin concentration and biological activity of the effluent samples collected. Abstract does not necessarily reflect U.S. Environmental Protection Agency views or policy.

Keywords: Phytoestrogens, Mycotoxins, Effluent

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S20

Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat? Perspectives from Fifteen Years with Prymnesium parvum

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Though environmental monitoring, assessment and management programs have been developed for chemical contaminants and other stressors in inland waters, the magnitude, frequency and duration of harmful algal blooms (HABs) may be increasing at the global scale. HABs are caused by complex factors that vary among algal species, but landscape modification, from anthropogenic activities ranging from effluent discharges, natural resource extraction and agricultural runoff to salinization and climate change influences on freshwater ecosystems, appear key forcing factors for HAB development in inland water of the U.S. When these HABs are observed, impairment of aquatic life, recreation, agricultural and potable uses of surface waters soon follows. However, impairments of such uses have not yet resulted in designations of exceedances of water quality criteria or standards for waterbodies under the U.S. Clean Water Act. For example, in the case of HABs from Prymnesium parvum (a.k.a., Golden Algae, or the Texas Tide), devastating fish kills have become so routine that fisheries managers no longer stock fish in affected lakes and reservoirs. In some inland habitats, the prevalence of HABs appears to represent more significant threats to sustainable environmental quality than conventional chemical contamination. In fact, toxins produced by HABs are now considered contaminants of emerging concern by the U.S. Environmental Protection Agency. Unfortunately, conventional approaches to resource assessment and management appear poorly prepared to address this question. With the prospects of environmental quality changes from urbanization and climate change continuing to be understood, such observations have recently prompted the question, Are HABs becoming the greatest threat to inland water quality? This question is considered through perspectives gained and lessons learned following 15+ years studying P. parvum in inland waters of the U.S.

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S21

A Review of Cyanobacteria and Cyanotoxins in Drinking Water Reservoirs in Ghana

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Cyanobacterial blooms and cyanotoxins represent a worldwide-occurring phenomenon, but dramatic differences exist among different countries in cyanotoxin-related human health risk assessment and management practices and policies. While national standards, guideline values and detailed regulatory frameworks for effective management of cyanotoxin risks have been implemented in many industrialized countries, the extent of cyanobacteria occurrence and cyanotoxin contamination in certain geographical regions is underreported and not very well understood. Such regions include major parts of tropical West and Central Africa, a region constisting of nearly 30 countries occupying area of 12 mil km2, with a total population of 500 milion people. Only few studies focusing on cyanotoxin environmental occurrence in this region have been published so far, and reports dealing specifically with cyanotoxin contamination in drinking water are being extremely scarce. Over a period of 15 years, from January 2004 to December 2019, a pioneering research into cyanobacteria and cyanotoxins in drinking water reservoirs in Ghana was initiated. The reservoirs are Weija, Kpong, Barekese, Owabi, Brimsu and Kwanyarko Reservoirs, supplying drinking water to Accra, Eastern, Ashanti and Central Regions of Ghana. Concentrations of microcystins were monitored during drinking water treatment process in the six treatment plants. HPLC was the analytical method for the detection of toxins in samples both extracellular and intracellular. Results showed that 65% samples of intake water contained intracellular microcystins whereas dissolved toxins were detected in 33% samples. Concentration of intracellular toxins in the intake water exceeded 1 µg/L (i.e. WHO guidance value for microcystin-LR) in 27% samples with the highest detected concentration 8.73 µg/L. 17% of samples of final treated water still contained intracellular or particulate-associated microcystins (maximal concentration 0.61 µg/L), and 14% samples of treated water contained dissolved microcystins (maximal concentration 0.81 µg/L). HPLC analyses of samples gave six variants of microcystin, MC-LR, MC-YR, MC-RR and MC-LA and two unknown microcystin variants monitored at 238 nm. Dominant cyanobacteria species found in these reservoirs among others are Microcystis aeroginosa and Planktothrix agardhii. Due to the chronic effect of these toxins it is recommended that drinking reservoirs with low levels of microcystins must be regularly monitored to keep it free from microcystin to ensure safeguarding human health.

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S22

Microcystin removal from lake water for potable water using Bio-fence around Nyanza Gulf of Lake Victoria

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The deterioration of water quality of Lake Victoria has been a threat for human health. Hence, in LAVICORD (The Lake Victoria Comprehensive Research for Development) project, we focused on the development of several appropriate technologies for safe and clean water. The studies were performed at beaches around Nyanza Gulf of Lake Victoria, because we found cyanobacteria blooms at several places in Nyanza Gulf of Lake Victoria. Therefore, first of all, we solved the cyanotoxin microcystin contamination problem, because we elucidated the remarkable microcystin contamination in potable water collected from beach of Nyanza Gulf. A Bio-fence system was installed at Ogal beach, at which the highest contamination of microcystin was found, to produce safe potable water. The cyanobacterial bloom at Ogal Beach was mainly consisted with Microcystis sp. The both width and length of the bio-fence was 1m. Effective depth was changed by the lake water level. The Bio-fence has a simple mechanism to produce clean and safe water by using crushed charcoal as a biological filtration medium. The residence time of water flow thorough the bio-fence was around from 8 hr to 12 hr. Chl-a, TSS and microcystin were mainly measured as water quality parameters. Under optimal conditions, the Bio-fence was effective in removing up to 94% of the Microcysits cells from the lake water. It also removed 97% and 93% of Chlorophyll-a and TSS respectively. Concentration of microcystins (MCs) was measured by PP2A method. The average concentration of MCs in the Bio-fence treated water was 0.92 µg/l which was below the WHO guidelines of 1 µg/l of MC-LR in drinking water. These results showed that the water quality was drastically improved by the installed Bio-fence. In addition, the Bio-fence technology was cost effective and efficient easy to operate technology for treatment of potable water around Lake Victoria. However, it was affected by the water level fluctuation of Lake Victoria. Therefore, we need to improve the Bio-fence for practical use, because the strength of water level fluctuation recently became larger by the effect of climate change.

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S23

Waterborne cyanobacteria in the air: exposure and effects

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Toxic compounds produced by cyanobacteria pose hazards to wildlife and human health. Detection of cyanobacterial (CB) taxa and cyanotoxins in aerosols and dust particles raises the question of potential hazards associated with human exposures via inhalation, especially during water-related recreational activities. Our study focused to estimate the inhalation exposure to cyanobacteria and cyanotoxins from several Czech waterbodies contaminated with CB bloom and their toxicity for human respiratory tract using Beas-2B cell line originating from normal human bronchial epithelium. In the warm and dry summer 2018, the air was sampled at waterbodies contaminated with CB bloom (4 localities) and at a reference locality using high volume active sampler equipped with total suspended particulates (TSP) inlet (Digitel, Switzerland). Additionally, grab water samples were taken into rinsed PET bottles and biomass was sampled from waterbodies and concentrated by using a plankton net (20 um) at the end of air sampling. Subsequent analyses included instrumental analysis of cyanotoxins (LC-MS/MS), endotoxin quantification, taxonomic analyses by macroscopy and genome analysis, and bioassays with human bronchial epithelial cell line Beas-2B. Microcystin-LR (MC-LR) and MC-RR were detected in the highest total water concentrations (filtered water + CB biomass), ranging 0.2–10.3 and 0.1–3.4 ug/l, respectively. Concentration of MC-LA, -LF, -LW, -LY, -YR, -WR, anatoxin, nodularin and cylindrospermopsin, ranged <LOQ–0.8 ug/l. No CB bloom and very low algae cell counts were observed at the reference locality that serves as a reservoir for drinking water supply. Indeed, concentrations of all measured cyanotoxins were low, in the range of LOQ-0.1 ug/l. The waste majority of cyanotoxins was present in the biomass (intracellular fraction, IC) and only minor amounts of cyanotoxins were dissolved in water or bound to other materials (extracellular fraction, EC). Accordingly, the IC fraction of cyanotoxins and other CB compounds extracted by methanol from waterborne cyanobacteria was more cytotoxic towards Beas-2B cells than the EC fraction, whereas lipopolysaccharides (LPS) isolated from the biomass had no effect on the cell viability. A significant dose-dependent increase in the production of interleukins IL-6 and IL-8, important cell signalling molecules involved in the immune system response, was observed in Beas-2B after exposure to LPS isolated from 2 sites, Brno and Sykovec reservoirs. Results of other planned analyses will be discussed. Thus far, our results indicate that inhalation of aerosolized cyanobacteria occurring in the air at CB bloom-contaminated waterbodies could result in the irritation of human respiratory tract.

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S24

Degradation and detoxification of cylindrospermopsin by hydroxyl and sulfate radical-based advanced oxidation processes

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Besides taste and odor compounds, cyanobacteria can produce a range of toxic secondary metabolites, i.e. cyanotoxins, such as cylindrospermopsin (CYN). Due to increased eutrophication of surface water and climate change in recent years, toxic cyanobacterial blooms occurred more frequently and to greater extent. As a consequence, the hepatotoxic and potentially carcinogenic CYN and other cyanotoxins became more abundant. Since cyanobacteria and its toxins pose a risk to human health, appropriate treatment technologies are required to assure removal of these toxins and to provide safe drinking water. Different approaches for the removal of CYN from drinking water have been studied, including biodegradation, activated carbon filtration, chlorination, ozonation and several advanced oxidation processes (AOPs). AOPs, i.e. methods which produce highly reactive chemical species such as hydroxyl radicals (HRs), encompass a vast range of available technologies for drinking and wastewater treatment. Most commonly studied AOPs include e.g. Fenton oxidation, ozone- and UV-based methods to generate HRs. Compared to HRs, sulfate radicals (SRs) were shown to be less prone to scavenging by natural organic matter and are more reactive as indicated by their redox potentials (HR: 2.7 V/SHE and SR: 3.1 V/SHE under acidic conditions). SRs can be produced from peroxymonosulfate (HSO5-) or peroxydisulfate (S2O82-) in similar mechanisms as HR is produced from H2O2. However, SR-based AOPs have not nearly been investigated as extensively as HR-AOPs, especially in the context of cyanotoxin removal from drinking water. Although several AOPs were shown to effectively degrade CYN, degradation products were tentatively identified and mechanisms proposed, residual toxicity of treated CYN was rarely assessed. In this study, HR and SR were generated in Fenton(-like) reactions using Fe2+/H2O2, Co2+/HSO5- and Ag+/S2O82-. In the first part, effects on the degradation efficacy of selected treatment parameters, i.e. metal to oxidant ratio, metal/oxidant to CYN ratio and solution pH were investigated. Following the optimization of these treatment parameters for highest CYN removal, CYN degradation kinetics and products were determined. Since SR-AOPs can produce HR as secondary radical, the contribution of HR and SR to CYN degradation was examined employing the selective radical quencher tert-butanol. Finally, residual toxicity of CYN treated by HR- and SR-AOPs was assessed using a human-relevant three-dimensional in vitro liver cell model.

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S25

Hepatotoxins in groundwater wells and their accumulation in edible plants through irrigation in an intensive agricultural region of Greece

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The wide spread of cyanobacteria and its many effects on aquatic ecosystems are well known. However, the presence of cyanotoxins in the aquifer and their accumulation in irrigated plant species is poorly studied. Karla Reservoir is an example of a lake ecosystem which was dried in 1960's and now has been restored. It is located in central Greece which is one of the most productive agricultural regions of the country and until now irrigation needs for the surrounding cultivations in the area are covered by the lake's underground aquifer. However, the reconstructed reservoir is facing the presence of extensive blooms that are dominated by toxin producing cyanobacterial species. Cyanotoxins in lake water have also been recorded. The aim of the present study was to estimate the presence of hepatotoxins (microcystins and cylindrospermopsins) concentrations in groundwater wells and in edible parts of plants irrigated with well waters in the Karla region. Ten groundwater wells and the respective neighboring crops irrigated with their water located in the Karla region were selected for the present study. Crops included melon, watermelon, industrial tomato, barley, wheat, corn, sunflower, garlic and onion. According to results, hepatotoxins were found in all groundwater samples. Microcystin concentrations in water ranged between 1.2 μgMC-LR eq/l and 1.8 μg MC-LR eq/l. Cylindrospermopsin concentrations in water ranged between 0.5 μg/l and 1.3 μg/l. All plants collected from fields using groundwater for irrigation were found to accumulate microcystins and cylindrospermopsins. Microcystin and cylindrospermopsin concentrations in plants correlated positively with their concentrations in well waters (r = 0.94). On the other hand, microcystin and cylindrospermopsin concentrations varied significantly among plants ($P < 0.05$). The highest concentration of microcystins was detected in industrial tomato (0.567 ng MC-LR eq/g fresh weight) and the lowest was in sunflower (0.137 ng MC-LR eq/g fresh weight). The highest concentration of cylindrospermopsins was detected in watermelon (0.321 ng/g fresh weight) and the lowest was in garlic (0.098 ng/ g fresh weight). The presence of hepatotoxins in the groundwater aquifer is probably related to the migration of these toxins from the surface water of Lake Karla. It has been proved that a significant amount of Karlas' surface water slides through the permeability of geological formations enriching the area's aquifer. All plants collected from fields irrigated with hepatotoxin-contaminated groundwaters were found to accumulate these toxins at concentrations that exceed the acceptable level based on the amount of plant tissues consumed by human. Groundwater wells and edible plants should be continuously monitored for the presence of cyanotoxins to protect the public against the exposure to such hepatotoxins.

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S26

Removal of natural toxins in biological sand filters for drinking water treatment

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Natural toxins comprise a large and chemically very diverse group of emerging contaminants. A considerable pool of these compounds is rather mobile, with possibility of leaching to surface and groundwater from terrestrial source. Hence, there are concerns whether several groups of natural toxins pose a risk to drinking water quality. In Denmark, 99% of water supply is abstracted from groundwater. Due to the good water quality, treatment of groundwater is usually a simple process, comprising aeration and subsequent biological rapid sand filtration. Indigenous microbial community present in sand filters is responsible for several biological processes (oxidation of ammonium, iron, methane, etc.). The aim of this study is to investigate whether substantial natural toxin removal is possible using rapid sand filters, a cheap and commonly applied water treatment method in Denmark. The study focusses on two groups of highly mobile and toxic phytotoxins: norsesquiterpene glucosides (ptaquiloside and caudatoside) and alkaloids (jacobine N-oxide, senecionine, sparteine, gramine and caffeine). Filter sand used in this study is collected from aerobic rapid sand filters at five Danish waterworks and investigated for their phytotoxin removal potential. Microcosms were set up with filter sand, groundwater and phytotoxins applied at concentration of 200 μg L-1. During the course of 14 days, degradation potential of the sand filters is investigated by collecting and analyzing water samples. For the toxin analyses, UPLC-MS/MS (alkaloids) and LC-MS (norsesquiterpene glucosides) methods are used. In addition, DNA analyses are included in this study to help understanding which microorganism are involved in toxin degradation processes. Preliminary results (from one waterwork) showed that concentration of all phytotoxins after 14 days is following: ptaquiloside decreased to 8% of the initial concentration, caudatoside to 4%, jacobine N-oxide to 86%, senecionine to 91%, sparteine to 50%, gramine 41-47% and caffeine to 94%. Alkaloids exhibit recalcitrant character in this study and further investigations of different sand filters are currently performed.

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S27

Monitoring and treatment of St. George lake to mitigate Cyanobacterial Harmful Algal Blooms (cyano-HABs)

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Cyanobacteria are phytoplankton microorganisms, also known as green-blue algae, named after their green-blue (Greek: cyano) color. They are an essential component of the food web in all aquatic ecosystems, but excess loads of nutrients into the waterbodies can cause their rapid and excessive growth which leads to the formation of Harmful Algal Blooms (cyano-HABs). In stressful conditions, toxic genera of cyanobacteria excrete and/or release into the water a broad variety of bioactive metabolites, also known as cyanotoxins. These metabolites can negatively impact the ecosystem and human health in various ways, making it an important environmental issue of concern [1]. With climate change being linked to global expansion of harmful cyanobacteria [2], it is imperative that we find both early detection systems but also efficient methods to mitigate harmful cyanobacteria blooms at source. This study aimed to monitor St. George lake in order to correlate its trophic condition with its water quality characteristics and identify the key environmental variables driving cyanobacteria blooming and their cyanotoxicity [3]. St. George lake is located in the National Park of Athalassas in Cyprus and serves as an aquatic life and bird habitat, making it an extremely important biotope for the island. Our research group requested and was granted permission from the Department of Forests to sample and monitor the lakes of the park for a 12-months period, between January and December 2019. During this period, ten water sampling events took place and samples were analyzed for T, pH, TSS, conductivity, nutrient content, photosynthetic activity of cyanobacteria and green algae, and cyanotoxins concentration. Monitoring has indicated that the cyanobacteria blooming period in St. George lake lasts for five months from May to September 2019, while microscopic observation of preserved samples showed that 99% of the phytoplankton biovolume was attributed to a single picocyanobacterial species, the Merismopedia sp. These species are reported in the literature as microcystin and nodularin producers which are the most detected cyanotoxin groups in surface waters [4]. Thus, collected samples from August, when the bloom had its peak density, have been analysed for cyanotoxins presence and concentration. Beside monitoring the lake, cyanobacterial contaminated water was also used to test novel oxidants that release hydrogen peroxide as a mitigation process with various efficiencies. During the blooming period, water was collected and used for bench-scale experiments in order to compare different oxidation processes in terms of efficiency and targeted treatment, while considering the environmental impact.

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S28

Tools to manage cyanobacteria agglomerations in freshwater ecosystems

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Increase of water temperature and eutrophication processes accelerate harmful algal blooms in freshwater ecosystems. If point sources of pollution are controlled in many European Union countries, the diffuse pollution of phosphorus and nitrogen compounds from agricultural land is still a challenge for the management. Undoubtedly, the primary goal is to cease pollution and block access to water ecosystems. However, the urgent measures are also necessary to be applied to already deteriorated lakes that suffer from harmful cyanobacteria blooms, especially those ecosystems which are used for the drinking water supply and recreation. Harvesting of cyanobacterial agglomerations can contribute in reducing of nutrient load and cyanotoxins concentrations from water bodies. Restoring water quality and biological health of a particular aquatic ecosystem allows to prevent cases of intoxication via recreation, diminish costs of drinking water treatment and ensure safe water for consumers. The project AlgaeService for LIFE - LIFE17 ENV/LT/000407 (2018-2023), proposes to use a system of tools to manage water quality and cyanobacterial blooms in freshwater ecosystems. Particularly, two technologies will be applied to harvest agglomerations of cyanobacteria in aquatic ecosystems of different size. The efficiency of harvesting of cyanobacteria using the specialised prototype with conveyor type system will be tested in lakes and ponds in 2020. Another prototype-harvester equipped with centrifugation system will operate in large water bodies, such as the Curonian Lagoon. Moreover, remote sensing methods, such as satellite images and images of unmanned aerial vehicle, will be applied to define timing and target area of cyanobacteria agglomerations for efficient operation of prototypes. The ArcGIS application "Mark a blooming water body", the interactive map with marked blooming locations and the questionnaire are available on the project website (http://algaeservice.gamtostyrimai.lt/). This tool encourages state institutions and society to contribute in filling the database of blooming water bodies and raises public awareness on issues related to harmful cyanobacteria blooms.

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S29

Evaluating in silico tools for estimating physico chemical properties of natural toxins relevant to water quality

authors

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Natural toxins could pose a risk for drinking water in Europe. The world of natural toxins encompasses a wide variety of chemical groups, such as alkaloids and terpenoids, which have been disregarded in monitoring of drinking water quality.[1] Physico-chemical properties that determine the PMT profile [2] of natural toxins could be estimated by QSAR models when experimental data is lacking. Either existing or new models can be used to study the persistence and mobility of natural toxins in the environment. Existing QSAR models for anthropogenic compounds have been widely applied in environmental sciences, However, they are based on measured property data for pesticides, industrial chemicals and hydrocarbons, which can be structurally distinct from chemicals that are natural toxins. The applicability domain of a QSAR model defines the region of chemical space in which predictions are expected to be more reliable since they are based on interpolations and avoid extrapolations.[3] To establish the applicability domain of a model different methods can be used [4], with similarity testing by distance in the chemical space being the most prevalent. We compared the Euclidean distance (dE) of 1586 toxins in the database of plant toxins provided by Günthardt [5] against 2447 compounds in the KOWWIN model and 589 compounds in BIOWIN5 and BIOWIN6 models. Thresholds had been defined in literature using dE average values of the chemicals in the training sets of the model; in this work, we developed a new method for defining model-specific thresholds, and found that 40.7% and 16.2% of natural toxins were outside of the applicability domain of the models KOWWIN and BIOWIN, respectively. A limitation of existing QSAR models like KOWWIN and BIOWIN is that natural toxins are not present in the model training sets. These models could produce large and unexpected errors when applied to natural toxins that have structural features that are not present in the training sets, which would be the QSAR modeling equivalent of a black swan event [6]. To begin to address this possibility, we developed a new QSAR model using experimental Koc values for natural toxins from the NaToxAq consortium and for other chemicals collected from the literature. Predictors to build the model were extracted from RCDK package and analyzed with R. Applying stepwise linear regression and partial least squares methods we achieve models with R2 values of 0.69 and 0.73, and q2 values of 0.56 and 0.54, respectively.

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S30

Simulation of Phytochemicals in water with the PEARL model

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There is a wealth of research on the fate and effects of xenobiotics in the environment, and legislation is in place to minimise their adverse impact on the environment and human health. However, toxins also occur naturally in the environment but there is currently no understanding of the extent to which they may, or may not, also pose a risk; whilst any risk may be lower than xenobiotics, natural toxins can contribute to toxicity mixtures. Although they are widely produced by plants, but our understanding about their fate and transport in soil and water is very poor. Recently the presence of natural toxins in soil and water has been reported in UK and Denmark. It is therefore necessary to understand the role of natural toxins in the potential contamination of drinking water sources. The objective of this study was to estimate the potential concentration of natural toxins that may transfer to drinking water sources from plants. Natural toxins produced by plants are as diverse as any other toxic chemicals in terms of physico-chemical properties and toxicity. Once they are released in the environment, the fate and transport of natural toxins mostly depend on their physico-chemical properties and factors such as soil type, organic matter and precipitation. Transport models are used to predict the concentration of e.g. pesticides in surface and groundwater. The FOCUS Pearl model was used to estimate predicted environmental concentrations (PECs) of natural toxins and to investigate the impact of different parameters (e.g. plant/toxin/soil/weather) on these concentrations. Physico-chemical parameters were taken from the Toxic Plants-Phytotoxins database developed by Günthardt et al., 2018. Some experimentally measured properties by NaToxAq project were also used in this study such as half-life and Koc. The results show that the highest risk is posed in catchments containing shallow soil with low organic content. The predicted concentrations were used to identify the geographical location of high risk areas, based on similarities of e.g. soil and climate. Results will be used to assess the relative risks and to identify where further research should be focussed with regards to model input parameters. This study will establish a base for investigations into potential contamination by natural toxins. Keywords: natural toxins, water sources, transport model, PECs

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S31

Degradation of Cyanotoxin Cylindrospermopsin in Water Using Ultrasonication: Role of Reactive Radical Species

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Cylindrospermopsin (CYN) is a cyanotoxin of increasing interest, since it has been identified as hepatotoxic, dermatotoxic and cytotoxic. The increasing occurrence and expansion of cyanobacterial blooms able to synthetize CYN in European water bodies has been frequently reported, which clearly creates the need for the development of efficient and effective treatment processes [1]. Ultrasonic irradiation of water (sonolysis), is an attractive advanced oxidation process (AOP), that has been considered as alternative for water treatment. When ultrasound waves pass through an aqueous medium, they generate various reactive radical species (HO▪, H▪, and HO2▪), and hydrogen peroxide [2]. In the past ultrasound irradiation has been used for the degradation of selected cyanotoxins (i.e., MC-LR and MC-RR) [3]. To the best of our knowledge, it is still unclear if this process is able to degrade CYN and what are the optimum operating conditions. This study aimed a) to investigate the sonolytic degradation of CYN in aqueous solutions under several design and operating parameters (position of reaction vessel, ultrasound intensity, initial CYN concentration, time of sonolysis and initial pH) and b) to determine and quantify the reactive species responsible for the degradation of CYN in water. Results demonstrated that sonolysis can effectively and rapidly degrade CYN, over a wide range of initial concentrations (0.1 – 1 mg L-1), since the toxin was fully degraded in the first 50 min of the reaction. Ultrasound intensity showed the maximum effect on the rate and efficiency of the process, while pH was also a significant factor, since at pH lower than 6, degradation rates were greatly reduced. The degradation followed the pseudo-first order reaction kinetic model. In the optimum operating conditions, initial reaction kinetic constants (kobs) reached a maximum of 0.192 min-1. Further investigation of the ultrasonication process, using coumarin and fricke dosimetry, revealed that the design and operating parameters that mainly influence the production of reactive species were: a) the position of the reaction vessel and b) the applied ultrasound intensity. Sonolysis appeared to produce mainly HO▪, which was indicated by the application of several radical scavengers (i.e. MeOH, Ter-butyl-alcohol). In order to quantify HO▪ radicals produced during sonolysis under various operating parameters a novel approach was followed: coumarin dosimetry was applied is the sonolysis system in parallel to a steady-state gamma radiolysis device (60Co Gamma Chamber source), which is a process with a well-known HO▪ yield. Comparison of the results provided an innovative quantification tool for the production of HO▪ during sonolysis.

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S32

A naturally occurring carcinogen in Pteridium aquilinum: I) modelling the fate of ptaquiloside in soils

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Plant toxins, also referred as phytotoxins, have in the last two decades been identified as emerging contaminants in the environment. These toxins are universal, chemically diverse, present in high amounts and some very toxic. This study presents a novel modelling approach for assessing the fate of plant toxins in the soil-plant-atmosphere system. The model has been developed for the case of ptaquiloside (PTA), a terpenoid carcinogen produced by bracken fern (Pteridium aquilinum). The crop module of the agro-hydrological model DAISY has been adapted to account for reproducing natural toxin dynamics in plants, covering processes such as toxin generation on the canopy, wash off by precipitation and mass recovery after depletion. The parameterization of the new processes has been based on data from a 2-year monitoring program and from the literature. A sensitivity analysis was also performed for ranking the importance of the newly implemented processes on two different quantities. The amount of the PTA reaching the soil surface and the amount of PTA which is leaching at 1 m soil depths. The analysis showed that PTA generation in bracken was the most important process, followed by the PTA wash-off. However, soil degradation was the only process defining leaching of PTA for the sandy soil. The model recreates realistic toxin production in the canopy, as well as realistic amounts of the toxin washed off by precipitation, in the same orders of magnitude that data from previous research. The simulations results show that PTA can leach to the groundwater, with a median value for the annual flux-averaged toxin concentrations at 1 m (PEC) depth equal to 16 and 135 ng l-1 for the sandy and loamy soil, respectively. The much higher PEC in the loamy soil was due to preferential transport of the toxin through macropores which resulted in a lower PTA net degradation, due to the by-pass of the biologically active layer of the soil. Leaching events are highly affected by the soil water content and rain intensity, and they present seasonal dynamics with highest PEC during the winter period and lower during the growing season. Based on the results, we conclude that PTA should be considered as an aquatic micropollutant and that it may reach water bodies and reservoirs in form of pulse events. These pulses can take place in the growing season during high intensity precipitation events, but predominantly in the beginning of autumn when the soil get water saturated. We identify a need for research on PTA dynamics and partitioning in the different phenological stages of bracken, as well as increasing our knowledge on the release of toxin from the plant to the surroundings.

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S33

Biotransformation of cylindrospermopsin by manganese-oxidizing bacteria

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Cylindrospermopsin (CYN) is a highly persistent alkaloid cyanotoxin with hepatotoxic and neurotoxic properties. Therefore, it is important to understand its fate in the environment and to search for strategies to remove CYN from aquatic systems. So far, little is known about the biological transformation of CYN that has been reported only for few microbial isolates and communities. Manganese-oxidizing bacteria (MOB) have been shown to remove a variety of pollutants and therefore have been proposed as a potential biological tool to remove diverse pollutants from water. However, the potential of MOB to remove cyanotoxins and possible transformation products had not been assessed so far. Here, we investigated CYN transformation by different MOB, isolated from natural and technical systems under diverse growth conditions. Tested MOB's included one Pseudomonas sp. strain, two Ideonella sp. strains, and one Comamonadaceae bacterium. The removal of CYN at an environmentally relevant concentration of 120 µg L-1 ranged from 25 to nearly 100% when MnCO3 was used as manganese source for MOB in the media after 3 to 28 days of incubation. In the absence of manganese, the removal was zero or lower than 20%.

To detect CYN transformation products, MOB were incubated 4 to 34 days, depending on the growth rate of each tested MOB, with 7 mg L-1 of CYN. All the tested MOB removed CYN to concentrations below the detection limit at different rates. Pseudomonas sp. strain OF001, was the fastest MOB among the tested strains removing nearly 100% of the toxin within three days under all tested conditions. Eight transformation products were detected and identified based on mass spectrometric analysis (LC-MS/MS), using an inclusion list of previously described transformation products of abiotic reactions and further analysis of their fragmentation patterns. For all tested MOB the same eight transformation products were detected, suggesting a general transformation mechanism catalysed in the presence of MOB. The cytotoxicity of the mixture of transformation products in two hepatic human cell lines HepG2 and HepaRG was negligible in comparison to pure CYN and comparable to controls without toxins. Considering the efficient removal of CYN by the tested strains, MOB could be used for water treatment to remove CYN and might contribute to the natural removal of CYN in freshwater systems.

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S34

Varietas Placuerit – Variability in Natural Toxin Environmental Mobility

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Natural toxins are multifunctional, ionizable organic compounds, some of which are found in surface waters. To assess which out of the total myriad may potentially pose a risk to overall water quality, the compounds' environmental mobility has to be assessed by quantifying sorption coefficients to major geosorbents. However, hardly any experimental data describing natural toxin mobility are available and in silico prediction tools show limited applicability for multifunctional, ionizable compounds. Hence, effective environmental exposure/risk assessment of natural toxins is difficult and systematic research on environmental distribution processes urgently needed. We established a column chromatography setup for systematic high-throughput analysis of sorption coefficients to different geosorbents (organic carbon, clay minerals) under changing environmental conditions (pH, aqueous solution composition). The investigated analyte set was based on an earlier perstistence and mobility priorization and comprised over 100 natural toxins with varying ionizability from 30 different subclasses. Organic carbon-water partitioning coefficients (pH dependent, Doc) were derived as primary toxin mobility indicator. All considered natural toxins were confirmed as highly mobile with log Doc values < 4 at pH 6. Terpenoids were generally the most mobile compound class with a geometric mean log Doc < 1.5, while pyrrolizidine alkaloids (PA) were one of the most mobile alkaloid subclasses with a geometric mean log Doc of 1.8. In contrast to most of the terpenoids, many alkaloids are protonated at environmental pH and as such particularly influenced by the presence of minerals in soils. Clay minerals immobilized the investigated alkaloids more strongly than terpenoids by enhanced sorption (e.g., pH dependent montmorillonite-water partitioning coefficient log Dmont ≈ log Doc + 1 for PA). Coefficients derived under changing conditions with regards to pH and eluent composition indicate that the varying sorption affinity of natural toxins is the result of a highly complex interplay of several interaction types that are not only dependent on the compounds' properties, but also the sorbent characteristics as well as the composition of the aqueous medium. Combined results from all experiments performed under varying conditions allow to disentangle those complex mechanisms to pinpoint key structural moieties dominating natural toxin mobility. Insights gained for natural toxins as model compounds for multifunctional, ionizable organics are of great value for understanding environmental transport and fate processes of this highly important class of compounds. As such, data will allow reliable exposure/risk assessment including the determination of the individual contribution of different geosorbents to the overall mobility of natural toxins on their path from source to tap. In addition, data provides the basis for the development of better in silico models for multifunctional, ionizable compounds

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S35

Fate of Quinolizidine Alkaloids from blue lupin (Lupinus angustifolius L.) - an Agricultural Field Test

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Lupin belongs to the Fabaceae family, which includes more than 450 species. The annual world production of lupin seeds exceeds one million tons. Lupin is used for producing functional foods and animal feed, but is also cultivated for soil improvement because of their ability to fix nitrogen. However, lupin contains toxic secondary metabolites namely quinolizidine alkaloids (QA), in all plant organs, being highest in the seeds. QAs are used as part of the plants biological defence mechanism against pathogens and herbivores. In Europe, mainly four lupin species are cultivated and Lupinus angustifolius (blue lupin) is one of them. In blue lupin, the main QAs reported are lupanine, sparteine and 13α-hydroxylupanine, with minor amounts of lupinine, angustifoline and gramine. QAs showed moderate to acute oral toxicity due to neurological effects leading to the loss of motor coordination and muscular control. The intake of total QA are regulated by health authorities in some countries. The environmental fate of QAs is largely unknown. Therefore, a field experiment (Agroscope Reckenholz-Tänikon, Research Station ART, Zürich, Switzerland) was conducted on lupin, to quantify i) the production of QAs in the plant, ii) the amount of QAs reaching the soil surface and iii) transported in the soil. The variables that were monitored during the period of study were QAs concentration in lupin, QAs concentrations in the soil at 50 cm depth and in the drain water. In addition, drainage water from the field was monitored during rain and irrigation events. Modelling of the daily dynamics of QAs in plant and water is performed using the agro-ecological model DAISY. The model was recently modified to account for the processes of toxin release from the plant canopy and describe the transport of QAs in the soil. The experimental results show that total QAs concentration increased gradually with a maximum concentration of 40 mg/kg dw at the harvest season. The mass of QAs in soil during harvest season (August) corresponded to 0.01% of what was present in the plant (g QAs g DW.1). Drainage flow-weighted event concentration of QAs in the drain water ranged from 0.05 to 2.2 ng/L. The data improved our understanding of QA dynamics in an agricultural field, proving that QAs in lupin can be released in pulses to from the plant and detected in the drainage water.

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S36

Photochemical transformation of emerging cyanobacterial peptides in surface waters

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Intensified cyanobacterial bloom events across the globe promoted by eutrophication and likely climate change are of increasing concern because of the adverse effects associated with the release of bioactive compounds, including cyanopeptides. Numerous studies have been conducted in the past decade regarding the occurrence, fate processes, and toxicity of microcystins - one cyanopeptide class that has been classified as hepatotoxic. However, to date, more than 1000 cyanopeptides from other classes including anabaenopeptins, cyanopeptolins, microginins, aeruginosins and cyclamides have been structurally identified. While it is known that a range of cyanopeptides are produced simultaneously by bloom-forming cyanobacteria, we lack essential information about exposure concentrations and toxicity for these emerging cyanopeptides. Here we focused on the exposure side of the risk equation for emerging cyanopeptide from common bloom forming cyanobacteria. We determined their direct and indirect photochemical transformation in sunlit surface waters and the effect of pH. As most cyanopeptides are not commercially available, we harvested them from laboratory cultures of Microcystis aeruginosa, Dolichospermum flos-aquae, and Planktothrix rubescens. We analyzed biomass extracts by suspect screening with LC-HRMS/MS and a vigorous data analysis workflow considering more than 700 structurally known cyanopeptides including cyclamides, cyanopeptolins, anabaenopeptins, microginins, aeruginosins and microcystins. We established cyanopeptide profiles for each species and inspected the photochemical fate of 39 cyanopeptides during exposure to simulated sunlight. In lake water matrix, two third of all compounds showed detectable decay but the photochemical loss rates ranged from 4 to >10 hours. Overall, a 3-hour exposure in sunlit surface waters reduced total cyanopeptide concentrations by 6-30% depending on the cyanopeptides from different species. Microcystis produced predominantly photostable microcystins, cyclamides and cyanopeptolins, while Dolichospermum and Planktothrix produced anabaenopeptins that showed significant degradation. For all 14 phenol-containing cyanopeptides including several anabaenopeptins, we observed an increase of the phototransformation rate constants with increasing pH. The absorbance spectrum of phenolates has a greater overlap with the sunlight spectrum compared the protonated form and is known to be more reactive towards photochemically derived singlet oxygen. We currently investigate the reaction mechanisms of selected cyanopeptides to provide system-independent reaction rate constants with photochemically produced reactive oxygen species such as singlet oxygen. These constants can later be applied to other exposure scenarios where the oxidant concentrations are known. Our results are among the first to quantify the environmental fate processes of these emerging cyanopeptides. Knowing which cyanopeptides are persistent in surface waters will also indicate which compounds can reach drinking water treatment plants and thus need further attention.

EAWAG

06

Poster Abstracts

P01

Does host microbiome play role in adverse outcomes of cyanotoxins?

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Harmful algal blooms are a global public health concern for drinking water quality. Over the past several years, primary attention has been focused on hepatotoxic and hepatocarcinogenic effects of cyanobacterial toxins, especially microcystins. However, other major clinically - relevant pathologies associated with cyanobacterial blooms include inflammatory gastrointestinal (GI) diseases (gastroenteritis), hemorrhage, nausea, vomiting, diarrhea, and abdominal pain. Episodes of severe human poisonings have been recorded after short-term exposure but the effects of repeated and chronic exposure to low levels of cyanotoxins on the GI tract remains a critical, largely unexplored issue. Despite wide recognition by public health professionals and government agencies that cyanotoxins pose a significant health concern, convincing data that identifies the specific mechanisms underlying gastroenteritis with cyanotoxin exposure is lacking. Little attention is given to cyanotoxins and the gastrointestinal system, and no study takes a comprehensive approach to link cyanotoxins to gut microbiome dysbiosis, a relevant target for dietary and waterborne exposures. Our scientific premise is that exposure to cyanotoxins induces gut dysbiosis by affecting the microbiome directly, in addition to negatively affecting host GI and immune responses. We will present the review of the accessible literature connecting cases of cyanobacterial poisonings with human gut distress. We will also present the state of the art of the effects of cyanobacterial toxins on gut microbiome and potential impact on human health. Scientifically, our knowledge of the contribution of cyanotoxins to gastroenteritis is limited, and our review, which is a part of a bachelor thesis of M.S., is poised to uncover the mechanisms linking link the exposure and gut distress. From a public health perspective, gastroenteritis associated with cyanobacteria may be major contributor to malnutrition and lesser quality of life in regions afflicted with contaminated water and our work aim to map this problem and propose research that is expected to bridge the knowledge gaps.

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P02

Elucidating mechanisms of cyanotoxin-induced immunotoxicity

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Mass occurrences of cyanobacteria are frequently reported in numerous freshwater lakes, that serve as direct sources of drinking water. There are many incidents of chronic exposure to cyanobacterial toxins, which have been attributed to inappropriate treatment of water supplies or contaminated food. However, aside from classic toxic effects including hepatotoxicity and tumor promotion, cyanotoxins are also known to modulate the immune system. Several in vitro and in vivo studies, indicate that cyanotoxins may alter proliferation and function of various immune cells, but many questions remain as to the true effects of these toxins on immunity. Here we will present the outcomes of our long-term research project that has been investigating the effects of a broad range of cyanobacterial peptides and alkaloid (e.g. microcystins, aeruginosin and cylindrospermopsin) on cells regulating innate immunity, specifically macrophages that represent key effector cells for human health. Our results indicate that specific cyanobacterial peptides may have significant immunomodulatory activities at very low, environmentally relevant concentrations. Importantly, this project has defined novel insights into the mechanisms and processes behind potential effects of cyanotoxins on immunity. However, the role of the surface receptors that trigger observed activation of immune cells is still questionable and warrants further investigation.

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P03

Commercial coated stir bar usage for microcystin bioavailability and bioaccessibility assays

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Microcystins (MC) pose threat to humans that have been in contact with it or consume contaminated products. To decrease health risks to the public, fast and efficient method for the sampling and monitoring needs to be developed.Considering its MCs heptapeptide chemical structure there are hydrophilic [4], hydrophobic [2], water replacement molecule and covalent bonding parts, which can be used for adsorption on specifically designed Gerstels ethylene glycol/polydimethylsiloxane (EG/PDMS) Twister/Stir Bar.EG/PDMS copolymer based stir bar sorptive extraction (SBSE) can be developed for bioavailability – non-depletable and bioaccessibility – depletable mode for aquatoxicological assays. Standardized theoretical concentration (500 µM) of selected MCs in aqueous solution 0.01 M NaN3/CaCl2 (S) can be used. For bioavailability mode 500 ml of S in beaker and 10 ml of S for bioaccesibility mode in glass centrifuge tube on shaker at 100 rpm. Experimental testing solution concentration should be measured before and after assay by taking 1 ml into the glass vial.Kinetic assay should be done at least with 8 time points (1- 64h). Theoretical calculation show for bioavailability and for bioaccessibility low losses in test medium with PDMS. Expected higher loss in this testing should occur for EG/PDMS as confirmation of our hypothesis. Stir bar could be taken for desorption in glass insert socketed in glass vial with 0,2 ml of AcN under sonication for 30 min and 25 °C 3x for complete desorption from stir bar. Before sending extract to HPLC analysis needs to be diluted 50x in order to fit calibration curve. Partition coefficients (P) needs to be calculated at every time point for EG/PDMS SBSE for MCs.Pseudo-first order, pseudo-second, Elovich, and partical diffusion kinetic models are expected to suggest chemisorption over physisorption which governs adsorption process. This will highlight hydrogen (H) bonding from MCs hydrophylic functional groups on EG part and hydrophobic interactions on PDMS part of EG/PDMS copolymer. For future studies pesticide functional groups for H-bonding, acceptor and donor separately, should be counted for future modeling and analysis.

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P04

Effects of the cyanotoxin microcystin-LR in human bronchial epithelial cells in vitro

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Changes in ecological and environmental factors lead to an increased occurrence of cyanobacterial water blooms. Many cyanobacterial species produce toxic secondary metabolites, known as cyanobacterial toxins or cyanotoxins, that pose a threat to both environmental and human health. The toxicity of cyanotoxins has been linked primarily to oral exposure scenarios. Recently, health risks of cyanotoxins are being re-evaluated considering their toxic potential to humans upon inhalation, as the lung may be an important recipient of direct toxic effects and an entry portal to systemic exposure. In fact, pneumonia-like and asthmatic symptoms have been linked to cyanobacterial blooms. We investigated the effects of a prevalent and environmentally abundant cyanotoxin microcystin-LR (MC-LR) using two different respiratory system-relevant human bronchial epithelial (HBE) cell lines. We observed both expression of several specific organic-anion-transporting polypeptides, potentially involved in MC-LR cellular uptake, and formation and accumulation of MC-LR protein adducts in exposed HBE cells. However, exposure of cells to MC-LR up to 20 µM caused no significant cytotoxic effects according to multiple viability endpoints and no disruption of cell layer integrity assessed by cell impendance measurement. Indeed, the time-dependent increase of putative MC-LR adducts with protein phosphatases was not associated with an activation of mitogen-activated protein kinases ERK1/2 and p38 in HBE cells. With respect to the evidence on the toxin uptake and its interactions with intracellular proteins of human bronchial epithelial cells, the effects of MCs in human airway epithelium should be further investigated using physiologically more relevant in vitro models and exposure scenarios.

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P05

Effects of the Cyanobacterial Strain Nostoc Z1 on Gene Expression in Daphnia Magna

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Cyanobacteria have, in recent years, received a lot of attention due to their ecological impact, and the ability of certain strains to produce highly active toxic compounds known as cyanotoxins, which have the potential to cause adverse effects in animals and humans. Chronic effects in humans are especially concerning due to the possibility of accidental ingestion of low concentrations of cyanotoxins in drinking water over a long period of time. Hence, a method for early detection of the incurred deleterious effects would be of great importance and can potentially be achieved by observing changes in the expression levels of responsive genes. The aim of this study was to analyze the effects of an intracellular cyanobacterial extract of Nostoc Z1 on Daphnia magna, a commonly used freshwater model organism, by examining the changes in the expression of five genes (cytochrome P450 – 314 family (cyp314), and from the 360 family (cyp360A8), glutathione S-transferase gene (gst), p-glycoprotein gene (p-gp) and vitellogenin (vtg)). Housekeeping gene β-actin was used as an endogenous reference gene, in order to normalize gene expression data across samples. After exposing D. magna neonates to three extract concentrations, relative changes in the expression of the selected genes were quantified using the real-time polymerase chain reaction (RT-qPCR) method. Results have shown a gradual change in the relative expression of most of the analyzed genes in a dose dependent manner. Significant changes were detected when examining the pgp, gst and vtg genes, particularly after exposing D. magna to the highest extract concentration, which led to a significant up regulation in the expression of these genes. The increase in the expression levels of p-gp and gst genes suggests that the detoxification pathways have been activated in response to the presence of a toxic substance in the organism, while the enhancement in the expression of vitellogenin indicated the presence of estrogenic compounds in the tested extract, which act as endocrine disruptors. Genes belonging to the cytochrome P450 superfamily were activated, however, their expression was not significantly different from the levels observed in the control group. These findings confirm that the selected cyanobacterial strain is potentially genotoxic to Daphnia magna. They also provide a basis for future investigation of specific genetic markers responsive to cyanobacterial toxicity.

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P06

Occurrence and stability of the Illudane glycosides ptesculentoside, caudatoside and ptaquiloside from Bracken ferns (genus Pteridium) in surface waters

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Bracken is a group of carcinogenic ferns comprising the fifth most abundant plant genus in the world, Pteridium. WHO has evaluated the evidence of bracken carcinogenicity in experimental animals as sufficient, and classifies the plant as possibly carcinogenic to humans. Proximity to bracken populations is widely reported observed risk factor for human gastrointestinal and oesophageal cancers. The carcinogenicity of the genus is associated with its highly reactive compound illudane glycoside ptaquiloside (PTA). Other studies demonstrate that the carcinogenicity is amplified by other bracken constituents structurally nearly identical to PTA: ptesculentoside (PTE) and caudatoside (CAU). Several earlier studies detected PTA and its hydrolysis product pterosin B in water. However, PTE and CAU are more water soluble than PTA. This study examined and detected PTE, CAU and their hydrolysis products pterosins G and A in surface water bodies (streams and lakes) for the first time. In addition, evaluation and comparison of the stability of the 3 glycosides in natural waters was performed. Four factors best representing natural conditions were investigated in a full factorial experimental design over a period of 3 weeks (water origin, pH, temperature and microbiological activity). The temperature and pH were identified as the most important factors affecting the compound degradation rates. The results demonstrated that the 3 glycosides degrade in a very similar rates which identifies their similar reactivity. The compounds were largely stable under all combinations of the studied natural conditions for at least 24 hours. This knowledge is important for representative sampling of waters after precipitation-driven leaching events.

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P07

Human 3D hepatic spheroids for in vitro assessment of hepatotoxic and steatogenic cell events in response to cylindrospermopsin and microcystin-LR

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Anthropogenic eutrophication of freshwater bodies and global warming increases the occurrence of toxic cyanobacterial proliferation or also called water blooms. Cyanobacteria produce potent toxins (cyanotoxins), which are diverse in their toxic effects and chemical structures. The hepatotoxic cylindrospermopsin (CYN) and microcystins (MC) are well-recognized hepatotoxic cyanotoxins. CYN is considered to be an emergent freshwater contaminant which is detected in the environment more frequently, which might be linked to the global change. MCs represent the most prevalent group of hazardous cyanotoxins in the environment, with structural variant MC-LR being the most frequently reported. CYN and MC-LR have been implicated in acute hepatotoxicity and chronic liver diseases in humans exposed to cyanobacteria contaminated water. Different approaches can be used to further investigate hepatotoxicity of CYN and MC-LR and particularly their ability to contribute to the development of nonalcoholic fatty liver disease (NAFLD), steatohepatitis, and eventually into irreversible liver diseases (fibrosis, cirrhosis and hepatocellular carcinoma). In this study, we optimized 3-dimensional (3D) in vitro model based on human hepatocarcinoma cell line HepG2 and cultures of human telomerase-immortalized adult liver stem cells HL1-hT1 to evaluate hepatotoxic potential and steatogenic activity of CYN and MC-LR. Hepatic spheroid cultures were adapted for 96-well microplate format and multiparametric evaluation of spheroid growth, morphology, viability/metabolic activity, membrane integrity and ATP content. Mature HepG2-derived spheroids showed increased expression of key hepatospecific genes, including drug-transporting proteins OATP1B1 and 1B3, which are responsible for cellular uptake of MC-LR, or drug-metabolizing enzymes (CYP1A2) possibly involved in CYN metabolization. Hepatic spheroid cultures of HepG2 and HL1-hT1 cell lines showed increased sensitivity to cytotoxic effects of CYN and MC-LR in contrast to monolayer cultures. These assays were complemented by assessment of cellular and molecular processes involved in development of liver steatosis and steatohepatitis, such as lipid accumulation and alteration of selected genes involved in lipid and fatty acid metabolism. This study demonstrates that such 3D hepatic spheroid model can be used for in vitro assessment of critical molecular and cellular events for development of chronic liver diseases such as hepatic steatosis and steatohepatitis, in response to hepatotoxic cyanotoxins or other environmental toxicants.

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P08

A naturally occurring carcinogen in Pteridium aquilinum: II) A field monitoring study

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Bracken fern (Pteridium aquilinum) is a cosmopolitan plant species which is present in wide array of environmental conditions and it is very adaptative and highly invasive. Ptaquiloside (PTA), a toxin from the terpene family, is a main toxic secondary metabolite produced by bracken. PTA has been identified as a biological hazard due to its correlation with different chronic diseases in animals and humans, including the promotion of cancer in humans. Previous research have detected PTA in different media such as plants, litter, soil, groundwater and surface water. The aim of this study has been to investigate the PTA dynamics in different environmental compartments at the pedon-scale, with special focus on release of PTA from the canopy of bracken fern to soil and soil solution. We conducted a 2-years field monitoring program with the objective to determine PTA concentrations in plant, canopy wash-off water and soil water. Soil water at 50 cm depth was monitored for an entire year, covering a growing season and the following winter period after senescence. The water content in the first 100 cm depth of the soil profile was monitored as well as different environmental variables such as temperature, relative humidity and precipitation. Results showed that PTA mass is up to 2 g m-2 in the bracken canopy and that it can be effectively washed by precipitation in high amounts. Ptaquiloside concentrations in the washed off water from the canopy reaching the surface after two rain events ranged from 16 -2,080 µg l-1. This process is highly influenced by leaf area index (LAI) and mass of PTA in the plant at the moment of the precipitation event. Estimations of the mass of PTA washed off by a single rain event ranged from 1 to 37 mg m-2¬, with an average deposition in the soil of 9.4 mg m-2. The litter has been found to be another possible source of PTA, with concentrations in the leachate ranging from 11 to 76 µg l-1. Soil water PTA concentrations are highly dependent on precipitation, with measured peak PTA concentrations up to 127 µg l-1. PTA in the soil solution (50 cm depth) seems to present a baseline concentration of 5 to 20 µg l-1 even few months after senescence, suggesting the existence of multiple and continuous sources in the soil. The results demonstrate that PTA might leach in relatively high concentrations to deeper soil layers with low microbiological activity, where previous research has found PTA to be relatively stable. This study is the first longer term monitoring work for bracken demonstrating that PTA can be present in soil water, in concentrations comparable to those found for industrial pollutants, such as pesticides.

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P09

Leaching of Pyrrolizidine Alkaloids from Ragwort (Senecio jacobaea L.) Plant to Corresponding Soil and Water

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Pyrrolizidine alkaloids (PAs) are secondary plant metabolites produced by plants. Plants use PAs mostly as a defence mechanism against predators. PAs present a serious health risk to human and livestock. Although these compounds have been extensively studied in food and feed, little is known regarding their environmental fate. To fill this research gap, PAs monitored in ragwort (Senecio jacobaea L.), soil and water from three Danish field sites from December 2017 to December 2018, with PAs detected throughout the entire sampling period. PA concentrations investigated in a Ragwort-impacted catchment during the growing season in response to rain events. Out of thirty only nineteen PAs were quantified in the soil and water, the total PAs concentrations in a range of 0.8 to 4 mg/kg dw in soil and 0 to 529 µg/L in surface water, this may indicate their stability in the environment. The highest PAs loads were substantially lower into water (around 5x10-3 kg/ha), compared to the average amounts present in ragwort (506 kg/ha), and soil (1.7 kg/ha). A clear temporal connection was observed between PAs concentration in the soil and corresponding rain events. This study documents that PAs are transported from ragwort plants to the surrounding soil with potential transport to proximal surface water. Leaching of PAs was positively correlated with the ragwort growth stage and amount of precipitation.

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P10

Are Natural Alkaloids Transported from Soybeans to Streams?

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Soybean [Glycine max (L.) Merr.] plant is an important economic crop globally. Soybeans are rich in protein, ω-3 fatty acids, carbohydrates, fiber, and many other micronutrients. Natural toxins such as isoflavones, saponins and alkaloids have been documented in soybean plants, but little research has been conducted regarding their potential prevalence in the environment. This study provides the first ever assessment to determine if soybean-derived alkaloids are transported from agricultural lands to aqueous ecosystems. The United States is the leading producer and exporter of soybeans globally with Illinois and Iowa by far being the states with the greatest production. Roughly 3.4 million ha of soybeans are produced in Iowa annually, Thus, Iowa was an ideal location to test the hypothesis whether soybean-derived alkaloids are transported off-field to corresponding stream systems. To understand the distribution of alkaloids in agricultural-impacted rivers and streams, sampling sites with varying basin sizes were selected in areas of substantial soybean production. Samples consisted of 1-L grab samples that were immediately frozen until sample processing could take place. Samples were collected from September 15, 2019 (pre-harvest) to February 20, 2020 (four months after harvest). Four streams (West Branch Wapsinonoc Creek, Muddy Creek, Clear Creek, and Old Mans Creek) were sampled using a hydrologic-based approach. Muddy Creek represents an effluent-dominated stream basin minimally impacted by agriculture. These streams were sampled both during storm events at or near peak flow and during base flow conditions. Two additional sites were sampled on a fixed schedule. The Mississippi River was sampled monthly from October to February and the Iowa River was sampled every other month from October to February on a fixed schedule. All samples for this study are being analysed for six indole alkaloids, eight phytoestrogens and two common herbicides (atrazine and metolachlor). The samples are analysed by a previously validated method that enables determination of all targeted compounds with low limits of detection (4-7 ng/L).

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P11

Cylindrospermopsin exposure causes attenuation of cell proliferation and elongates the metaphase in mitotic human airway epithelial cells

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Cylindrospermopsin (CYN) is produced by cyanobacteria (esp. Cylindrospermopsis raciborskii) and accumulates in the water during algal blooms. It may get airborne as a component of aerosols and inhaled by humans during recreational or professional activities at lakes or estuaries (Backer et al. 2010; Wood & Dietrich 2011). CYN has been characterized as a potent cytotoxin in eukaryotic cells mediating genotoxicity (Straser et al. 2013), inhibition of protein synthesis or induction of oxidative stress (Pichardo et al. 2017), degradation of cytoskeletal components (Gacsi et al. 2009) or irregularities in kinetochor function during mitosis resulting in errors in chromosome distribution in the daughter cells (Humpage et al. 2000). In human airway epithelial cells, CYN disrupts normal signal transduction and compromises cell survival as well as the integrity of the epithelial barrier (Kubickova et al. 2019). Using immortalized human airway epithelial cells and time lapse microscopy (Nikon Biostation II) we set out to study the cellular effects of CYN focussing on proliferation rate and the duration of the visible phase of mitosis, the metaphase. We generated time lapse movies (20 images per hour for 24 h) of confluent cultures of 16HBE14o- cells in the absence or presence of CYN (0.5-7.5 µmol/l in 20 % methanol; maximum methanol concentration in the culture medium was 0.1 %). The results of image analyses of stills and analyses of movie sequences of these time lapse movies indicated that cell proliferation rates in CYN-exposed 16HBE14o- cells decreased in a time- and concentration-dependent manner. CYN-concentrations > 1.5 µmol/l significantly reduced cell proliferation rate starting at 8 h of exposure and thereafter. Starting at the same time and at the same concentrations of CYN, we observed an elongation of the metaphase of the mitosis in proliferating cells as judged by measuring the time from chromosome condensation and the starting point of sister chromatid separation at the onset of anaphase. These observations indicate that CYN or one of its metabolites in these cells disrupts the normal function of the spindle apparatus. We are currently investigating the molecular mechanisms underlying these cellular effects.

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P12

Open access knowledge of cyanobacterial metabolites for suspect screening by LC-HRMS/MS

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Over the past decades, a legion of cyanobacterial metabolites has been identified. However, we lack essential information about concentrations in surface waters and environmental behaviour of most compounds. The question arises, whether these compounds are of human and ecological concern regarding their abundance, persistence and toxicity. The key challenge presents their large structural diversity, lack of reference standards, and thus the high analytical requirements for identification and quantification before further testing becomes feasible. Liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) is the state-of-the-art analytical method for cyanopeptides. Typically, identification by LC-HRMS/MS relies on compound specific information of reference materials that are available in databases and libraries. This information is available for micropollutants and allows to cover a large chemical space for each analyzed sample. Such information does not publicly exist for 98% of known cyanopeptides. In 2019, we initiated a collaboration of international experts in the cyanobacteria research community to create one comprehensive and publicly available database of cyanobacterial metabolites. We have established a comprehensive suspect list of cyanopeptides by merging, expanding, and validating existing private and public data by merging and augmenting our and other publicly available information. The list currently contains complete structural and literature information for more than 1400 entries including aeruginosins, microginins, anabaenopeptins, cyanopeptolins, microviridins, cyclamides, microcystins and other cyanobacterial metabolites. We are currently making the list publicly available to encourage more researcher to perform suspect screening of cyanopeptides and are working on including MS spectral data as the next step.

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P13

Toxin production by cyanobacteria in altitude gradient in small water bodies of Pamir mountain desert

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Production of toxins and other secondary metabolites are of high importance, especially when ubiquitous occurrence of cyanobacteria is considered. Ever-new cyanotoxins are being discovered, with planktonic cyanobacteria being the most studied communities, although records of cyanotoxins from benthic communities are becoming increasingly common. Still not much is known about cyanobacteria and cyanotoxins in aridic and high altitude environments, especially Central Asia. Our recent study of microbial mats in small water bodies in high mountains' deserts of Eastern Pamir (Tajikistan) suggested that toxin production by cyanobacteria in these stressful environments is very limited.

Here we present further study of cyanobacteria, cyanotoxins and other biologically active compounds in microbial mats and water above them, collected from Eastern Pamirs' deserts water bodies in 2019. We investigated ten sampling sites in altitude gradient from about 1000 to 4000 m a.s.l., using microscopic as well as genetic identification based on NGS of V3-V4 16S rRNA amplicon and toxin assays using LC-MS/MS. The analyses demonstrated that studied microbial mats contained potentially toxic cyanobacteria (Anabaena, Lyngbya, Nostoc, Oscillatoria and Phormidium), but the production of toxins and other secondary metabolites in natural environments was limited, with no clear correlation with altitude. In only one microbial mat from 2000 m a.s.l. debromoaplysiatoxin was discovered, while three samples of water taken from above the mats (at 1000, 2000 and 3000 m. a.s.l.) contained 2-Methylisoborneol (MIB) and/or Geosmin.

We also tested strains isolated from microbial mats coming from similar locations, which we studied in previous years. Two strains originating from mats, in which no cyanotoxins or other secondary metabolites were identified, produced debromoaplysiatoxin and microginin in laboratory conditions. The first compound was produced by not yet phylogenetically identified Leptolyngbya sp. and the second by Nostoc paludosum. The study shows that despite the presence of potentially toxigenic cyanobacteria, the production of toxins and other secondary metabolites in small water bodies in Pamir mountain desert was limited, with no clear correlation with altitude. Interestingly, strains obtained from microbial mats in which no toxins or other secondary metabolites were identified, produced such substances in the laboratory. Further studies are needed to determine whether, and to what extent, altitude or other factors limit toxin production in this stressful environment.

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P14

Automated In-Situ Cyanotoxin Assessment Toolbox For Real-Time Surface Water Monitoring

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Cyanobacteria (green-blue algae) are phototrophic microorganisms and an essential component of the food web in all aquatic ecosystems. However, eutrophication of surface waters can lead to the formation of cyanobacteria harmful algal blooms (cyano-HABs) that directly affect water quality by producing undesirable colour, taste, odour, and by releasing harmful cyanotoxins into the water [1]. Besides the tendency to form mats during their blooming phase, the toxic strains of cyanobacteria produce an array of compounds that differ in both structure and mode of action. Lethality of cyanotoxins is higher than most of the compounds currently regulated in the Drinking Water Directive of EU as it can be harmful to plants, invertebrates and vertebrates (including humans) at naturally occurring concentrations [2]. Cyanotoxins assessment toolbox - CYANOBOX, is a 2-year project, coordinated by innovation and technology company CyRIC, with interdisciplinary research activities that combine surface water monitoring, electrical and mechanical engineering, material science, remote monitoring, development of novel biosensors, and reliable at-source detection of toxic metabolites. The project aim is to deliver an autonomous, affordable, and easy to operate water monitoring system as an early-warning tool for surface waters affected by cyano-HABs. Along with CyRIC, we are collaborating in building a unique water processing system that can remotely filter and lyse the cyanobacterial cells in-situ so that an accurate measurement is taken for both extracellular and intracellular concentration of one of the most important groups of toxic metabolites, the microcystins [3]. Part of the research is the development of novel biosensors for the detection of the heptapetide microcystin-LR, which is the most detected in surface waters variant of the group [4]. Our focus is on optimizing the method to lyse the cells in a way that maximizes the recovery of MC-LR, followed by the development of biosensors for identification and quantification of cyanotoxins. Being able to detect accurately and remotely the concentration of cyanotoxins in the water without the physical presence of humans, it will be beneficial to water bodies, administrators, local communities and researchers. This system will remotely evaluate the severity of a bloom based on its toxicity and track changes in water that traditional discrete monitoring activities usually miss. We expect that communities that apply this system, will have an early warning system and will achieve reduction in monitoring costs as well as waterbody restoration costs.

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P15

Cyanobacterial water blooms dominated by species from different orders produce teratogenic retinoid-like compounds into surface waters

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Cyanobacteria are common inhabitants of surface waters. However, massive cyanobacterial water blooms connected with eutrofization and increasing temperature represent worldwide problem, also due to their ability to produce significant amount of various bioactive compounds, some of which are toxic and may adversely affect animal and human health. Our research has documented that various cyanobacterial species can produce compounds with retinoid-like activity that could adversely affect development and cause teratogenic effects. We have sampled water bloom biomass and surrounding water affected by cyanobacterial blooms with different taxonomy composition and various dominant species from different freshwater ponds and reservoirs. The study documents that retinoids are commonly produced by environmental cyanobacterial blooms dominated by species belonging to various widespread genera such as Microcystis, Dolichospermum, Planktothrix, Woronichinia, Pseudanabaena and others from different cyanobacterial orders. We determined their retinoid-like activity by in vitro bioassays focused on the activation of retinoic acid receptor, which plays an important role in early development. The retinoic acid receptor-mediated response was tested on the reporter-gene mammalian cell lines with reporter gene under the control of retinoic acid responsive element. We also determined the concentrations of main identified retinoid compounds (such as all-trans retinoic acid (ATRA), 9/13cis retinoic acid (RA), 5,6epoxy-RA, 4keto-ATRA, 4keto-retinal and retinal) and cyanobacterial toxins (such as microcystins, anatoxin and cylindrospermopsin) by LC-MS/MS analyses. Retinoids were detected in most field biomass and water samples affected by cyanobacterial bloom. 4-keto all trans retinoic acid and retinal were the most common forms detected in the samples. This study provides environmentally important information about the common presence of mixtures of retinoids in various water bodies associated with the occurrence of cyanobacterial blooms dominated by many different species.

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P16

Cyanobacterial toxins and the intestines – What are we missing?

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Gastrointestinal disturbances (e.g. nausea, vomiting, abdominal pain, diarrhea) are one of the most frequently reported symptoms upon human exposure to cyanobacterial blooms. The most frequent exposure route to water-borne cyanobacteria is via accidental consumption of contaminated water, either intended (insufficiently treated drinking water) or accidentally (recreation). Subsequently, cells of the gastrointestinal tract (GIT) are the first to come into contact with cyanobacterial water bloom components, and the epithelial lining of the GIT is the first barrier to be overcome by cyanobacterial toxins and metabolites to reach and affect internal organs. Despite the relatively high frequency of adverse GIT effects in response to cyanobacterial bloom exposures, the role of cyanobacterial toxins or other water bloom components, such as (cyano-)bacterial lipopolysaccharides, are not fully characterized yet. Our systematic literature review revealed significant data gaps in the understanding of metabolites breaching the gastrointestinal barrier and the role of the immune system in the establishment of clinical symptoms. Furthermore, microcystins and cylindrospermopsin were specifically linked to gastrointestinal symptoms, immune system effects, or both. Moreover, cyanobacterial bloom lipopolysaccharides, other less studied metabolites and their mixtures have been also implicated to have a role in gastrointestinal inflammation. Despite the lack of available data on less studied cyanobacterial bloom metabolites, the adverse effects of microcystins on GIT tissues are likely to be magnified by co-action with other virulence factors inherent to the complex mixture of cyanobacterial blooms, for example, the inflammation mediating LPS. This may possibly lead to enterocolitis rather than liver failure upon exposure. In addition to acute GIT symptoms, there is evidence that frequent and prolonged exposure of the GIT to low levels of potentially carcinogenic and tumor-promoting cyanobacterial toxins (e.g. microcystins) may contribute to colorectal cancer incidence. In conclusion, the intestines as the first barrier and the entry of cyanotoxins into the organism need to be considered as recipients of cyanobacterial toxicity. Furthermore, other, often highly abundant bioactive metabolites such as aeruginosins, have to be toxicologically evaluated at distinct levels also accounting for (sub-)chronic exposure to low concentrations and in combination with naturally co-occurring metabolites, which can be expected in drinking water supplies.

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P17

Effects of the cyanobacterial toxin cylindrospermosin on human airway epithelial cells in vitro

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Occurrence of cyanobacterial blooms is fueled by climate change and anthropogenic eutrophication of freshwater ecosystems. Recently, cylindrospermopsin (CYN), originally reported from (sub-)tropical regions, is being detected in temperate climates with increasing frequency. This increases the concern of its potential human health hazard and drives scientific effort to investigate health risks linked to CYN-producing blooms. The primary exposure route for humans to CYN is oral, leading to hepatotoxic effects. Nevertheless, extrahepatic manifestations of CYN toxicity have been reported and cyanobacterial blooms have been linked to pneumonia-like symptoms and other adverse respiratory conditions. Besides respiratory exposure via toxin-containing aerosols, detection of cyanotoxins in loess crust dust raises the question of a potential hazard of human exposure via inhalation. We investigated the susceptibility and vulnerability of human bronchial epithelial cells (HBE1 and 16HBE14o-) to 0.1-10 µM CYN in vitro. Cytotoxicity was evaluated morphologically, by real-time cell analysis and by three metabolic assays with EC50 values ranging between 0.7-1.8 µM (HBE1) and 1.6-4.8 µM (16HBE14o-). Subsequently, the sub-cytotoxic concentration of 1 µM CYN was tested for its impact on intracellular mitogen-activated protein kinase (MAPK) signaling by western blot detection of ERK1/2 and p38 phosphorylation levels. While only a slight increase in p38 phosphorylation was induced in HBE1 cells, ERK1/2 and p38 activation increased gradually and significantly (p38) in 16HBE14o- cells upon exposure for 8-48 h. This study implies possible hazards of cyanotoxin inhalation, which may have a severe impact on epithelial barrier integrity and airway inflammation, thus facilitating the manifestation of respiratory diseases.

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P18

Stressing cyanobacterial risk in drinking water supply by using eutrophic freshwater as source

authors

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In the last decades, the frequency and intensity of cyanobacterial blooms are of increasing concern. They have become a direct threat to the drinking water supply by clogging filters, bringing odour and unpleasant taste to the treated water and worst of all, causing elevated cyanotoxins which can be difficult to remove, yet lead to severe health issues. I would like to give a summary of my PhD dissertation on NaToxAq conference and exchange knowledge and tools for water managers and operators to understand cyanobacterial risk in their water so that bloom problems can be prevented or mitigated. My thesis proposes an adaptive Cyanobacteria Management Tool (CMT) in drinking water supply. It starts with an overview of this problem and a conceptual management tool design, i.e. Cyanobacteria Management Tool (CMT by which multi- indicators for actions are provided. Secondly, a picture of this problem in Swedish freshwater was studied both on a national and local scale, including their geographical distribution, species dynamics, seasonal pattern and their connection with eutrophication statues, land use and other factors. Thirdly, my study highlights nutrient's impact on cyanobacteria formation, including testing two hypothesises, 1) if Total Phosphorus (TP) can be used to predict cyanobacteria risk and 2) if the Dissolved Inorganic Nitrogen and Phosphorus ratio (DIN/TP) is a better indicator for cyanobacteria risk than TN/TP.

The results were also verified by a full-scale on-site experiment study of pre-treating eutrophic water at a local water treatment plant. Lastly, cyanotoxin detection challenges and strategies are presented. The key findings are summarized below:

- Max Cyanobacteria growth capacity in response of TP can be achieved by quantile regression and target TP limit for drinking water WHO alert level 1 is suggested < 15 µg L-1; and DIN/TP < 10 indicates cyanobacteria peaks.
- Most problematic lakes that experience intensive cyanobacterial blooms are in southern Sweden; and the lakes are eutrophic or hypereutrophic due to intensive land use
- Clear seasonal pattern for cyanobacteria biomass and dominating condition shows that attention should be paid to September to November regarding cyanobacteria risk
- Cyanotoxin screening tools such as immunoassay test kits or Lateral flow immunoassay (LFIA) are useful for indicating certain toxins; advanced analytical tools are required for toxin profiles confirmation. Findings from this thesis can be used to update a local based CMT and used as a workflow for water operators to improve their monitoring routines and develop their strategies and at the end, our work emphasizes again that nutrient control is the key to protect our drinking water from intensive cyanobacterial blooms.

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P19

Early warning system for on-line monitoring of water resources

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Water quality monitoring is very important for collecting date to have a better overview of water characteristics. Our newly developed "invisible sensor" serves as an early-warning system of water pollution occurrence. Based on our experiences was proved that the data from this system might be used not only for regular judgement of water quality, but even for real-time control of accidental emergency conditions and events. Sensor can monitor the dynamic of space and temporal biomass occurrence of cyanobacterial development, especially in early phase of water blooms.

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P20

First insight into the aetokthonotoxin's biosynthesis

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Avian vacuolar myelinopathy (AVM) constitutes a prominent example of therisk that cyanobacteria pose to aquatic environments. This fatal neurological disease causes severe motor impairment of bird individuals that havebeen exposed to Aetokthonos hydrillicola (a true-branching cyanobacterium) through the food chain or by direct feeding. Recently, a novel cyanobacterial secondary metabolite (aetokthonotoxin) has been found to be likely responsible for AVM. This toxin is a penta-brominated compound constituted by two indole moieties, whose presence in both natural A. hydrillicola colonies growing in the AVM seasonas well as in unusual cultures supplemented with bromide has been corroborated by mass spectrometry imaging (MSI). Considering the highly brominated nature of the compound, we screened for the toxin´s putative gene cluster through bioinformatic analysis of a newly sequenced draft genome. We identified a putative gene cluster (9.2 Kbp) composed of six predicted genes, five with an identifiable function (a tryptophanase, a cytochrome P450, a cyclase/dehydrase, and two halogenases) and a single hypothetical protein. To provide first insights into the aetokthonotoxin biosynthetic pathway, six Golden Gate plasmids containing these candidate genes were designed and propagated to competent E. coli cells. This allowed us to induce the production of the cluster's encoded enzymes and to characterize their activity through in-vitro enzymatic assays. Here we present our advances on the characterization of enzymatic activities of the proteins encoded in the aetokthonotoxin gene cluster as well as the status of the elucidation of its biosynthesis.

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P21

Effect of cyanobacterial retinoids on nuclear receptor activation and neural differentiation in vitro

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During the early stages of life, many differentiation processes occur that can be disrupted even by minor changes in metabolic regulation. Retinoic acid controls the differentiation and patterning of various stem and progenitor cell populations. Also, neural tube and neural system formation is dependent on retinoic acid signalling pathways. Retinoid-like compounds are produced by cyanobacteria in surface waters, and in high concentration, have embryotoxic, teratogenic and neurotoxic effects on fish, amphibians, and humans as well. Developmental neurotoxicity (DNT) is commonly tested on rodents; non-animal in vitro tests are still under development. In concordance with the European Union's Centre for Alternative Methods (ECVAM), a model relevant to human DNT was successfully implemented. The neural stem cell line (NSC H9) we use was exposed to non-toxic retinoid concentrations and monitored for their in vitro differentiation for 22 days. Additionally, relative potency of retinoids to activate retinoic acid and thyroid hormone signalling pathways were evaluated using mammalian cell-based reporter gene assays. Both, all-trans retinoic acid (atRA) and 9-cis retinoic acid (9cRA) showed high potency in activating the human retinoic acid receptor and human retinoid-X receptor (effective concentrations in nM range). Interestingly, high concentrations (≤300 nM) of both retinoids also indicate an antagonistic effect on thyroid hormone signalling. Acute toxicity concentrations for selected retinoids and consequently the effects of lower concentrations (8-1000 nM atRA and 9cRA) on neural differentiation were determined in NSC H9 cells. While the structural organization of the network is significantly impaired at higher concentrations of retinoic acids (≥200 nM), this is not reflected in the abundance of committed neurons, as assessed by TBR1 biomarker detection with qPCR. In addition, retinoic acid exposure appears to decrease the number of cells committed to the glial lineage (GFAP expression) in favour of neural maturation. The tested retinoids appear naturally in the environment due to water blooms. Besides atRA being the model ligand for retinoic acid receptors, our studies show a similar potency of other retinoids, like 9cRA, on this receptor. Additionally, the crosstalk between retinoid and thyroid hormone signalling, as indicated by the reporter gene assay, stresses the importance of retinoids during early development. The effects of cyanobacterial metabolites on human health and development are still unknown, but the dose-dependent alteration of network patterning may play a role in neurodegenerative diseases and learning disabilities. We are shedding light on this issue, also investigating binary mixtures of retinoids and cyanobacterial toxins in the future.

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P22

Detection of marine biotoxins in Bulgarian coastal waters in 2018-2019

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Recommendations about healthy diet include consumption of seafood because of its nutritional qualities. Bulgarian Black Sea mussel has been documented to contain beneficial values of polyunsaturated fatty acid, proteins, vitamins etc. Though the Black Sea is exposed to various discharges (rivers, municipal and industrial sources, agriculture etc.) which could cause its pollution. Recently, persistent organic pollutants and heavy metals have been registered in edible species collected from Bulgarian coast. Marine biotoxins are another concern regarding seafood safety. Yearly, producers of marine toxins (Pseudo-nitzschia, Alexandrium, Dinophysis) are registered in Bulgarian coastal waters throughout the national monitoring program. The aim of this study is to assess the levels of marine biotoxins in most consumed marine mollusks – Black Sea mussels and to evaluate the human exposure. In total 55 wild and cultivated mussel samples are collected in the period 2018-2019 from the north Black Sea coast respectively from recreational harvesting sites and farming areas. Hepatopancreas homogenate of each sample was subjected to methanol extraction. Domoic acid and lipophilic toxins were determined via LC-MS/MS and paralytic toxins via high-performance liquid chromatography with fluorescent detection followed by postcolumn derivatization. Domoic acid, pectenotoxin-2, yessotoxin and a novel for Bulgarian coastal waters marine biotoxin-spirolide-1 are detected in few mussel samples. Highest values are registered for yessotoxin- 12.4 ng/g and for domoic acid – 1.9 ng/g. Spirolide-1 is the most common marine biotoxin – it is detected in six samples. Paralytic toxins are not detected. Comparison of the toxin composition of mussel samples with toxin profile of plankton net samples collected in the same period shows that domoic acid, pectenotoxin-2 and sporolide-1 are determined in both mussels and plankton. Concentrations of detected marine biotoxins that are regulated in the EU are recalculated for whole mussel meat. A conversion factor 5 proposed by EFSA is applied. Safety assessment of investigated mussels is achieved by comparison of calculated levels with legislative limits, acute reference dose and tolerable daily intake. Results indicate that no adverse effects are expected if studied mussel samples were consumed.

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P23

Occurrence of natural toxins. An LC-HRMS/MS approach for their tentative identification in surface water.

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Aquatic environment can present an extreme variety of contaminants. Among pesticides and other anthropogenic pollutants another class of chemicals are being studied regarding natural compounds released by the environment. These compounds called natural toxins can be produced by different organisms, plants, bacteria and fungi. Harmful effects vary with different targets such as liver, skin, nerves and other diseases. The current World Health Organization's legislation only regulates the presence of microcystin LR in drinking water with a limit of 1 µg L-1, however most of the other toxins have not been included in any regulation protocol. Once natural toxins are released in water, hydrolysis, photolysis, oxidation, and microbial metabolism can potentially generate secondary transformation products that can be even more harmful and concentrated than their precursor. Liquid chromatography–high resolution mass spectrometry (LC–HRMS) plays a central role for their identification even when certified standards are unavailable. In this case the approach is also called suspect screening which is a growing trend to analyse environmental samples that present a wide range of compounds with lack of standards. Suspect approaches reported for environmental samples have been based on semi-quantitative analysis (using peak intensities) when the standards are not available. Anyway, this approach seems to be still poorly accepted by environmental scientists due of the challenge that represent the inability to provide quantitative information of the suspect compounds. In this work is presented an LC-HRMS/MS approach using Orbitrap that combines sensitive high resolution full-scan spectrum with data dependant (ddMS2) acquisition. To extract a wide range of natural toxins we used a triple-stage solid-phase extraction developed in our laboratory The method was validated with a mix of representative standards with recoveries in the range between 53 and 95%, and method limits of detection (LOD) between 0.02 and 1.22 µg/L. This approach was then applied to real samples from 4 sampling sites along the Ter River in Catalonia (NE Spain). 23 natural toxins were tentatively identified, and 9 of them confirmed with standards (aflatoxin B1, anatoxin-a, nodularin, microcystin-LR, baicalein, kojic acid, cinchonine, B-asarone and atropine). The results of the quantification of these compounds showed concentrations below 1 µg/L in all cases, that is considered safe according to the actual legislation. Tentative identification level 2 was achieved following the approach proposed by Schymansk et al., 2014 (Environ. Sci. Technol. 2014, 48, 4, 2097-2098).

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P24

Stability of the fern carcinogen ptaquiloside in water resources

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Ptaquiloside (PTA) is a prominent genotoxic illudane glycoside causing cancer and acute intoxications in farm animals. Brackens are used for food, and are under suspicion of causing human cancers. As such, bracken ferns are classified as Possibly carcinogenic to humans by WHO/IARC. Under natural conditions, PTA degrades to the stable pterosin B (PtB), which are used as indicator of previous presence of PTA. PTA and/or PtB are found in more than 20 fern species but with a possible distribution in thousands of ferns. In addition to PTA several other illudane glycosides exist, having potentially the same toxicity (e.g. caudatoside, ptesculentoside and ptaquiloside Z). Ptaquiloside contaminates the immediate surroundings of bracken populations. Ptaquiloside is readily released from the ferns, and contaminates soils and water recipients, such as surface waters, lakes and upper groundwater. Here, we present the stability of PTA under near-natural conditions in 10 different natural waters from Denmark (lakes and groundwater). Under sterile conditions, dissipation (= hydrolysis) of PTA in aqueous solution follows classical hydrolysis kinetics: kobs = kacid[H+] + kneutral + kalkaline·kwater[H+]. The rate constants obtained from lab experiments using buffered solutions are: kacid = 25.7±1.0 M-1 h-1; kneutral = 9.5±6.0·10-4 h-1 and kalkaline 4.8±0.0·104 M-1 h-1. The activation energy for PTA hydrolysis at pH 4.6 is approx. 75 kJ mol-1. Hence, environmental stability is a function of both pH and temperature. Degradation is rapid in natural non-sterilised biological active lake waters with half-lives from 5 to 25 h. All PTA disappeared within 200 h. However, sterile controls showed no degradation. During winter, slow degradation took place (half-lives up to 100 h). Degradation under sterile conditions could be explained by the existing model for hydrolysis as function of pH and temperature while microbial degradationactivity is significant in non-sterilised waters. PTA were more persistant in groundwater with half-lives from 7 to 50 days and with a good fit by abiotic hydrolysis in agreement with low microbial activity in groundwater. The existing model for hydrolysis did not perform well at high pH (approx. 7.5/8.0). PtB was formed as the end product by hydrolysis in the tested groundwaters while no PtB was observed in the surface waters reflecting fast degradation of PtB by aquatic microorganisms. Our results indicate that the risk of general contamination of surface waters is highest during winter where microbial activity is low. The risk of contamination of aquifers is expected to be highest at near-neutral conditions in aquifers where PTA can persist for months.

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P25

Modeling Koc of natural toxins

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Existing Quantitative Structure-Property Relationship (QSPR) models for prediction of organic carbon-water partition coefficients (Koc) are known to have limited applicability for multifunctional, ionizable organic compounds such as natural toxins. EPISuite's KOCWIN for example, cannot determine any phase preference for the majority of basic ionizable compounds, giving a constant Koc of 1. Using experimental data for neutral natural toxins to evaluate overall model performance, root mean square errors (RMSE) are also rather high (RMSE = 1.1), stressing the general need for better models for multifunctional compounds. Experimental Koc data was gathered for natural toxins and additional anthropogenic chemicals from literature resulting in a final set of 185 compounds. The set comprised 87 truly neutral and 98 ionizable chemicals, with predicted log Kow between -2 and 6. All data was obtained using the same experimental approach (column chromatography) and analysis conditions with regards to pH, eluent composition and temperature. The latter show particularly strong influences on the sorption of multifunctional, ionizable compounds. We aim to build a new model that can be used for predicting Koc values for most known natural toxins, and ultimately be implemented to predict partially or fully charged compounds as well. We first included compounds measured in neutral form and calculated molecular descriptors with AlogP and CDK library in R. Final descriptors were chosen after pairwise evaluation of correlated descriptors and finally the whole dataset split into training and test sets for model building. Different linear regression based models (stepwise linear regression and partial least squares) were tested giving performances of RMSE between 0.27 and 0.37. Assessment of the applicability domain of the model by similarity testing is further needed to ensure the reliability of the predictions for any given new compound.

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P26

Dynamic bioreactor 3D hepatospheroid cultures to study cyanotoxin-induced chronic hepatotoxicity and liver disease

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Among the in vitro models, the emergence of 3-dimensional (3D) models have proved to be quite advantageous when compared to 2-dimensional (2-D) monolayer cultures. The 3D liver models was seen to better mimic the in vivo-like tissue microenvironment and physiological cellular interactions including regaining of hepatocyte-specific characteristics and functions over longer periods, making them a better model in hepatotoxicity studies unlike 2D cultures which poorly recapitulates the major hepatocyte-specific functions. 3D liver models can range from various static cultures of simple hepatospheroids or stem cell-derived liver spheroids/organoids (scaffold-free,hydrogel scaffold-based or co-cultures with non-parenchymal cells) upto growing them in dynamic bioreactors. Dynamic bioreactor cultures due to their capability to provide adequate long term nutrient supply, platform for achieving higher cellular densities, long term survivability and stability, seem to play a vital role in 3D in vitro modelling, useful for different applications including toxicity studies. In this study, we used Rotary Cell Culture System (RCCS) based on simulated microgravity, thereby scaling up the experiments and facilitating long term studies. The RCCS bioreactors were found to offer very unique features such as lower shear forces and turbulence and higher mass transfers of nutrients and oxygen, which minimizes cellular damage and necrosis and can be effectively utilized, especially for growing simple to stem cell-based hepatospheroids/organoids of multiple cell types mimicking intact tissues and organs. Here, we used hTERT immortalized human adult liver stem cell line HL1-hT1 and human liver cancer cell line HepG2 for generating hepatospheroids in RCCS bioreactors. We characterized these bioreactor cultures in comparison to 2D and 3D static cultures (microtiter plate-based cultures). Different types of analysis and functional characterization of the hepatospheroids were aided by the capability of bioreactors for scaling-up the cultures to obtain an adequate amount of hepatospheroids for different downstream process analysis. We used potent hepatotoxic cyanotoxins, microcystin-LR (MC-LR) and cylindrospermopsin (CYN) as model hepatotoxic compounds. These cyanotoxins are linked both to acute human intoxications and liver injuries and associated with the development of chronic liver diseases, such as fatty liver diseases or liver cancer. In our study, using RCCS bioreactor-based 3D hepatospheroid cultures, we were able to assess both liver cell damage induced by these cyanotoxins as well as alterations of key cellular and molecular events involved in the development of liver steatosis/steatohepatitis. This RCCS bioreactors thus allow for detection of minute changes in dynamic, physiologically more relevant spheroid or organoid culture system, making this technology a promising tool for providing 3D liver models for toxicological applications.

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P27

Degradation of a cyanobacterial toxin by non-thermal plasmas

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Cyanobacteria can produce various toxic secondary metabolites, e.g. the hepatotoxic and potentially carcinogenic cylindrospmeropsin (CYN). Due to increased eutrophication of surface waters, toxic cyanobacterial blooms occur more frequently, consequently increasing the abundance of CYN and other cyanotoxins. In order to mitigate the risk these toxins pose, appropriate treatment technologies are required to assure their removal and provide hazard-free drinking water. Recent research on CYN removal from drinking water includes different approaches such as biodegradation, activated carbon filtration, ozonation and advanced oxidation processes (AOPs). Amongst AOPs, non-thermal plasmas (NTPs) increasingly received more attention for their potential application in water treatment due to their capability to produce a vast range of reactive species such as ozone, hydroxyl radicals, photons and electrons in situ. NTPs are generated by electric discharges in water or gas under atmospheric or low pressure. Despite their potential, the application of NTPs for cyanotoxin removal from drinking water has scarcely been investigated and is limited to very few cyanotoxins like the prototypical microcystin with a focus on gas-phase discharge systems. This study is the first to provide a basic understanding of the interaction of different NTPs with CYN and to compare gas- and liquid-phase discharge systems in this context. In the presented study, CYN was treated by six different plasma sources which can be distinguished by their physico-chemical plasma characteristics, i.e. type of discharge and associated plasma-chemistry. After 60 min of plasma treatment, the plasma sources could be ranked according to their CYN degradation efficacy as follows: spark discharge > gliding arc discharge > dielectric barrier discharge (DBD) > corona-like discharge > surface discharge > plasma jet. Based on these results, the reactors' usability and plasma-chemistry, the corona-like discharge and DBD sources were selected for further studies. For the corona-like discharge, the degradation efficacy increased with increasing operating voltage and solution pH. After 15 min of plasma treatment at pH ≥ 7.5, ongoing CYN degradation was observed even without further plasma application, indicating a residual oxidative effect of the plasma treated water. For the DBD in air around water droplets, increasing the operating voltage decreased the degradation efficacy, while the solution pH was not observed to affect CYN removal. CYN degradation by the corona-like discharge submerged in water followed pseudo first order reaction with an efficacy of 60.0 ± 5.3 mg/kWh, while the DBD in air with water droplets was less effective with 7.1 ± 0.8 mg/kWh and pseudo zeroth order kinetics. Degradation by the corona-like discharge was mainly promoted by hydroxyl radicals as well as other long-lived reactive species and degradative mechanisms, while the DBD mainly produced ozone and lower quantities of NOx.

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P28

Fighting over Alkaloids – Clay versus Organic Carbon

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Alkaloids are one of the largest phytotoxin subgroup that is categorized as persistent and mobile in the environment. Based on experimentally derived organic carbon-water partition coefficients (pH dependent, Doc), all alkaloids are indeed highly mobile with an average log Doc < 2.5. However, sorption to organic carbon alone does not fully describe the behavior observed for alkaloids in real soils. For the alkaloids, which are largely protonated under environmental conditions, electrostatic interactions with other soil components such as clay minerals may highly influence their behavior. Thus, we derived partition coefficients for alkaloids to montmorillonite and kaolinite as exemplary clay mineral sorbents with high and low cation exchange capacity. Overall, partition coefficients for alkaloids were bigger for clay minerals than those for organic carbon. Single sorbent partition coefficients for pyrrolizidine alkaloids (PAs) were additionally compared to soil sorption data generated for two sandy soils with a clay fraction between 2 and 20% and an organic carbon fraction of 1 to 3%. Thus, new insights were gained pointing towards the dominant geosorbent responsible for the PAs soil behavior.

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P29

Degradation of cyanotoxins produced by the cyanobacterium Microcystis aeruginosa using chlorine-based compounds: implication for drinking water management

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Cyanobacteria or "blue-green algae" are photosynthetic prokaryotes distributed worldwide in different environments, including natural or artificial drinking water reservoirs. In freshwater, massive proliferations of cyanobacteria or CyanoHABs may occur, deeply altering the surrounding ecosystem and affecting aquatic biota. Recently, CyanoHABs occurrence has increased, causing general awareness for public health, as some cyanobacteria species produce toxic molecules named cyanotoxins. Human exposure may also occur, e.g. consumption of contaminated or untreated water, so that cyanotoxins presence in water for drinking purpose is considered as a major health risk. In drinking water plants, chemical oxidation with chlorine-based compounds is usually applied for the removal of cyanotoxins, nonetheless, there are some concerns about the efficacy of such treatments. In this study, the effectiveness of two chlorinated oxidants commonly used in Italy, sodium hypochlorite (NaClO) and chlorine dioxide (ClO2), was assessed on cultured cyanobacterium Microcystis aeruginosa, one of the foremost common species in freshwater. The two oxidants were tested at increasing concentrations, and cell viability was evaluated measuring photosynthetic efficiency. Value of EC50 (i.e. concentration inducing a 50% reduction of photosynthetic efficiency) was calculated, showing that NaClO exerted its effect on cyanobacterial cells at a lower concentration than ClO2. NaClO and ClO2 were then applied at chosen concentrations, and total and extracellular cyanotoxins content, specifically microcystins (MCs), before and after treatments were analysed. NaClO showed a significantly higher removal efficacy after 1 h treatment even at low dose with respect to ClO2, which, on the contrary, didn't affect MCs content, while determined an increase of extracellular fraction. When applied at higher concentration, ClO2 lead to a decrease of total MCs, but non-degraded toxins were found in water, suggesting that ClO2 could promote release of cyanotoxins, and subsequently increase water hazardousness. These results show that oxidant choice and dose are critical steps towards optimization of drinking water treatments, needed to ensure safety and quality of water supplies.

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P30

Cyanobacterial LPS induces inflammatory response in epithelial cells in vitro

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Cyanobacteria water blooms (WB) represent global ecological problem. They are a source of wide range of toxins including lipopolysaccharides (LPS), which are a part of cyanobacterial cell wall. One of the ways of human exposure may be recreational usage of freshwater reservoirs contaminated by WB. In this case, skin irritation and gastroenteritis may occur as skin and intestinal epithelia are the interfaces getting to the direct contact with toxins in the water. We studied possible pro-inflammatory effect of LPS originated from four cyanobacteria species (Microcystis aeruginosa, Dolichospermum flos-aquae, Planktothrix aghardii, and Aphanizomenon flos-aquae) on in vitro model. CaCo-2 and HT-29 cell lines were used as models of intestine, HaCaT cell line was chosen as a skin model. LPS isolated from WB predominated by cyanobacteria of interest (environmental sample) and axenic culture (AX; pure cyanobacteria) was tested for each species. Levels of interleukin (IL) 8 (CaCo-2, HT-29, HaCaT cells) and IL-6 (HaCaT cells) were measured as markers of inflammation. Pyrogene test was carried out for all WB samples. Cytokine array was performed for two most potent WB samples, M. aeruginosa and D. flos-aquae, on CaCo-2 cells. We observed three of four tested WB LPS caused inflammatory response at least in one intestinal model and two of them also in skin model. Two WB LPS showed this effect despite low response in the Pyrogene test. Moreover, pro-inflammatory effect was observed in three AX LPS in intestinal models and one AX LPS in skin model. Cytokine array revealed intestinal cells to produce wide range of mainly pro-inflammatory cytokines upon LPS activation. In conclusion, results suggest that not only cyanobacterial exotoxins but also certain cyanobacterial LPS may possess risk for human health and their harmful effect should not be underestimated.

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P31

Monitoring of the natural toxin ptaquiloside in groundwater

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Ptaquiloside (PTA) is a toxin naturally occurring in several ferns of which bracken is the most widespread. It is a norsesquiterpene glycoside with carcinogenic, mutagenic and clastogenic properties. Ptaquiloside is highly water soluble with almost no sorption to soil and sediment, hence leaches from bracken fern to the aqueous environment. In turn, PTA can potentially contaminate surface as well as groundwater. Due to possible Human exposure via drinking water, PTA is considered as an emerging contaminant. Ptaquiloside is chemically unstable under acidic as well and alkaline conditions and is prone to microbiological degradation, making it challenging to collect and preserve for analysis. Thus, controlled and well-designed sample preparation method is needed for reliable investigations of PTA and its degradation product pterosin B (PtB). Here we present a sensitive, simple and robust method for analyses of PTA and PtB in groundwater. The preservation step was developed by applying a Plackett-Burman experimental design testing the following variables: Bottle type, filtering, test time, water type, pH, temperature and transportation conditions. Combination of factors that ensured best preservation was defined: Amber glass bottles, buffer at pH 6, storage at 4°C, no filtration, no ice, analyses within 2 weeks. The recovery obtained was 94 to 100% for different groundwater types. The sample purification step had a pre-concentration factor of 240 and recovery percentage of the entire method is 85 ± 2 (PTA) and 91 ± 3 (PTB) at 95% confidence limit. The limit of detection for the full method is 0.0008 μgL-1. The method was used to monitor bracken toxins in drinking water wells in Denmark, Sweden and Spain. PTA was found in several shallow drinking water wells (0.06 – 0.12 μgL-1). However, no bracken toxins were found in deep drinking water wells. The method enables sensitive and trustful monitoring of PTA and PtB in groundwater. We offer a reliable method that can be used for scientific investigations of PTA and PtB and by water supply companies for monitoring of these compounds.

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P32

Cyanobacterial neurotoxin 2,4-diaminobutyric acid produces three-fold higher depolarization of leech Retzius neurons than other excitatory amino acids implicated in neurodegeneration

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2,4-diaminobutyric acid (DABA) is produced by numerous Cyanobacteria widely present in various aquatic and terrestrial ecosystems, so there is pronounced risk of global human and animal exposure to this amino acid. Environmental amino acids are significant agents for neurodegeneration and a potential risk factor in non-familial cases of neurodegenerative diseases. While ß-N-methylamino-L-alanine (BMAA), a structural isomer of DABA also produced by Cyanobacteria, has received a lot of attention, 2,4-diaminobutyric acid had not been thoroughly investigated. In the light of scarcity of electrophysiological studies on DABA and ubiquitous presence of Cyanobacteria, we have investigated the effects of this amino acid on the cell membrane potential of Retzius neurons. We also provide comparison of these effects with the effects elicited by other environmental and endogenous amino acids implicated in neurodegeneration, namely BMAA, β-N-oxalylamino-L-alanine (BOAA), glutamate and aspartate. Experiments were conducted on Retzius neurons of isolated segmental ganglia of the leech H. sanguisuga. Classical intracellular recording using glass microelectrodes was performed. Exposure of Retzius neurons by perfusion with solutions of amino acids (DABA, BMAA, BOAA, glutamate and aspartate) in increasing concentrations was made over a period of three minutes each. All investigated amino acids elicited a concentration-dependent excitatory response of Retzius neurons. When applied in concentrations of 3 mM and higher, DABA induced membrane depolarization evolving through two distinct stages. This excitatory phenomenon was not present during exposure to other amino acids of same concentrations. The first stage of depolarization elicited by DABA had an amplitude of 14.41±0.18 mV, which matched up to maximal depolarizations elicited by BMAA (13.10±0.03 mV), BOAA (15.70±0.91 mV), glutamate (17.93±0.53 mV) and aspartate (17.74±0.39 mV). Maximal amplitude of complete membrane depolarization induced by DABA was extensive at 47.08±0.03 mV, showing that this amino acid is the most effective of all tested here. A noteworthy contrast between DABA and other amino acids are the irreversible electrophysiological changes and/or loss of neuronal functionality of cells treated with DABA. Only half of Retzius neurons exposed to 5 mM DABA showed recovery upon washout and none of the cells recovered after exposure to 10 mM DABA, while same concentrations of other amino acids tested here led to complete recovery of all treated cells. DABA elicits substantial membrane depolarization, several times greater than other investigated amino acids, making it a potentially significant environmental factor in neurodegenerative diseases through excitotoxicity. It also induces irreversible functional changes of neurons, also indicating a prominent neurotoxic potential. These effects, together with ubiquitous presence, warrant further research of this cyanobacterial amino acid.

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P33

Toxicity of Plant Alkaloids in Daphnia magna

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Plant secondary metabolites are not essential for plant growth or survival but play an important role in attraction of pollinators and deterrence of herbivores. Of these secondary metabolites, one of the largest classes is the alkaloids, which comprise at least 21,000 different nitrogen-containing heterocyclic molecules, distributed throughout 20% of known vascular plants. Alkaloids include quinolizidine and indole alkaloids found in the lupin (Lupinus spp.), an ornamental flower and emerging protein crop, as well as pyrrolizidine alkaloids in the ragwort (Senecio spp.), an invasive, weed-like flower. As many alkaloids are toxins, there is concern they can leach into freshwater sources, where they may affect non-target organisms, such as the crustacean Daphnia magna. This study aimed to investigate the toxicity of selected quinolizidine alkaloids (lupanine, lupinine, sparteine), pyrrolizidine alkaloids (heliotrine, monocrotaline, monocrotaline N-oxide, senecionine) and an indole alkaloid (gramine) in D. magna. Acute toxicity, measured as the 48-hour EC50 for immobilisation in D. magna, was between 6.6 and 18.5 mg/L for the quinolizidine alkaloids, 1.36 mg/L for the indole alkaloid gramine, and 1.92 to 51.3 mg/L for the pyrrolizidine alkaloids. The N-oxidised form of monocrotaline was less toxic than the free form of monocrotaline (51.3 mg/L compared to 1.92 mg/L), in keeping with rodent toxicity data. Acute toxicity is a useful benchmark of toxicity, however chronic exposures are often more environmentally relevant in the aquatic environment. Daphnid mothers exposed to 525 µg/L sparteine took significantly longer to produce their first brood, and produced fewer and smaller offspring after 21-day exposure. Furthermore, mothers exposed to 250, 350 and 525 µg/L sparteine produced significantly smaller offspring. Mother size was initially reduced after 7-day exposure to 165, 350 and 525 µg/L sparteine, but recovered by 14 and 21 days. Considering the natural expansion of invasive pyrrolizidine alkaloid-containing plants, together with increasing culturing of quinolizidine and indole alkaloid-containing lupins for protein, it is likely that certain areas may contain high levels of alkaloids. This study shows alkaloids can elicit acute toxicity and chronic reproductive toxicity in D. magna, however, it is not known if environmental alkaloid concentrations reach this toxic threshold. Hence, there is a great need to investigate the stability and mobility of alkaloids in soil and water, to better elucidate their persistence and, ultimately, their risk to non-target organisms.

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The cyanobacterial community compositions and bacteria-bacteria interactions related to their potential impact on human health

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Lipopolysaccharides (LPS) are the main component of the cell wall of Gram-negative bacteria, but they occur also in cyanobacterial cell walls (CyanoLPS). Thus, surface waters with a massive growth of planktonic cyanobacteria, so-called cyanobacterial water blooms, contain a complex mixture of CyanoLPS as well as LPS from the cell wall of heterotrophic Gram-negative bacteria associated with cyanobacterial bloom. LPS of Gram-negative bacteria are recognized as potent inflammatory agents (endotoxins) causing serious human health effects through activation of Toll-like receptor 4 (TLR4), which is a critical component of innate immunity and functions as a rapid pathogen sensor. Although various inflammatory effects have been associated with human exposures to cyanobacterial water blooms, there is only limited knowledge about a biological activity of both CyanoLPS and complex mixtures of (cyano-)bacterial LPS from natural cyanobacterial water blooms (CWB-LPS). Our results showed that CWB-LPS isolated from complex environmental water bloom samples dominated by Microcystis aeruginosa trigger human innate immune response. CyanoLPS isolated from axenic M. aeruginosa strains PCC7806 and HAMBI/UHCC130 also induced pro-inflammatory effects in human cells. Therefore, pro-inflammatory effects of CWB-LPS are likely caused not only by LPS from bloom-associated G-negative heterotrophic bacteria but also by CyanoLPS. Within this study, we aim to investigate how pro-inflammatory effects of CWB-LPS from cyanobacterial water blooms depend on the cyanobacterial community composition and bacteria-bacteria interactions. To answer this question, we use 16S rRNA gene amplicon sequence analysis and TaqMan Probe qPCR to qualitatively and quantitatively characterize a microbial composition of cyanobacterial blooms, bloom-associated heterotrophic bacteria, as well as free-living bacteria from localities with different dominant cyanobacterial species and then compare these results with pro-inflammatory in vitro effects of CWB-LPS isolated from these blooms/localities.

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P35

Influence of environmental factors on Microcystins degradation bacteria and toxigenic cyanobacteria bloom: a Bayesian approach

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Microcystins (MCs), which are produced by toxigenic cyanobacteria, have been concerned as a serious global problem due to the risk of carcinogenesis and toxicity to human. Several researches have recently reported on the global warming effect upon the proliferation of toxigenic cyanobacteria and production character of MCs. The toxic production characters, of course, have been affected by not only water temperature but also other factors such as nutrients (N and P). Moreover, the effect of MCs degradation bacteria, which possess mlrA gene, has been investigated in terms of fluctuation of MCs in water environment. These factors are entangled with each other. In order to untangle such complex phenomena, modern statistical analysis has a strong power. Therefore, we aimed to investigate the relationships among environmental factors such as water temperature and nutrients (N and P) on cyanobacteria, toxigenic cyanobacteria, MCs degradation bacteria, and MCs using Bayesian statistical modelling as a powerful statistical tool. In our study, we used the survey data for small ponds in northern and central of Thailand from December 2013 to December 2014. The ponds were selected at different elevations from 30 m to 600 m to elucidate the temperature effects. Chlorophyll-a and nutrients parameters for N and P were measured. The number of total cyanobacteria, potential MCs producing cells, and MCs degradation bacteria were quantified by real-time PCR for PC, mcyB, and mlrA gene, respectively. The concentration of MC-RR, -LR, -YR were determined by HPLC. By using Bayesian statistical analysis, we elucidated the positive temperature effect on the abundance of cyanobacteria and toxigenic cyanobacteria. However, water temperature interestingly exhibited negative relationships with MCs and dissolved MCs (DMCs) concentration. The results suggested that higher water temperature might inhibit MCs production and release process as several researches mentioned. Furthermore, we considered how temperature might enhance MCs biodegradation by indigenous bacteria. Logistic regression model showed that water temperature, MCs, and DMCs accelerated the growth of MCs degradation bacteria. Nevertheless, mlrA gene did not significantly explain the variance in MCs and DMCs concentration. The result suggested the possible presence of MCs degraders lacking mlr gene in our water samples. Further investigation should be considered to uncover the underlying mechanisms that gave the present responses in this study.

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P36

The Influence of pH in Water Samples on the Recovery of Microcystin Congeners by MS/MS

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Cyanobacterial blooms are becoming more prevalent in western Europe due to eutrophication and increasing temperatures. To guarantee the safety of our drinking water and of recreational water activities, It is necessary to use adequate methods to detect hepatotoxic cyanotoxins. Thus, we adapted an LC-MS/MS method based on Turner et al (2018) to detect and quantify microcystin (MC) congeners and nodularin1. Additionally, we extended the method by providing an indication of the matrix effect (ME) for each sample without using a standard addition curve. Therefore, our analytical method is more compatible with monitoring programs that are needed to mitigate the risk for public health. These programs deal with a variety of matrix types with diverse physicochemical properties, of which water is the most important. Water can cause contamination through ingestion or MC accumulation in animals and plants. One of its variable physicochemical properties is the pH. While the Ohio EPA DES 701.0 method suggests a 5 to 11 pH range for their analysis of extracellular and intracellular toxins in different water sources2, EPA method 544 (LC-MS/MS) buffers the water samples at pH 7 during collection3. However, our validated method with simpler extraction (vs EPA 544) and LC-MS/MS detection method does not include buffering, resulting in a wide pH range during extraction (similar to Ohio EPA). This wide pH range can cause low extraction yield and recovery of some MC congeners and thus, in turn, imprecise total MC concentrations. Consequently, we have assessed the impact of pH differences on the recovery of MC congeners using our validated LC-MS/MS method. We determined the recovery and ME of the MC's in spiked samples of bottled spring water after adjusting the pH to different values between 5 and 11. During the experiment, we found that the recovery of multiple congeners was influenced by a difference in pH. This prompted us to elaborate a uniform screening and quantification technique for all congeners.

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P37

LipiD droplEts aS a TaRget fOr cYanotoxins (DESTROY): Application of systematic review framework to validate this association

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A systematic review is a method for answering a specific research question using a predefined, multistep process to identify, select, critically assess, and synthesize pieces of evidence from scientific studies to reach a conclusion. In our work, we used this concept to answer a question regarding lipid droplets as a target for environmental contaminants focusing on cyanotoxins. Lipid droplets (LDs) are important cytosolic organelles found nearly in almost all organisms, from bacteria to mammals. The role of LDs goes far beyond the energy storage as they are involved in multiple intracellular processes highly variable in different types of organisms, tissues, or cells. Recently, it has been suggested that disrupted structure and functions of LDs are involved in numerous pathological conditions in humans manifested as atherosclerosis, obesity, fatty liver diseases, as well as infertility and reproductive disorders. One of the highly discussed factors associated with all these pathologies is chronic exposure to environmental chemicals such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or cyanotoxins. Thus, compounds targeting LDs might be involved in the pathophysiology of these health issues in humans. In this poster, I will introduce a systematic review framework as a powerful tool in toxicology and risk assessment of cyanotoxins emphasizing the role/ function of LDs in acute or chronic liver diseases associated with cyanotoxins. I will also present available bioinformatics or other tools used/availed at each step of a systematic review platform to accelerate the entire process including Rayyan QCRI for excluding or including papers based on predefined criteria, SciRap tools for assessing the quality and susceptibility of in vivo or in vitro studies to bias and the Health Technology Assessment and STROBE guidelines for epidemiological or other types of studies. The findings from our systematic review will be discussed in connection with our outcomes from the studies focused on the effects of microcystin-LR and cylindrospermopsin in advanced in vitro 3D liver models to confirm the mechanistic link between cyanotoxins and LDs in the liver.

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Cyanobacterial diversity, biomass and cyanotoxins across the latitude in European freshwaters

Cyanobacterial blooms increase in frequency, intensity and duration around the world, as a result of progressing eutrophication and climate change. The effects of increased lake water temperature due to the global warming are predicted to drive changes in the structure of cyanoHABs and distribution of cyanobacterial toxins. However, these consequences of the global warming are anticipated to differ among geographical regions and to be more pronounced in the northern latitudes. Therefore, the aim of our study was to compare the species composition, biomass and toxin content of cyanobacterial blooms in southern (warm), central (temperate) and northern (cool) part of Europe in present climatic conditions. We hypothesized that cyanobacterial species composition, their biomass and toxins concentration differ in European freshwaters across latitude, and cyanoHAB's intensity and toxicity increases with the latitude. Ninety lakes were sampled during one of the hottest summers in Europe, in 2015, across a latitudinal gradient: southern Europe (Croatia), temperate Europe (Poland), northern Europe (Lithuania). More than 110 cyanobacteria taxa were identified in the studied water bodies along the latitude. RDA analysis showed that latitude was important factor for cyanobacterial biomass and distribution of some potentially-toxic

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cyanobacteria species, such as Aphanizomenon gracile and Dolichospermum spp. Moreover, the analysis showed that nutrients concentration and temperature were significant factor affecting biomass of another cyanobacteria species: Aphanizomenon flos-aquae, Planktothrix agardhii and Raphidiopsis (Cylindrospermopsis) raciborskii, accordingly. Additionally, logistic regression revealed a significant effect of latitude on cyanobacterial toxin distribution (increase of amount of toxins). Probability of occurrence of different variants of microcystins (MCs), anatoxin-a (ATX) and cylindrospermopsin (CYN) significantly increased along the latitudinal gradient, as well. P. agardhii was associated with the occurrence of dmMC-RR, dmMC-LR and MC-YR, and Microcystis species (M. viridis and M. wesenbergii), with the presence of MC-LR and MC-RR in the water bodies. ATX content in the lakes was related with the dominance of Cuspidothrix issatschenkoi and Dolichospermum species, mainly D. spiroides, D. smithii and D. viguieri. Thus, it is postulated that the currently recorded changes in weather conditions will cause presence of more intense and toxic cyanobacterial blooms in the higher latitude.

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P39

Characterization of new anabaenopeptins from cyanobacteria of Greek lakes

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Cyanobacteria are well known producers of bioactive and toxic compounds which are released in water during blooms and are harmful to aquatic organisms and public health. Anabaenopeptins (APs) is a class of cyclic cyanopeptides with over 100 congeners characterized so far. Their structure consists of a five-amino acid ring with a side-chain amino acid attached to lysine via a characteristic ureido linkage. Based on the limited studies for their bioactivity, APs are able to inhibit the activity of serine endopeptidases such as trypsin, chymotrypsin, thrombin and elastase and exopeptidases such as carboxypeptidase A (CPA), B (CPB), U (CPU) and TAFIa that could be beneficial in development of drugs for the treatment of cardiovascular diseases. On the other hand, APs are also protein phosphatases inhibitors (i.e. PP1 & PP2), similar to the well-known microcystins. APs are produced not only by cyanobacteria but also by other organisms and they can occur in freshwaters, brackish and sea waters. In this study, we investigated the presence of APs in cyanobacterial blooms and isolated strains from Greek freshwaters. Analysis was performed with liquid chromatography coupled to hybrid triple quadrupole/linear ion trap mass spectrometer (LC-qTRAP MS/MS), using Turbo Ion Spray probe in positive ionization mode. Detection of APs was carried out in information dependent acquisition (IDA) mode and enhanced ion product (EIP) mode was applied for the collection of fragmentation spectra. The structures of thirteen APs were elucidated based on obtained fragmentation spectra with four new congeners among them. Anabaenopeptin F, Oscillamide Y, and Anabaenopeptin B were the most frequently detected variants in the bloom samples collected from nine different Greek lakes. The ability of several cyanobacterial strains isolated from freshwaters of Greece to produce APs was also evaluated. Our results contribute to further understanding of the metabolic potential of cyanobacteria from the Mediterranean region.

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