

# 14th EuCheMS International Conference on Chemistry and the Environment



ICCE 2013, Barcelona, June 25 - 28, 2013

## Book of abstracts

### Satellite Event

### Alternative Flame Retardants: Analysis, Occurrence and Exposure

## ORAL PRESENTATIONS

13.30-14.00 - Ake Bergman - *Abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals*

14.00-14.30 - Martin Scheringer - *Persistence, long-range transport and bioaccumulation potential of emerging FRs: comparison with classical PBDEs*

14.30-15.00 - Lourdes Ramos - *Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the identification of organobrominated compounds in biotic samples*

15.30-16.00 - Eva Brorström-Lundén and Peter Haglund - *Sources, pathways and occurrence of brominated flame retardants (BFRs) and polybrominated dibenzofurans (PBDFs) in Sweden.*

16.00-16.30 - Enrique Barón - *Occurrence of halogenated flame retardants (BFRs and Dechloranes) in Spain*

16.30-17.00 - Adrian Covaci - *Belgium Alternative flame retardants in indoor dust: human exposure assessment*

17.00-17.30 - Cayo Corcellas - *Human exposure to halogenated norbornenes*

## Abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals

Åke Bergman<sup>1,2</sup>

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The number of environmental contaminants has increased dramatically over the past 50 years. One group of chemicals of concern is flame retardants (FRs) such as brominated-, chlorinated- and phosphorous FRs (BFRs, CFRs and PFRs). The European Food Safety Agency (EFSA) has over the last two years produced six adopted EFSA opinions<sup>1-6</sup>. These documents deal with food and feed exposure and the toxicity of the BFRs discussed, i.e. PBBs<sup>1</sup>, PBDEs<sup>2</sup>, HBCDDs<sup>3</sup>, TBBPA<sup>4</sup> and its derivatives, phenolic BFRs including their derivatives (not TBBPA)<sup>5</sup> and the final document<sup>6</sup> deals with emerging and novel neutral BFRs. In addition, Eljarrat and Barceló edited a book on BFRs that came out 2011<sup>7</sup>.

The large number of established, emerging, potential and novel FRs and the increasing number of publications dealing with all of those call for harmonized and simple abbreviations to avoid confusion in the area. In the literature it is not uncommon to find that several abbreviations is used to describe the same chemical or that one abbreviation may even be used to describe two different chemicals. In a rather recent article<sup>8</sup>, we propose abbreviations for halogenated and organophosphorus FRs using a two step procedure to establish Practical Abbreviations (PRABs). In the first step, Structured Abbreviations (STABs) are derived using specific STAB criteria based on the chemical structure of the FR. The STABs are helpful since they give useful information about the FRs structure. However, since some of the STABs are complicated and long, we proposed instead the use of the shorter PRABs. The PRABs are in most cases derived from the STAB, although, we also consider abbreviations extensively used in previous literature. Importantly, the methodology for abbreviating the FRs is applicable to other classes of true or potential environmental contaminants. Hence STABs and PRABs can be created also for such compounds.

### References

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2. EFSA., 2011a. *Scientific opinion on polybrominated diphenyl ethers (PBDEs) in food*. *EFSA Journal*. 9, 2156.
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6. EFSA., 2012b. *Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food*. *EFSA Journal*. 10, 2908.
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8. Bergman Å, Rydén A, Law R.J, de Boer J, Covaci A, Alaei M, Birnbaum L, Petreas M, Rose M, Sakai S, van den Eede N, van der Veen I., 2012. *A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals*. *Environ. Int.* 49, 57.

## Persistence, long-range transport and bioaccumulation potential of emerging BFRs: comparison with PBDEs

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In the last years, polybrominated diphenyl ethers (PBDEs) as a major class of brominated flame retardants (BFRs) have been regulated at the national and international level. Because of the restrictions on PBDE use, in many cases PBDEs have been or will be replaced by other BFRs; there is a group of approximately 40 alternative brominated and chlorinated flame retardants that may be of increasing interest as PBDE replacements [1]. Therefore, it is important to assess these emerging brominated and chlorinated flame retardants with respect to their environmental hazards, i.e. persistence, toxicity, bioaccumulation potential and potential for long-range transport. Here we focus on a set of 36 alternative BFRs and first investigate the availability and quality of chemical property data that are required for an environmental hazard assessment of these substances (octanol-water partition coefficient (Kow), bioconcentration factor (BCF), degradation half-lives in different media, EC50 or LC50 values for acute and chronic toxic effects in aquatic species). Second, we estimate the overall persistence and long-range transport potential of the 36 alternative BFRs using the OECD Screening Tool for Overall Persistence (Pov) and Long-Range Transport Potential (LRTP). For the assessment of the quantity and quality of the chemical property data, we retrieved data from 25 publicly accessible databases, including the database of the European Chemicals Agency (ECHA), and the OECD eChemPortal, which provides links to 24 participating data sources. For 17 of the 36 BFRs, no data at all were found in any of the databases. For the other 19 substances, the majority of the data that were found is for acute and chronic toxicity (around 60% of the data points for all 19 substances taken together); 35% of the data points is for Kow and BCF, and only 5% for persistence. The number of data available per substance varies strongly; for a few substances, including tetrabromobisphenol A, tribromophenol, and tris (2,3-dibromopropyl) phosphate, several data points are available for each property, but for other substances, the data are scarce. In the cases with multiple data points per property and substance, the data points often span a wide range (several orders of magnitude). In conclusion, the problems related to data availability and data quality are that (i) no or only very few data are available at all or (ii) the available data are so diverse that it is difficult to identify a best estimate. To run the OECD Pov and LRTP tool for the 36 BFRs, it was necessary to estimate the missing property data from the chemical structure. On this basis, results for Pov and LRTP indicate that many of the alternative BFRs are similarly persistent and prone to long-range transport in the environment as PBDEs. Our results lead to two main conclusions: (i) most alternative BFRs are only poorly characterized and the data that would be needed for hazard and risk assessment are not sufficient; (ii) at the same time, chemical property data estimated from the chemical structure of the alternative BFRs indicate that the environmental hazards of many of the alternative BFRs are similar to those of the PBDEs. This indicates that the current PBDE substitution process may perpetuate the environmental risks initially caused by PBDEs.

[1] Bergman Å, Rydén A, Law R J, de Boer J, Covaci A, Alaee M, Birnbaum L, Petreas M, Rose M, Sakai S, Van den Eede N, van der Veen I. (2012) Environ. Int. 49: 57–82

14.30-15.00

## **Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the identification of organobrominated compounds in biotic samples**

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Comprehensive two-dimensional gas chromatography (GCxGC) is a powerful separation technique particularly suitable for unravelling the composition of complex mixtures. Its combination with a time-of-flight mass spectrometer as detector (GCxGC-ToF MS) provides additional identification capabilities by incorporating the mass spectrum information to the information derived from usually structured chromatograms. Under these conditions, GCxGCToF MS becomes a powerful analytical technique for both target and non-target analysis even when appropriate standards are not available which also allows the tentative identification of unknown compounds.

This study evaluates the feasibility of GCxGC-ToF MS for the simultaneous analysis of several classes of organobromines (OBs) in bluefin tuna muscles prepared using a non-selective sample treatment procedure. Targeted OB classes included polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), methoxylated PBDEs (MeO-PBDEs), several halogenated naturally produced compounds (HNPs) and eight novel brominated flame retardants (NBFRs), polybrominated hexahydroxanthene derivatives (PBHDs), 2,4,6-tribromoanisole and a mixed halogenated compound (MHC-1). Special attention has been paid to solve co-elution problems observed during the analysis of OBs with one-dimensional GCbased techniques. Satisfactory separation among several relevant PBDEs and MeO-PBDEs has been obtained allowing their unambiguous determination in a single run. Additional studies were conducted to identify selected NBFRs and HNPs. 2,4-Dibromoanisole, a dibromophenol isomer and hexabromobenzene were identified in the studied samples. Several new tri- and tetra-BHD derivatives were also identified, indicating that these compounds could apparently exist as structured families in nature. In addition, a tetrabrominated diMeO-biphenyl and two tetrabrominated diMeO-BDEs not previously described in the literature were tentatively identified.

15.30-16.00

## Sources, pathways and occurrence of brominated flame retardants (BFRs) and polybrominated dibenzofurans (PBDFs) in Sweden

*Eva Brorström-Lundén and Peter Haglund*

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The overall results of a recent Nordic screening showed that emerging brominated flame retardants (BFRs) were regularly found in many different sample matrices indicating a widespread use of these substances in the Nordic countries (Schlabach et al., 2011). A previous Swedish screening of polybrominated dibenzofurans (PBDD/Fs) showed that these substances were widely distributed in the environment and that further studies were needed to find the sources of PBDFs (Brorström-Lundén and Haglund et al 2010).

A follow up screening study of BFRs and PBDFs was initiated by the Swedish Environmental Protection Agency. The overall objective of this screening was to determine the concentrations of selected BFRs and PBDFs in a variety of media in the Swedish environment and to assess possible emission sources and to highlight important transport pathways in the environment including large scale transport. Sampling was carried out at in e.g. ambient air, at potential point sources as well as in indoor air and dust. The presences of the substances in biota were also investigated to give an indication of whether they pose an environmental risk. Possible human exposure was investigated by analysing BFRs in human blood samples and PBDFs in human milk samples.

The emerging BFRs included in the screening were hexabromobenzene (HBB), pentabromotoluene (PBT) and pentabromoethylbenzene (PBEB), decabromodiphenyl ethane (DBDPE) and 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH). These BFRs were frequently found in biota in the previous Nordic screening. Additional substances in the screening were polybrominated diphenyl ethers (PBDEs), polybrominated dibenzodioxins (PBDDs) and chlorinated dioxins and furans (PCDDs and PCDFs).

The results from this screening will be presented.

### References:

Schlabach, M., Remberger, M., Brorström-Lundén, E., Norström, K., Kaj, L, Andersson, , Herzke, D., Borgen, A., Harju, M. , Bouttrup, S., Dam, M., Mannio, J., Jörundsdóttir, H. Ó., Hedlund, B., (2011). "Brominated Flame Retardants (BFR) in the Nordic Environment". NMR TemaNord 2011:528.

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## Occurrence of halogenated flame retardants in Spain

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Classical flame retardants (FRs) such as PBDEs have been widely used for many years to increase the fire resistance of different materials. However, it has been demonstrated that they can act as endocrine disruptors and have a high bioaccumulation capacity. For that reason they have been banned and other FRs have been developed to meet the fire safety regulations. Some of these compounds are halogenated norbornenes (Dec 602, Dec 603, Dec 604 and DP) and other brominated FRs such as pentabromoethylbenzene (PBEB), hexabromobenzene (HBB) and decabromodiphenylethane (DBDPE). These compounds are considered emerging FRs and the knowledge of their occurrence and behaviour is still limited. Moreover, most of the studies are carried out near to the production areas (USA and China), where they are present in higher amounts than in other areas.

Classical PBDEs have been detected in Spain in different environmental and biological matrices such as sediment, air, sewage sludge, aquatic and terrestrial biota. In spite of the less number of studies regarding the emerging FRs, they have been also detected either in environmental and biological matrices such as sediment, sludge, bird eggs, fish or dolphin bubbler.

PBDEs and halogenated norbornenes have been detected in sediment from two Iberian river basins, with levels ranging from not detected (nd) to 45 ng/g dw. DBDPE was found in the same sediments with levels ranging from nd to 32 ng/g dw. DBDPE levels were similar to those found for BDE-209, which may indicate that it has been used as substitute to the already banned BDE-209.

PBDEs and halogenated norbornenes have been also detected in bird eggs from the Doñana National Park, in the Southern Spain. Despite the fact that PBDEs are the most abundant compounds (from 2.1 to 90 ng/g lw), the presence of halogenated norbornenes is significant (from 0.9 to 60 ng/g lw). Moreover, halogenated norbornenes have shown bioaccumulation capacity on a terrestrial trophic chain.

In addition, both PBDEs and halogenated norbornenes have been detected in dolphins from the Gulf of Cadiz and the Strait of Gibraltar, with levels from 12 to 2660 ng/g lw and 0.5 to 29.7 ng/g lw, respectively. Again, classical PBDEs were the most abundant compounds, but the presence of the DP and its analogues should be taken into account. Besides, Dec 603 showed biomagnification potential, which is barely reported in the literature.

Currently, the FRs profile in Spain is dominated by PBDEs. However, since they have been

banned in EU, halogenated norbornenes and other emerging FRs may become more important in the near future. Therefore, more information about their occurrence and behaviour is needed.

## Indoor exposure of humans to organophosphate flame retardants

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Phosphorous-based flame retardants (PFRs) represent 24% of the market share in flame retardants. PFRs are used in polymers, such as cellulose esters, polyvinyl chloride, etc. from which they are released in their environment by abrasion, volatilization and leaching. As people spend in average 90% of their time in the indoor environment, including at home, at work, and during transportation, the monitoring of PFR concentrations in indoor matrices (e.g. air and settled dust) is a valuable tool to evaluate the daily exposure to these contaminants. Recent measurements of PFRs in dust show that PFRs are much more abundant than brominated FRs, with concentrations varying from several  $\mu\text{g/g}$  in Western European countries to a few  $\text{mg/g}$  in Japan or to only a few  $\text{ng/g}$  in developing countries, such as Pakistan or Egypt. Not only do the concentrations differ among countries, but also the profiles of PFRs vary. For example, TDCPP is much more abundant in US dust compared to European or Asian dust. Another phenomenon is the different patterns observed between microenvironments, such as work, transport and home. The estimates of daily dust intake are still being discussed, but generally it is assumed that adults ingest a daily amount of 10 to 50  $\text{mg dust/day}$ , while toddlers may ingest up to 200  $\text{mg/day}$ . For PFRs, this means that intakes may range from less than 1  $\text{ng/kg body weight}$  to up to 50 and 300  $\text{ng/kg body weight per day}$  for adults and toddlers, respectively. For the purpose of human biomonitoring, other sources need to be investigated as well, e.g. indoor air, dermal contact, and diet. Recent publications showed that PFRs reach concentrations of several  $\text{ng/m}^3$  in indoor air, and consequently inhalation of suspended particles could have an equal contribution to daily exposure as dust ingestion for adults.

## Human exposure to halogenated norbornenes

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Nowadays, chlorinated norbornenes are widely used as flame retardants (FR). These compounds, generally called dechloranes (DECs), have been used as substitutes of other banned halogenated flame retardants such as mirex and polibrominated diphenylethers (PBDEs). Recently, the presence of those substances in atmosphere, environment, biota and even food, makes important to understand their toxicity and the real human exposure to them. Concretely, the congener known as Dechlorane Plus (DP) is the most studied.

Traditionally, human exposure pathways are classified in three types: dermal, inhalation and ingestion. Given DECs properties, dermal absorption is negligible in front of the other two. Diet is the principal gateway for other FR, for example it means more than the 95% of PBDE intake. However, dust ingestion in children is calculated as far as 4 times the value in adults. That could imply differences between adult and child exposures to FRs. Moreover, nurslings represent a population risk group because of a lot of reasons. For instance, their only food is breast milk, they had the biggest food intake per kg body weight relation, they are in full neurological and physical development and, besides, their metabolism could not be completely prepared to eliminate toxics.

For all those considerations, studying atmosphere (gas and particulate fraction) and diet contamination is very important in order to determinate the human exposure to DECs. Specially, the foodstuff which would be of most interest could be breast milk.

Recently, there are some authors who study the DECs contamination in atmosphere of different cities around the world, their distribution between gas and particulate fractions and other important parameters to understand their behaviour. It seems that DP could have preference for the particulate phase, being its presence in air of some pg/m<sup>3</sup> and, generally, lower than hundreds of ng/g dry weigh in home dust. In spite of this, most of that knowledge is about the tendency of those contaminants but it has to be well-proved yet, so more complete studies would be done.

About DECs determination in human breast milk, there are only two published works. That reflects an important gap in the DECs exposure assessment. However, they agreed that DECs levels could be ten times lower than PBDE contamination levels in the same samples. It means values from 0.94 ng g<sup>-1</sup> lipid weight (lw) to 38 ng g<sup>-1</sup> lw, depending on the studied region. Finally, all that information together show a general view of distribution of chlorinated norbornenes contamination and it lets point out some needs in future investigations in human exposure assessment.

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**Satellite Event**

**Mining and Environment: Old Problems and New  
Solutions**

## ORAL PRESENTATIONS

9.00-9.45 - Kirk Nordstrom - *Baseline and premining geochemical characterization: The issue of natural background*

9.45-10.30 - Jose Miguel Nieto - *The genesis of acid mine drainage: the case of the Iberian pyrite belt*

10:30-11:15 - Bernhard Dold - *Biogeometallurgical pre-mining characterization of ore deposits: a new approach to increase sustainability in the mining process*

11.45-12.30 - David Arcos - *Mine rock drainage: characterization and modelling for assessment of waste rock dumps*

12.30-13.15 - Ricardo Amils - *Bioleaching, the future of mining*

15.00-15.45 - Rafael Pérez-López - *Delaying sulfide oxidation: long-term treatment of acid-producing mine wastes using coal combustion fly ash*

15.45-16.15 - Adam Jarvis - *The importance of chemical and biological processes in the passive remediation of iron-dominated coal mine waters compared to zinc-dominated metal mine waters*

16.15-17.00 - Tobias W. Rötting - *Passive treatment of acid mine drainage: conventional treatment systems and new developments based on inorganic processes to remove high metal concentrations*

## **BASELINE AND PREMINING GEOCHEMICAL CHARACTERIZATION: THE ISSUE OF NATURAL BACKGROUND**

D. Kirk Nordstrom

*US Geological Survey, Boulder, CO, USA*

A rational goal for environmental restoration of new, active, or inactive mine sites would be “natural background” or the environmental conditions that existed before any mining activities or other substantial anthropogenic activities. In a strictly technical sense, there is no such thing as natural background, hence the terms “baseline” and “premining” are preferred. Protocols for geochemically characterizing premining conditions are not well-documented but should be based on existing studies for new mines and analog studies for active or inactive mine sites. Examples will be described based on the Pebble mine in Alaska for new mines and the Questa mine for active or inactive mines.

## **THE GENESIS OF ACID MINE DRAINAGE: THE CASE OF THE IBERIAN PYRITE BELT**

Jose Miguel Nieto

*Department of Geology, University of Huelva, Spain*

Mining operations often induce acid or neutral drainage with high metal concentrations that potentially could affect adversely the environment and human health. Generally mine rock drainage occurs through tailing dams, stock piles, waste rock piles and leach pads and in mine pits or tunnels. The extent and the effects of this phenomena depend heavily on specific mine site characteristics. It is therefore necessary to develop and implement a plan to prevent, control, mitigate and prepare for the mine rock drainage.

Acid Mine Drainage (AMD) in the Iberian Pyrite Belt is probably the worst case in the world of surface water pollution associated with mining of sulphide mineral deposits. The Iberian Pyrite Belt is located in SW Iberian Peninsula and it has been mine during the last 4.500 years. The oxidative dissolution of the sulphides exposed by mining releases large amount of AMD. The central and eastern part of the Iberian Pyrite Belt is drained by the Tinto and Odiel rivers, which receive most of the acidic leachates. As a result, the main channel of the Tinto and Odiel rivers are very rich in metals and highly acidic until reaching the Atlantic Ocean.

10:30-11:15

**BIOGEOMETALLURGICAL PRE-MINING CHARACTERIZATION OF ORE DEPOSITS: A NEW APPROACH TO INCREASE SUSTAINABILITY IN THE MINING PROCESS**

Bernhard Dold

*Sustainable Mining Research & Consult. EIRL, Santiago de Chile*

Based on the knowledge obtained from acid mine drainage formation in mine waste environments (tailings impoundments and waste-rock dumps), a new methodology is applied to characterize new ore deposits before exploitation starts. This gives the opportunity to design optimized processes for metal recovery of the different mineral assemblages in an ore deposit and at the same time to minimize the environmental impact and costs downstream for mine waste management. Additionally, the whole economic potential is evaluated including rare earth elements (REE). The methodology integrates high-resolution geochemistry by sequential extractions and quantitative mineralogy by QEMSCAN® or MLA in combination with kinetic bioleach tests. The produced data set allows to define biogeometallurgical units in the ore deposit and to predict the behaviour of each element, economically or environmentally relevant, along the mining process.

## **MINE ROCK DRAINAGE: CHARACTERIZATION AND MODELING FOR ASSESSMENT OF WASTE ROCK DUMPS**

David Arcos

*Amphos 21 Consulting S.L., Barcelona. Spain*

The hydrogeochemical processes involved in MRD generation are complex. It is necessary to characterize the processes and parameters involved at different scales varying in orders of magnitude both in volumetric samples and representative times. Characterization of mineralogy and geo-chemical processes through laboratory scale tests provide accurate results of the composition of the drainage as a result of the interaction of sulfide minerals with water and oxygen and the potentially buffering minerals of the rock sample. This characterization phase however, needs to be combined with field scale in-situ pilot tests which characterize the hydrological as well as bio-geo-chemical processes occurring under site specific conditions. The plan must consider a phase to integrate all the information in a conceptual model that accounts for the entire previous characterization step.

The prediction of MRD generation and its impacts at basin scale are attained with numerical models. Reactive transport models of varying complexity are used to predict the impacts of the facilities in soils, aquifers and spring downstream. Numerical models should then be used to evaluate technically the effectiveness of different alternatives to prevent and/or mitigate MRD. A cost-benefit and/or multicriteria analysis of the different alternatives may then be developed in order to present the decision maker a tool to take informed decisions.

12.30-13.15

## **BIOLEACHING, THE FUTURE OF MINING**

Ricardo Amils

*Department of Molecular Biology, UAM; Astrobiology Center, CSIC, Madrid, Spain*

Since the discovery that chemolithotrophic microorganisms were responsible of the generation of AMD in the forties, bioleaching has gone over a long way. The introduction of molecular ecology tools for the analysis of bioleaching operations has resulted in important advances in the field.

## **DELAYING SULFIDE OXIDATION: LONG-TERM TREATMENT OF ACID-PRODUCING MINE WASTES USING COAL COMBUSTION FLY ASH**

Rafael Pérez-López

*Department of Geology, University of Huelva, Spain*

Flotation processing of sulfide ores for obtaining base metals produces highly-reactive tailings that are stored in impoundments and, if not managed properly, exposed to weathering conditions. Exposure of residual sulfides to air and water leads to the release of extremely acidic leachates with high concentrations of sulfate and potentially toxic elements, known as Acid Mine Drainage. Consequently, the tailings impoundments are an almost inexhaustible source of contamination by sulfide oxidation. Hence, an effective treatment requires environmentally and economically sustainable solutions that can prevent the production of these acidic leachates over long time. To this aim, the addition of a cover of coal combustion fly ash on sulfide mine tailings seems to meet the necessary requirements, minimizing the release of chemical contaminants to the environment.

**THE IMPORTANCE OF CHEMICAL AND BIOLOGICAL PROCESSES IN THE  
PASSIVE REMEDIATION OF IRON-DOMINATED COAL MINE WATERS  
COMPARED TO ZINC-DOMINATED METAL MINE WATERS**

Adam Jarvis

*School of Civil Engineering & Geosciences, Newcastle University, UK*

In the UK the construction and operation of passive treatment systems for remediation of coal mine drainage has been ongoing for nearly 20 years, and there are now more than 50 full-scale systems in operation. These systems primarily consist of aeration, settlement lagoons and aerobic wetland units, in which the primary remediation process is the abiotic oxidation of ferrous iron and precipitation of ferric iron. The performance of these systems is discussed, and some of the limits of their application, and potential difficulties of long-term operation, are discussed. In contrast, drainage from abandoned metal mines in the UK continues to discharge to streams and rivers largely unabated. Measurements of the scale of pollution from these metal mines shows that they are a major source of freshwater pollution in the UK, and therefore in recent years there have been increased efforts to develop full-scale passive treatment systems for their remediation. Much of this effort has been directed into compost-based bioreactors. The performance of laboratory-scale and pilot-scale systems is discussed, highlighting both the performance of the systems and their potential limits, and illustrating the importance of biotic process such as bacterial sulphate reduction in such units.

16.15-17.00

**PASSIVE TREATMENT OF ACID MINE DRAINAGE: CONVENTIONAL  
TREATMENT SYSTEMS AND NEW DEVELOPMENTS BASED ON INORGANIC  
PROCESSES TO REMOVE HIGH METAL CONCENTRATIONS**

Tobias W. Rötting

*Dpt. of Engineering and Geophysics, Catalonia Polytechnics, Barcelona, Spain*

Acid mine drainage (AMD) and other heavy metal-polluted discharges from active or abandoned industrial sites are a major cause of water contamination world-wide. Conventional treatment plants are expensive to operate, and application at remote sites may be impractical. Passive treatment systems which once built only require naturally available energy sources and infrequent maintenance may be an economical option to decontaminate these waters. Nevertheless, they are prone to clogging and passivation (loss of permeability or reactivity, respectively) when used to treat water with high metal concentrations or high acidity loads. This talk will give an overview on existing passive treatment systems and explain new designs based on inorganic processes that were tested in the Iberian Pyrite Belt to remove high metal concentrations from AMD.

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**Illicit Drugs in Wastewaters**

## ORAL PRESENTATIONS

13.30-14.10 - Juan V. Sancho - *Analytical strategies for investigation of illicit drugs in wastewaters. Potential of HR MS in this field*

14.10-14.50 - Barbara Kasprzyk-Hordern - *Illicit drugs in wastewater: chirality and other under-investigated phenomena*

15.30-16.10 - Kevin Thomas - *Comparing illicit drug use in 19 European cities through sewage analysis*

16.10-16.50 - Caleb Banta-Green - *Drug consumption estimation from sewage water analysis versus epidemiological studies*

## Analytical strategies for investigation of illicit drugs in wastewaters. Potential of HRMS in this field

Juan V Sancho<sup>1</sup>, Félix Hernández<sup>1</sup>, Lubertus Bijlsma<sup>1</sup>, Maria Ibáñez<sup>1</sup> and Eduardo Beltran<sup>1</sup>

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The determination of specific biomarkers of drugs of abuse in urban wastewater can be used for estimate their consumption in a particular population. The success of this approach (Sewage epidemiology) relies on the availability of advanced analytical methodologies to identify and quantify these compounds in high complex matrix, like influent wastewater.

Based on the chemical properties of the compounds to be searched and the sample complexity, liquid chromatography coupled to tandem mass spectrometry has become the analytical workhorse in this application field. Despite its high selectivity and sensitivity, sample treatment must be performed yet in order to reach the extremely low levels expected in the samples, at the sub-ppb range. As an example, a typical method based on off-line SPE prior to UHPLC-ESI-MS/MS determination will be discussed, focussing on critical aspects such as matrix effects or confirmation of identity.

One of the disadvantages of tandem mass spectrometry is the need for pre-selecting a precursor ion to be fragmented in the collision cell. This implies that other compounds of interest (illicit drugs in this case), even at high concentrations, are missed and therefore significant information from the samples are overlooked. In order to overcome this handicap, full spectrum acquisitions at high sensitivity and mass accuracy are required to have a complete overview of the compounds present in the samples. In this sense, high-resolution mass spectrometry (HRMS) could fill this gap. Hybrid quadrupole-time of flight (QTOF) mass analyser coupled to ultra performance liquid chromatography can be an efficient approach for discover and identify unexpected illicit drugs and metabolites in wastewater samples.

We can use UHPLC-QTOF MS within this application field in two approaches. First, to perform a wide screening for a huge list of illicit drugs and known metabolites based on the expected presence of the (de)protonated molecule as well as fragment ions in the low and high energy TOFMS spectra, respectively, when using the so-called MS<sup>E</sup> acquisition. In this sense, we can even discover and identify unexpected compounds without reference standards.

A second approach involves the use of UHPLC-QTOF MS in metabolism and degradation studies. The availability of control samples together with specialized software platforms (i.e., Metabolyx) allows us to highlight the presence of related compounds (metabolites and transformation products), which can be tentatively elucidated based on their accurate-mass product ion spectra. As an example, the identification of several transformation products of cocaine and cannabis after different conditions (hydrolysis, chlorination, photolysis,...) will be discussed.

## Illicit drugs in wastewater: chirality and other under-investigated phenomena

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The aim of talk is to discuss recent developments in the analysis of illicit drugs in wastewater. The talk will consist of three sections:

1. Targeted analysis of >60 illicit, abused drugs and their metabolites in wastewater with the usage of multi-residue solid-phase extraction–ultra performance reversed phase liquid chromatography–positive electrospray ionisation tandem mass spectrometry, triple quadrupole (SPE–RPLC–QqQ) method. The results from the analysis of samples from the UK will be presented. Particular emphasis will be put on possible analytical pitfalls.
2. First comprehensive targeted analysis of illicit and abused drugs on suspended particulate matter in wastewater with the usage of a combination of pressurised liquid extraction, solid phase extraction and reversed phase liquid chromatography coupled with tandem mass spectrometry using triple quadrupole (PLE-SPE-RPLC–QqQ).
3. First enantiomeric profiling of chiral drugs in wastewater using multi-residue solid-phase extraction–chiral liquid chromatography–positive electrospray ionisation tandem mass spectrometry using triple quadrupole (SPE–chiral LC–QqQ) and quadrupole-time-of-flight (SPE-chiral LC-QTOF) method. Results from both field study and laboratory microcosm bioreactors will be presented.

The main emphasis will be put on under-investigated aspects of the analysis of drugs in wastewater with an aim of providing reliable estimation of community-wide drug use. They include, among others, stability of illicit and abused drugs, their sorption to solids and their stereoselective fate in humans and in wastewater. The emphasis will be put on chiral analysis, which provides for a distinction between consumption and direct disposal of chiral drugs to wastewater.

### *Acknowledgements*

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Funding to support SEWPROF MC ITN entitled '*A new paradigm in drug use and human health risk assessment: Sewage profiling at the community level*' from the People Programme (Marie Curie Actions) of the European Union's 7th Framework Programme FP7/2007-2013/under REA grant agreement n° [317205] is also acknowledged.

## Comparing illicit drug use in 19 European cities through sewage analysis

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In 2011, a collaborative, Europe-wide study on the analysis of wastewater for estimating the use of illicit drugs on a European scale was performed for the first time. For the comparative study to be successful, a best-practice consensus document and an analytical intercalibration study was performed, and all of the sewer networks and sampling systems characterized by the use of a questionnaire. In 2011, intercalibration showed that the analytical data could be safely compared, with sampling and sewer differences not likely to result in major uncertainties. The approach was thereafter simultaneously applied in 19 European cities, making it possible to directly compare illicit drug loads in Europe over a 1-week period. Our main findings from 2011 were distinct temporal and spatial patterns in drug use across Europe. Cocaine use was higher in Western and Central Europe and lower in Northern and Eastern Europe. The total consumption for Europe as a whole is extrapolated to 356 kilos daily, which would account for approximately 10 – 15 % of the global supply of cocaine (as estimated by the United Nations Office on Drugs and Crime). High per capita ecstasy loads were measured in Dutch cities, as well as in Antwerp and London. In general, cocaine and ecstasy loads were significantly elevated during the weekend compared to weekdays. Per capita loads of methamphetamine were highest in Helsinki, Turku, Oslo and Budweis, while per capita loads of cannabis were similar throughout Europe. Additional studies have been performed in 2012 and 2013 with increasing number of cities (25 and 50 respectively) and repetitive intercalibration exercises. These studies demonstrate that through the use of best-practice and inter-laboratory intercomparison it is possible to spatially and temporally compare differences in drug use through sewage analysis at the international scale.

16.10-16.50

## **Drug consumption estimation from sewage water analysis versus epidemiological studies**

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Wastewater drug testing is a growing area of research particularly in the areas of chemistry and engineering. The data however are often interpreted in the context of human behaviour and population health. Drug epidemiology is an established field whereby various indicator data sources, about a largely hidden phenomenon, are analysed and interpreted to inform public health, public safety and public policy. This presentation will discuss the ultimate, societal goals of wastewater drug testing and subsequent interpretation. The components of a good drug epidemiology major will be reviewed. The attributes of wastewater derived drug use estimates will be examined from an epidemiological perspective. Several examples of classic indicator drug data sources will be reviewed and contrasted with wastewater derived data. The motivations and methods for comparing wastewater and other drug epidemiology data will be detailed. Conclusions and implications will be reviewed.

# **14th EuCheMS International Conference on Chemistry and the Environment**



**ICCE 2013, Barcelona, June 25 - 28, 2013**

## **Book of abstracts**

### **Satellite Event**

**Cyanobacteria and Cyanotoxins in Aquatic  
Environments.**

ICCE 2013 Satellite Event ‘Cyanobacteria – Cyanotoxins in Aquatic Environments’ – Barcelona, 25

June 2013

Program

[http://www.icce2013.org/satellite\\_cyan.html](http://www.icce2013.org/satellite_cyan.html)

09:00	Dionysios Dionysiou, Univ. of Cincinnati : Welcome of participants – Introduction to the event	
09:05	Deniz Karaca, COST Office, and Triantafyllos Kaloudis, EYDAP SA : CYANOCOST: A European network for cyanobacteria and cyanotoxins	
<i>Session I : Ecology of Cyanobacteria – Occurrence of cyanotoxins</i>		
09:20	<b>Linda Lawton</b> , Robert Gordon University, UK	<b>Natural diversity of cyanobacteria and the toxins they produce</b>
09:50	Leonardo Cerasino, IASMA, Italy	Cyanotoxins in the Italian Deep Subalpine Lakes (DSL): distribution and controlling factors
10:10	Bryan Brooks, Baylor University, USA	Cyanobacterial and Other Biological, Chemical and Physical Influences on Harmful Blooms of <i>Prymnesium parvum</i>
10:30	Hanna Mazur-Marzec, Univ. of Gdansk, Poland	Chemotype diversity of the toxic cyanobacterium <i>Nodularia spumigena</i>
Coffee break		
<i>Session II : Health and ecosystem effects</i>		
11:15	<b>Vitor Vasconcelos</b> , Porto University, Portugal	<b>Routes and bioavailability of cyanotoxins and risks for human health</b>
11:45	Antonio Quesada, Universidad Autónoma de Madrid, Spain	Cyanobacterial health risks in inland bathing waters
12:05	Zorica Svircev, University of Novi Sad, Serbia	Epidemiology of primary liver cancer in Serbia and possible connection with cyanobacterial blooms
12:25	Maria Moustaka, Aristotle University, Greece	Harmful Cyanobacterial Blooms in Mediterranean Freshwaters
Poster Session (facilitator: Xuexiang He, University of Cincinnati)		
<i>Session III: Detection of cyanobacteria and cyanotoxins</i>		
13:45	<b>Kaarina Sivonen</b> , University of Helsinki, Finland	<b>Methods to detect producers of cyanobacterial toxins and odorous metabolites</b>
14:15	Cintia Flores, IDAEA-CSIC, Spain	Mass spectrometry techniques for the analysis of cyanotoxins: MALDI-TOF/TOF, LC/ESI-MS/MS AND LC/ESI-HRMS
14:35	Rainer Kurmayer, University of Innsbruck, Austria	Molecular ecology and evolution of toxin-producing cyanobacteria in drinking and recreational water
14:55	Peter Hunter, University of Stirling, UK	Monitoring mass populations of toxic cyanobacteria in lakes using airborne and satellite remote sensing
15:15	Luc Brient, University of Rennes I, France	Detection of benthic cyanobacteria in rivers and lakes
Coffee break		
<i>Session IV: Prevention and treatment</i>		
16:00	<b>Kevin O’Shea</b> , Florida Int. University, USA	<b>Advanced Oxidation of Cyanotoxins: Reaction Pathways and Mechanistic Details.</b>
16:30	Hans Matthijs, Univ. of Amsterdam, Netherlands	Hydrogen peroxide as a biocide for selective suppression of harmful cyanobacteria
16:50	Anastasia Hiskia, NCSR Demokritos, Greece	Recent advances towards water purification from cyanotoxins and taste & odor compounds using photocatalysis with TiO <sub>2</sub> and polyoxometalates
17:10	Konstantinos Kormas, University of Thessaly, Greece	Natural born degraders of cyanobacterial toxins
17:30 Antonio Quesada: Concluding remarks – closing of event		

## **A. ORAL PRESENTATIONS**

## CYANOCOST : A European network for cyanobacteria and cyanotoxins

The Management Committee of CYANOCOST<sup>1,2</sup>

(1) Names - affiliations in: [http://www.cost.eu/domains\\_actions/essem/Actions/ES1105?management](http://www.cost.eu/domains_actions/essem/Actions/ES1105?management)

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CYANOCOST (COST Action ES 1105) is a European network for “Cyanobacterial Blooms and Toxins in Water Resources: Occurrence, Impacts and Management”, operating in the framework of European Cooperation in Science and Technology (COST). The extensive network is active from 2012 to 2016 and currently encompasses research, academia, industry and stakeholder partners from 28 European countries plus the USA. The main objective of CYANOCOST is to increase, disseminate and harmonize capabilities across Europe for the risk management of cyanobacteria and cyanotoxins in water bodies, by establishing strong and synergistic links between academia, authorities, industry and citizens. The network compiles and integrates experience, identifies research needs and gaps, focuses on solutions and disseminates data, results and best practices to end-users and stakeholders to protect public health, utilities, facilities and enterprises and hence contribute to European science, society and the economy. Participants are organized into four Working Groups (WGs): WG1 – occurrence of cyanobacteria and cyanotoxins; WG2 – fates, impacts and effects; WG3 – prevention and control measures and WG4 – end-user and outreach tools, materials and products. Currently, handbooks and special issues on cyanobacterial and cyanotoxins monitoring and analysis, molecular biological methods, in-lake control and prevention measures and water treatment processes are under preparation. A database to include relevant information is also under development. CYANOCOST additionally offers grants for Short Term Scientific Missions (STSMs), especially targeted to Early Stage Researchers (ESRs), which aims to promote the dissemination of know-how and expertise throughout Europe. As the CYANOCOST network expands to embrace more countries, researchers and stakeholders, there are numerous opportunities for interested parties to be involved in its activities. Information about CYANOCOST can be found in [www.cyanocost.com](http://www.cyanocost.com).

## Natural diversity of cyanobacteria and the toxins they produce

Linda A Lawton, IDEAS Research Institute, School of Pharmacy & Life Sciences, Robert Gordon University, Aberdeen, UK

Cyanobacteria (blue-green algae) are an ancient group of photoautotrophic bacteria which are ubiquitous in aquatic environments, found in many terrestrial locations and co-exist as symbiotes with a number of higher organisms. Their success in colonising diverse habitats, including extreme environments, can be attributed to their prolonged existence on Earth (c. 3.5 billion years) and their ability to adapt. One characteristic that may support their survival under diverse conditions could be linked to their ability to produce a significant array of bioactive compounds. Many of these compounds have been found to be toxic to other organisms including plants, animals and humans, hence they have been collectively labelled cyanotoxins. The function of these compounds has yet to be fully elucidated but considerable work has been carried out to characterise, monitor and limit the adverse impact they may have. Of the bioactive compounds produced, peptides have been found to be particularly numerous. Microcystins (cyclic hepatotoxic peptides) are the most widely studied and are recognised as a potent hazardous contaminant of freshwaters including those used for recreation, agriculture and drinking water supplies. The structurally related peptide, nodularin, was the first of the cyanotoxins to be reported in the scientific literature almost 150 years ago and still presents a hazard particularly in brackish waters. Other compounds of concern have been collectively grouped as neurotoxins, namely, saxitoxins, anatoxin-a and anatoxin-a(s) and although structurally unrelated they have been observed to cause rapid fatality in domestic animals and pets, following consumption of contaminated water. In the last 20 years or so, the detection and occurrence of cylindrospermopsin, a polycyclic uracil derivative that inhibits protein synthesis, has also resulted in concern for human and animal health. With further potentially hazardous cyanobacterial metabolites continuing to come to light, sustained vigilance is required.

# Cyanotoxins in the Italian Deep Subalpine Lakes (DSL): distribution and controlling factors

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Toxic cyanobacteria blooms represent a serious threat for many aquatic ecosystems worldwide. The most common potentially toxic cyanobacteria in temperate waters belong to the genera *Microcystis*, *Planktothrix*, and *Dolichospermum*, which have the ability of producing microcystins and anatoxins. Toxins can produce severe effects on humans directly (contact or ingestion of contaminated water) or indirectly (consumption of contaminated food).

Deep Subalpine lakes Garda, Iseo, Como, Maggiore, and Lugano are important water resources in the Italian Northern district. We have conducted a sampling campaign in these lakes with the aim of compare the cyanotoxin diversity. We have applied advanced analytical techniques based on LC-MS/MS technologies. Two main classes of toxins have been found: microcystins (MCs) and anatoxins. A big variability in MC variants and concentrations has been found among the lakes. In the largest lake (Garda) we have also investigated in detail the temporal dynamics of the toxin production. The concentration of total MCs was strictly linked to the temporal dynamics of *Planktothrix rubescens* in the lake, irrespective of the season and vertical depth. These findings suggest a constitutive MC production relatively independent from environmental factors. It is stressed how the estimation of models linking MCs and cyanobacteria abundances should provide a greater reliability in the definition of management strategies aimed at minimising the risks due to cyanobacteria development.

# **Cyanobacterial and Other Biological, Chemical and Physical Influences on Harmful Blooms of *Prymnesium parvum***

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Environmental assessment and management faces challenges associated with contaminants for which limited information is available for their fate and effects. Though regulatory programs have developed approaches to characterize risks from chemicals of historical concern and related chemical management programs, such approaches are not as widely defined or employed, however, for chemicals of emerging concern (CECs) and harmful algal blooms (HABs), whose increase in frequency and magnitude over the past few decades has occurred at the global scale. Though much attention has been given to coastal HABs, inland waters often experience HABs, which can be exacerbated by anthropogenic activities. Cyanobacterial HABs remain a critical important threat in inland water quality. In fact, their toxins represent CECs that can alter the effectiveness of traditional surface water quality management frameworks, and influence HAB development of other harmful algae. For example, HABs of *Prymnesium parvum*, an invasive haptophyte historically observed in estuarine and coastal habitats, have increasingly produced devastating fish kills in inland waters of the United States and other parts of the world. This presentation will examine influences of cyanobacteria on *P. parvum* blooms within the context of defining biological, chemical and physical factors forcing the development and termination of *P. parvum* HABs in inland waters.

# Chemotype diversity of the toxic cyanobacterium *Nodularia spumigena*

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The bloom-forming cyanobacterium, *Nodularia spumigena*, is known as a producer of the hepatotoxic cyclic peptide called nodularin (LD<sub>50</sub> = 50 µg/kg). This toxin, as many other cyanobacterial peptides, is synthesized non-ribosomally by multifunctional enzyme complexes. Due to specific structure of the complexes, a variety of congeners within one class of peptides is usually biosynthesized.

Despite the increasing interest in cyanobacterial non-ribosomal peptides, the ecological function of these metabolites is still unknown. However, many studies focus on their important biological activity and potential biotechnological application.

In the work, liquid chromatography – tandem mass spectrometry was used to determine the diversity of peptides produced by *N. spumigena* from the Baltic Sea (17 strains) and from the coastal waters of southern Australia (8 strains). To characterize the structures of the compounds, their enhanced ion product spectra were collected with QTRAP5500 system. Altogether 50 peptides, including 25 new congeners, were detected. The compounds belong to nodularins and spumigins, which are almost exclusively produced by *N. spumigena*, and to aeruginosins and anabaenopeptins, also produced by some other cyanobacterial taxa.

The analyses of peptide profiles showed that some of their features were characteristic only for strains isolated from a given geographical region. Additionally, the principal component analysis of *N. spumigena* peptides placed the Baltic strains in metabolically diverse chemotypes with 11-24 peptide structures. The Australian strains were distantly related to the strains from the Baltic Sea. They produced lower number of peptides (9-12) and were grouped into one chemotype.

The grouping of *N. spumigena* strains based on peptide profiles corresponded well with the genetic diversity of the species determined based on the sequence analyses of the phycocyanin intergenic spacer region (cpcBA-IGS).

The study showed that oligopeptide profile of *N. spumigena* is a stable and specific chemotaxonomic feature and can be used to discriminate the metabolically diverse sub-populations within the species.

## Routes and bioavailability of cyanotoxins and risks for human health

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Cyanobacteria toxins have been studied for the last decades in special in what concerns with diversity, chemical characterization, physiological effects, detection methods and risk characterization. In this last aspect, most of the approaches deal with the main route for intake of cyanotoxins which is via contaminated drinking water. In fact this maybe the main route worldwide due to the extensive eutrophication of freshwater systems, but other routes should be considered. In this talk the diversity of routes will be discussed together with the main cyanotoxins that pose in our days problems to human health. Dialysis, dermal contact, nasal and oral intake (water and food items) will be ranked and the needs for future research in order to have comprehensive risks assessments will be highlighted. A special emphasis will be given to food items contaminated with cyanotoxins, including plants, food supplements, and aquatic invertebrates and vertebrates. Some cyanotoxins such as microcystins may be found bound to proteins in contaminated food items, and the preservation and cooking processes may change the bioavailability of the toxins. Recent works show that this may happen, so the risk assessment should include this bioavailability approach.

## Cyanobacterial health risks in bathing waters.

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The European Directive on Bathing Waters considers cyanobacteria as one of the variables to be included when creating the safety profiles of these recreational waters. Non-marine bathing waters are quite numerous and popular in Europe and consist on rivers and water reservoirs. Every country produces a list of the official natural inland bathing sites and presents a particular strategy to assess the health risks derived of the massive growth of cyanobacteria. Our group has investigated the official inland bathing sites, analysing the occurrence of potentially toxic cyanobacteria and the concentrations of microcystins, cylindrospermopsin and anatoxin-a. Our results indicate that cyanobacteria were quite abundant in about 25% of sites and, while anatoxin was present in only one sampling site, microcystins and cylindrospermopsin were detected in 13 and 6 sampling bathing sites respectively.

This nation-wide study represented a good opportunity to investigate the distribution of cyanobacteria and cyanotoxins in a large number of ecosystems in an extensive geographical scale and along the complete summer season (July-September). Apparently there are not clear relationships between the climatic and lithological variables and the occurrence of cyanobacteria and or cyanotoxins. In that way, only trophic status seems to govern cyanobacterial blooming. *Microcystis* appears as the most abundant potentially toxic genus in the country. *Aphanizomenon ovalisporum* can be considered as a real risk for human health, because it always produces its toxin, cylindrospermopsin, which seems to be degraded very slowly in Spanish waters. On the other hand anatoxin-a seems to represent only a marginal risk for human health in these waters. Other compounds as PSPs (saxitoxins) or BMAA have not been included in this study but new data indicate that they should be included for future works

# EPIDEMIOLOGY OF PRIMARY LIVER CANCER IN SERBIA AND POSSIBLE CONNECTION WITH CYANOBACTERIAL BLOOMS

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Nowadays, the occurrence of harmful cyanobacterial blooms becomes a common phenomenon. Cyanobacteria can produce metabolites highly toxic to humans. More than 80% of reservoirs used for water supply in Central Serbia have bloomed over the past 80 years. Ten-year epidemiological study shows a significant increase in the incidence of primary liver cancer (PLC) in the regions where water from the blooming reservoirs is used for human consumption. At the same time, no correlation was found between the incidence of PLC and other risk factors, such as cirrhosis and hepatitis viruses. It is also possible that acting as initiators and/or cofactors, cyanotoxins disguise a positive correlation between incidences of PLC and its risk factors, making it even unexpected: higher incidence of risk factors - lower incidence of PLC and vice versa. As one of the most effective methods for breakdown and removal of MCs from raw water during water treatment, ozonation might be considered as preventive measure in drinking water pretreatment processes, but together with possible negative side effects, like expressive incidence of non-Hodgkin's lymphoma in districts with ozonation management. Therefore, additional assessment of the health risks that arise from human exposure to cyanotoxins is advisable.

**Key Words:** Cyanotoxins; epidemiology; primary liver cancer (PLC); cirrhosis; HCV; HBV; ozonation; Serbia

# Harmful Cyanobacterial Blooms in Mediterranean Freshwaters

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Cyanobacterial blooms are a frequent phenomenon in eutrophic freshwaters worldwide as in the Mediterranean region having a major impact on quality of freshwater ecosystem services, particularly on drinking water supply and recreational use. The objectives of this work are: (a) to give a review of the published information on cyanobacteria blooms occurrence and the presence of toxin producing species in lakes and reservoirs of 21 countries bordering the Mediterranean and (b) to use original and published data from studies on cyanobacterial blooms in Greek freshwaters of different type (hydrological regime and morphometric/topographic characteristics) to highlight the compositional diversity of the blooms, the ecology of the bloom-forming cyanobacteria and the link of cyanobacterial blooms to water quality, providing knowledge for setting ecological restoration targets. Studies on cyanobacterial blooms were often restricted to individual freshwaters and which may not be representative as a whole of the country. Nevertheless, in Spain, France, Italy, Greece, Turkey, Israel, Morocco and Algeria information on toxic cyanobacterial blooms is sufficient to demonstrate the need for cyanotoxin risk assessment and ecological water quality management. In contrast, there is no published information on cyanobacterial blooms in 9 Mediterranean countries. The most common bloom-forming genera in Mediterranean freshwaters were *Microcystis*, *Planktothrix*, *Aphanizomenon*, *Anabaena* and *Cylindrospermopsis/Raphidiopsis*. All these genera have been found in toxic blooms. Potentially toxin producing species of these genera were found in all of the studied Greek freshwaters. Phylotypes and morphotypes belonging to the genera *Microcystis*, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis/Raphidiopsis*, *Limnothrix*, *Planktothrix*, *Anabaenopsis* and *Cuspidothrix* comprised most of the diversity of the bloom-forming cyanobacteria in Greek freshwaters. The bloom-forming species showed different tolerances and sensitivities to lake water flushing, light conditions, nutrients and salinity. Different cyanobacteria coexisted in a lake by shifting their niches in time and vertically in the water column. In this work, we emphasize the link of harmful cyanobacterial blooms to water quality according to both Water Framework Directive and World Health Organization guidelines.

# Methods to detect producers of cyanobacterial toxins and odorous metabolites

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Mass occurrences (blooms) of cyanobacteria are common worldwide and can be highly toxic or cause odour problems in water and fish. Blooms are often hepatotoxic containing peptide toxins (microcystins and nodularins) or neurotoxic having anatoxin-a, anatoxin-a(S) or saxitoxins. Toxic blooms have caused number of animal poisonings and are health risk for human beings. Toxin producers can not be differentiated with microscopy since toxic and non-toxic strains of the same species occur. The same is true for the producers of cyanobacterial odorous metabolites geosmin and 2- methylisoborneol (2-MIB). Presently, biosynthesis of many of these compounds is known. Detection of genes responsible for toxin/odorous metabolite production offer excellent way to identify potential producers of these compounds. We have developed PCR, qPCR methods and chip assays to study presence of potentially hepatotoxin (microcystin and nodularin) producing strains. The analyses can be carried out based on the isolated DNA and RNA from the environment and reveal which genera of the producers are present and in the case of qPCR can be also give quantitative information. DGGE based microcystin/nodularin synthetase gene analyses reveal unknown diversity. Most recently genome project of *Anabaena* strain 37 revealed the anatoxin-a synthetase genes and the method to detect various anatoxin-a producers was developed. Examples of the use of these methods will be given. Cyanobacteria are the major producers of geosmin and 2- MIB in aquatic environments and their biosynthesis was recently discovered. We screened 100 cyanobacterial strains from 20 different genera to identify possible producers of these odorous metabolites. Identification was made with solid-phase microextraction coupled with gas-chromatography/mass-spectrometry (SPME GC-MS). We designed cyanobacteria-specific primers to detect the *geoA* gene involved in the biosynthesis of geosmin and the 2- MIB synthase gene. Geosmin producers (21) were found from six genera: *Nostoc*, *Aphanizomenon*, *Oscillatoria*, *Planktothrix*, *Cylindrospermum* and *Calothrix*. Two 2- MIB producers were found belonging to genera *Oscillatoria* and *Planktothrix*. PCR results were consistent with SPME GC-MS detection. Developed molecular tools can be used in monitoring both toxin and odorous metabolite producing cyanobacteria in environmental samples and reveal their associations to environmental factors.

# MASS SPECTROMETRY TECHNIQUES FOR THE ANALYSIS OF CYANOTOXINS: MALDI-TOF/TOF, LC/ESI-MS/MS AND LC/ESI-HRMS

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Cyanotoxins have become a serious ecological and health issue due to the massive cyanobacterial water blooms that have developed in eutrophied waters worldwide. Consequently, various water authorities have developed cyanobacterial alert levels and cyanotoxin threshold values for drinking water production and recreational activities to protect water consumers.

To date the analysis of cyanotoxins has been approached from different perspectives (bioassays, ELISA and PPA kits, molecular techniques and different HPLC methodologies). The different methodologies have been updated in order to increase the specificity, selectivity and sensitivity of analytical methods and to reduce the analysis time and sample handling. In this regard we have developed different methodologies for the determination of cyanotoxins based on mass spectrometry. Mass spectrometry techniques are a powerful tool for the identification and quantitation of environmental pollutants. The study of fragmentation pathways by tandem mass spectrometry analysis, allows crucial structural information. On the other hand, accurate mass measurements by high resolution mass spectrometry (HRMS) allow knowing the molecular formula of an analyte, if working with the appropriate resolution and accuracy.

In the present work, SPE *on-line* LC/ESI-MS/MS, MALDI-TOF/TOF and LC/ESI-HRMS techniques are applied for the analysis of cyanotoxins in natural *blooms*, mainly focused in microcystins and other cyanotoxins. SPE *on-line* offers practical advantages over *off-line* methods: reduction of the sample volume (even 1mL), decrease of the time; improvement of the sample throughput; and the automation of the method. MALDI-TOF/TOF methodology allows the rapid and direct analysis of all together analytes in the aqueous samples and biomass, without prior chromatographic separation. Moreover, Orbitrap technology enables to work in HRMS for both, precursor and product ion, in tandem mass spectrometry (HRMS/HRMS). Additionally, it is possible to make retrospective analysis of samples in Full Scan mode (MALDI-TOF and LC/ESI-HRMS) without re-injecting. The fragmentation pattern of detected toxins, in positive and negative MALDI and ESI-modes, has been studied. Finally, results from the all analytical methodologies (QqQ, MALDI-TOF/TOF and Orbitrap) were combined.

# **Molecular ecology and evolution of toxin-producing cyanobacteria in drinking and recreational water**

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Harmful algal blooms formed by cyanobacteria (cyanoHABs) are a global phenomenon observed both in fresh and brackish water. The human society is facing a couple of toxic compounds of different chemical structure that are produced by several genera, with some of them leading to outbreaks and diseases in livestock and humans. In addition an impressive number of bioactive (related) secondary metabolites are produced, while genotypes differing in the production of these compounds frequently co-occur.

It is a major challenge for ecology to identify the factors leading to toxic outbreaks which is due to the development of toxic ecotypes carrying the genes for the production of a certain toxin. In both theoretical and experimental ecology a certain toxin-producing phenotype is considered relatively static, i.e. costs and benefits of toxin synthesis are related to its ecological fitness.

I argue that this approach represents a single snapshot sampling event and cannot address the diversification of toxic and nontoxic ecotypes driven by selective factors also not directly linked to toxin production. As lakes and reservoirs are ephemeral in terms of geological time scales when compared with marine ecosystems, the current success of some toxic bloom-forming species may rather result from opportunistic abilities than from an adaptation to a specific freshwater ecosystem.

The elucidation of the genes involved in the synthesis of these toxins paved the way to

- (i) Explore time scales and mechanisms in the evolution of responsible genes involved in cyanoHAB formation.
- (ii) Postulate hypotheses about factors driving the innovation of toxin synthesis
- (iii) Forecast the ecological fitness of toxic/non-toxic genotypes during cyanoHAB development

These questions are addressed by integrating of the ecology and the genetic analysis of ecotypes through a comprehensive field and laboratory research program. The ultimate aim is to use these results for a more efficient protection/restoration of aquatic ecosystems.

# Monitoring mass populations of toxic cyanobacteria in lakes using airborne and satellite remote sensing

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Mass populations of toxin-producing cyanobacteria occur almost globally in nutrient-enriched inland and coastal waters and are a major concern for the ecological status of these water bodies and for animal and human health. It is therefore crucial that effective tools are developed to gather timely and reliable data on the occurrence of cyanobacterial blooms and their responses to environmental change. This talk will explain, using case studies from lakes in the United Kingdom and Lake Balaton in Hungary, how airborne and satellite remote sensing can be used to detect and quantify cyanobacterial populations in lakes and coastal waters. Further, it will outline how forthcoming advances in satellite technology, particularly the European Space Agency's Global Monitoring for Environment and Security (GMES) programme, will move us closer towards operational monitoring of algal and cyanobacterial blooms from space.

## Detection of benthic cyanobacteria in rivers and lakes

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Over the last few years, mortality of animals near waterbodies have led scientists to highlight the presence of neurotoxic epiphytic or benthic cyanobacteria as the cause of their death. These benthic cyanobacteria are present in all aquatic environments and also in terrestrial ecosystems living in symbiosis with lichen. They are constituents of biofilms on stones in rivers, on macrophytes, in surface sediments in water bodies at shallow depth, on mudflats and on the bark of trees inundated during dam filling. Now that their presence begins to be affirmed, these benthic cyanobacteria raise management problems given the potential of toxins production. Fluorescence probes for the detection of phycocyanin, a pigment specific to cyanobacteria, are practical tools that provide a semi-quantitative proxy for their biomass. Their simplicity of use allow the sampling of multiple data points in the field together with other physico-chemical environmental parameters to be representative of rapidly changing conditions. This relatively new approach to the world of benthic cyanobacteria requires further investigations to assist water managers with practical protocols.

# Advanced Oxidation of Cyanotoxins: Reaction Pathways and Mechanistic Details.

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Advanced oxidation technologies (AOTs) are effective for the remediation of a wide variety of pollutants and chemical toxins. The recent focus of our research projects has been on the treatment of naturally occurring toxins, specifically cyanotoxins produced in surface waters during harmful algal blooms. These naturally occurring toxins contaminate drinking water sources and create a unique challenge for drinking water suppliers because they do not come from a point or industrial sources. We have conducted detailed kinetic and product studies on the advanced oxidation of problematic cyanotoxins, microcystin-LR (MCLR) and cylindrospermopsin (CYN). A characteristic feature of all MCs and the analogous cyclic pentapeptides, nodurlins, is the hydrophobic ADDA side chain with diene and aromatic functionalities. The ADDA chain is critical to the biological activity of MCs and highly susceptible to oxidation by a variety of AOTs. MCs are readily degraded and detoxified by a variety of AOTs. Radiolytic and UV TiO<sub>2</sub> photocatalytic treatment of CYN leads to rapid degradation of the toxin with the formation of typical hydroxyl radical oxidation products. Detailed studies on the oxidation of cyanotoxins by different AOTs will be presented.

# Hydrogen peroxide as a biocide for selective suppression of harmful cyanobacteria

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In the environment abundant growth of harmful cyanobacteria is a world-wide problem. Using progress in photosynthesis research as a clue, a method has been developed for selective suppression of cyanobacteria with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Applications have been successful in a number of natural lakes, where a single treatment with homogeneously distributed H<sub>2</sub>O<sub>2</sub> prohibited blooming of cyanobacteria during the season. The method is based on the surprising observation that cyanobacteria with surplus availability of electrons at the stromal side of photosystem I make use of a Mehler type reaction that makes H<sub>2</sub>O and not H<sub>2</sub>O<sub>2</sub>. The latter is normally produced in plant and green algal chloroplasts during light and nutrient stress. In designing of the method the reasoning has been that lack of earlier exposure to H<sub>2</sub>O<sub>2</sub> makes expression for anti-oxidative stress capacity less required in cyanobacteria than in green algae. Hence, cyanobacteria when suddenly exposed to H<sub>2</sub>O<sub>2</sub> will be sensitive, and green algae are not.

# Recent advances towards water purification from cyanotoxins and taste & odor compounds using photocatalysis with TiO<sub>2</sub> and polyoxometalates

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Effective and environmental friendly methods for water treatment are being developed as alternatives to conventional disinfection-oxidation methods. They are generally referred to as Advanced Oxidation Processes (AOPs) and involve mainly UV light in the presence of hydrogen peroxide or ozone, UV-near visible light in the presence of TiO<sub>2</sub> or polyoxometalates, Fenton reagent, sonolysis, etc. These methods can achieve mineralization of pollutants and are based mainly on the formation of the highly oxidizing ·OH radicals at ambient temperature and atmospheric pressure. In the frame of our studies, the degradation of a potent cyanobacterial toxin, microcystin-LR (MC-LR) in water was investigated using TiO<sub>2</sub> based photocatalysts, i.e. nitrogen doped TiO<sub>2</sub> (N-TiO<sub>2</sub>) and reduced graphene oxide-TiO<sub>2</sub> composite (GO-TiO<sub>2</sub>) under UV-A, solar and visible light. Commercial Degussa P25 TiO<sub>2</sub>, Kronos and reference TiO<sub>2</sub> nanopowders were used for comparison. It was found that under UV-A irradiation, all photocatalysts were effective in toxin elimination with Degussa P25 TiO<sub>2</sub> to be the most effective. Under solar light irradiation N-TiO<sub>2</sub> and GO-TiO<sub>2</sub> photocatalysts presented similar photocatalytic activity with commercially available materials such as Degussa P25 and Kronos TiO<sub>2</sub> for the destruction of MC-LR. Especially, upon irradiation of N-TiO<sub>2</sub> with visible light, Degussa P25 practically did not show any response, while the N-TiO<sub>2</sub> displayed remarkable photocatalytic efficiency. These results indicate that appropriate doping of TiO<sub>2</sub> exhibits a remarkable photo-efficiency in the solar or visible light that could be promising for the degradation of toxic organic pollutants in water, overcoming limitations concerning UV light utilization. Additionally, the photocatalytic degradation and mineralization of cyanobacterial off-odor compounds geosmin/2-methylisoborneol (GSM/MIB), was achieved under UV-A in the presence of polyoxometalates (SiW<sub>12</sub>O<sub>40</sub><sup>3-</sup>). A plethora of intermediates were identified and degradation pathways are proposed. Similarities with TiO<sub>2</sub> photocatalysis suggest that in both cases ·OH is mainly responsible for the photodegradation mechanism.

# Natural born degraders of cyanobacterial toxins

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Cyanobacteria are among the most charismatic microorganisms on the planet. Although they are ubiquitous in nature and their ecological significance has been recognised for long, they pose conflicting interest to humans. On one side, some Cyanobacteria can provide beneficial services to humans like food supplements, bioactive compounds, etc., while others can produce toxic compounds as secondary metabolites, i.e. toxins. These compounds are of variable toxicity to plants, animals and humans and cause major problems even at the ecosystem level, especially in freshwaters with intense and prolonged cyanobacterial blooms. To date, there are several ways to remove, degrade or transform these toxins by chemical methods, due to the satisfying chemical characterization of the majority of the known cyanobacterial toxins. This contradicts with our knowledge on the biodegradation of these compounds, which is markedly lacking mostly due to the limited number of available prokaryotes with proven toxin degrading metabolic features. As a first step towards the discovery of such new microorganisms, in this paper we present an overview based on the 16S rRNA and *mlrA* (microcystinase) genes diversity of all the known isolates that are known to degrade cyanobacterial toxins in freshwaters. The available data revealed that there is a rather limited number of Bacteria, that can degrade cyanobacterial toxins, bearing in mind the high species richness occurring in freshwaters. These Bacteria belong primarily to the Proteobacteria, with several strains from the sphingomonads, and one from each of the *Methylobacillus* and *Paucibacter* genera. Other strains belong to the genera of *Arthrobacter*, *Bacillus* and *Lactobacillus*. No members of Archaea have been found to date with some kind of cyanobacterial toxin degrading association. By combining the ecological knowledge on the distribution, abundance and ecophysiology of the Bacteria that co-occur with toxic cyanobacterial blooms and the newly developed molecular approaches, it is plausible to discover not only more strains with cyanobacterial toxin degradation features, but also to reveal their metabolism and to effectively test their degradation ability.

## **B. POSTER PRESENTATIONS**

# Magnetic Iron Oxides as a Novel Strategy for the Removal of Microcystin-LR from Water

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The incidences of cyanobacterial blooms in freshwater bodies have increased due to the eutrophication and global warming. Microcystins (cyclic heptapeptides) are produced by cyanobacteria and pose serious chronic and acute toxic effects on human beings and animals. While approximately 80 different variants of microcystins have been identified, microcystin-LR (MC-LR) is one of the most toxic and problematic variants. MC-LR is chemically stable and conventional water treatment methods are often not effective for its removal from water. In this study, a number of magnetite materials were synthesized with different co-precipitation methods and applied for the removal of MC-LR. Select magnetite materials exhibit high adsorption efficiency of MC-LR and can be easily separated by applying an external magnetic field after the treatment. The adsorption efficiency increased as the increase of loading of magnetite particles. The adsorption of MC-LR follows Freundlich adsorption isotherm nicely. The addition of hydrogen peroxide can increase the removal of MC-LR via a Fenton reaction, and the removal efficiency increased as the increase of hydrogen peroxide. Combination of ultrasound, hydrogen peroxide, and magnetite particles as an advanced oxidation technology showed a promising performance for the removal of MC-LR from water. Our results indicate that this novel strategy is promising for the removal of MC-LR from aqueous solutions.

# Photocatalytic Degradation of Cyanobacterial Metabolites in Water using Reduced Graphene Oxide-TiO<sub>2</sub> Composite

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Microcystins (MCs) are natural toxins produced during cyanobacterial blooms that occur in freshwaters worldwide. Microcystin-LR (MC-LR) is the most common and toxic MC variant. Geosmin (GSM) and 2-methylisoborneol (MIB) are other cyanobacterial metabolites that can taint water causing undesirable taste and odor. The photocatalytic degradation of MC-LR, GSM and MIB in water under both UV-A and solar light in the presence of reduced graphene oxide-TiO<sub>2</sub> composite (GO-TiO<sub>2</sub>) was studied. Two commercially available TiO<sub>2</sub> materials (Degussa P25 and Kronos) and a reference TiO<sub>2</sub> material prepared in the laboratory (Ref-TiO<sub>2</sub>) were used for comparison. Under UV-A Degussa P25 was the most efficient photocatalyst for the degradation of all target analytes followed by GO-TiO<sub>2</sub> and then by Ref-TiO<sub>2</sub> and Kronos. Under solar light irradiation GO-TiO<sub>2</sub> presented similar photocatalytic activity to Degussa P25 followed by Kronos and Ref-TiO<sub>2</sub>. Intermediate products formed during the photocatalytic process with GO-TiO<sub>2</sub> under solar light were identified and were generally identical to those observed by Degussa P25 under UV-A. Assessment of the residual toxicity of MC-LR at the course of the GO-TiO<sub>2</sub> photocatalytic process showed that toxicity is proportional only to the remaining MC-LR in solution. The applicability of GO-TiO<sub>2</sub> was evaluated under solar light illumination in real surface water samples and GO-TiO<sub>2</sub> proved to be effective in the degradation of all target cyanobacterial metabolites.

# Monitoring of Microcystins in Lake Marathonas, a Water Reservoir of Athens, Greece

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The formation of toxic cyanobacterial blooms and the contamination of water bodies by cyanotoxins is a very serious concern, especially in countries in which surface water is used as the main source for human consumption. Among cyanotoxins, the hepatotoxic cyclic heptapeptides microcystins (MCs) seem to be the most widespread class of toxins that are present in fresh and brackish waters. Lake Marathonas, formed by the construction of Marathon Dam near the town of Marathon in the south east of Athens, is one of the drinking water reservoirs of Athens. This study presents the results of the quantitative monitoring of four MCs, i.e. MC-LR, -RR, -YR, -LA and Nodularin (a similar to MCs toxic cyclic pentapeptide) in Lake Marathonas, from 2007 to 2010. Identification and quantification of the target cyanotoxins was carried out by LC-ESI-MS/MS. Monitoring included several sampling points and it was divided into four time periods, each starting from July and ending to June next year. Of the compounds investigated, all target MCs were detected and identified. NOD was not detected in any sample. During the first three time periods, the predominant toxin was MC-RR ranging from 2-60, 3-65 and 3-63 ng L<sup>-1</sup>, respectively, followed by MC-LR detected in a range from 2-15, 2-12 and 2-25 ng L<sup>-1</sup>, respectively. MC-YR and MC-LA were found in traces during these periods. Higher concentrations of MCs were detected in the fourth period from 7/2010- 12/2010. Contrary to previous results, MC-YR was the predominant toxin in almost all samples at concentrations up to 717 ng L<sup>-1</sup>. MC-LR and MC-RR was also detected in almost all samples in concentrations up to 452 and 174, respectively. MC-LA was not detected during this period. Monitoring results indicate a seasonal trend with an increase from September to the end of each year. Samples from the treated drinking water were also analysed during these periods and none of the target compounds was detected in the finished water. The results of this study constitute the first report on the presence, concentration levels and seasonal variations of MCs in Lake Marathonas.

## Cyanotoxins in natural waters of North-West part of Russia

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During last decades, a lot of attention has been directed to cyanobacteria as potent producers of toxic substances during cyanobacterial water blooms. Harmful Algal Blooms (HAB) have been also noted in waterbodies of North-West part of Russia. The important contribution to the phytoplankton was made by cyanobacteria, producing from 30 up to 99% of total biomass. *Aphanizomenon flos-aquae*, *Planktothrix agardhii*, species of *Microcystis* (*M. aeruginosa*, *M. wesenbergii*, *M. viridis*) has been found as dominated species in plankton.

Since 2008 SRCES RAS has obtained the information on the seasonal dynamics of cyanobacteria, their distribution and concentrations of different cyanotoxins in natural waters of European part of Russia (lakes in Saint-Petersburg area and coastal zone of the Gulf of Finland).

The information about specificity of cyanobacteria seasonal dynamics in these water bodies was received by analyzing the phytoplankton structure. The information obtained by hydrobiological and biology-chemical methods (such as calculated outcome of the biomass of phytoplankton, determination of the dominating species of algae and biogenic elements of natural samples) helps to suppose the presence of cyanotoxin species.

Determination of the cyanobacterial toxins has been carried out using high-performance liquid chromatography - tandem mass-spectrometry with high-resolution on LTQ Orbitrap instrument with linear and orbital traps in the electrospray-ionization (ESI+). Dissolved toxins (in filtrated water) and intracellular toxins (in biomass) were analyzed in each sample. Clean up procedure of filtrates included the solid phase extraction - SPE (Oasis HLB). Extraction of intracellular toxins from biomass was done by US-extraction. The data of chromato-mass-spectrometry analysis of water and phytoplankton samples of investigated waterbodies of Northwest part of Russia showed the presence of different cyanobacterial metabolites in definable concentrations during warm periods of 2010-2012. The list of cyanotoxins and anabaenopeptides producing by microalgae was determined.

Maximum total concentration of detected dissolved microcystins (including demethylated forms) in natural water was 1,2 mkg/l noted in the middle of July of 2012 when the cyanobacteria biomass was also the highest. Maximum total concentration of detected intracellular microcystins was 2,3 mg/g dry weight, detected in August of 2012. The presence of anatoxine was detected in several samples only in one of investigated water body (Suzdal lake). Maximum concentration of anatoxin-a in water (540 ng/l) has been detected in the middle of July in 2010.

It was shown that the set of toxins and their concentrations varied significantly between investigated water bodies.

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### **Removal of Cyanobacterial Toxin Cylindrospermopsin by UV-mediated Advanced Oxidation Processes**

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With the increasing occurrence of cyanobacterial harmful algal bloom events around the world, it is vital to develop an efficient process to remove such toxins that could be produced and released into drinking water sources. Cylindrospermopsin (CYN), although not detected until recently, is considered as a highly toxic cyanobacterial toxin that presents a potential health risk to humans. In this study, the effective removal of CYN from sources of drinking water supply by UV-mediated advanced oxidation processes (AOPs) was investigated. It was found that there was nearly no degradation of CYN at a UV-254 nm fluence as high as 640 mJ/cm<sup>2</sup>; however, when the AOPs were applied, a significant improvement was achieved, suggesting the important roles of radicals, i.e., hydroxyl radicals and sulfate radicals. The presence of dissolved organic matter, as presented by TOC, UV-254 nm absorbance and fluorescence index, and alkalinity showed an inhibition on the destruction of CYN by the AOPs. The formation of hydroxylated-CYN confirmed the successful transformation of CYN, which provides us a better understanding of the mechanisms by the AOPs.