## WORKSHOP

### Cyanotoxins in Fresh Waters
### Advances in Analysis, Occurrence, Treatment
### May 5th 2014, Athens, Greece

# BOOK OF ABSTRACTS

**Organized by:**

**NCSR Demokritos**, Institute of Nanoscience and Nanotechnology, Laboratory of Nanomaterial-Based Photocatalytic Processes and Environmental Analysis

**CyanoWater** - “Cyanotoxins in Fresh Water. Advances in Analysis, Occurrence and Treatment”, Operational Programme "Education and Lifelong Learning", Action “ARISTEIA”.

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CyanoWater

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CyanoWater

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## Cyanotoxins in Fresh Waters

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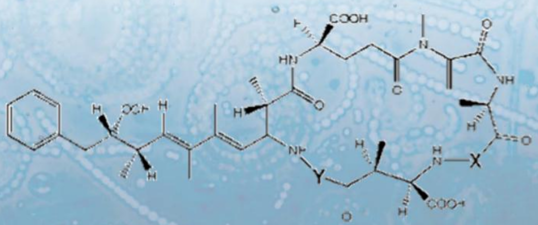

## Monday, May 5th 2014

09:00—16:00

## NCSR Demokritos Conference Center

P. Grigoriou & Neapoleos, Ag. Paraskevi Attikis

**Free admission — Registration on-site**

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**CyanoWater** - "Cyanotoxins in Fresh Water. Advances in Analysis, Occurrence and Treatment", Operational Programme "Education and Lifelong Learning", Action "ARISTEIA".

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**Cyanotoxins** comprise a large group of potent toxins (e.g. peptides, alkaloids, amino-acids) produced by cyanobacteria that are common in surface waters and tend to form blooms in lakes and other freshwater bodies. Exposure through drinking or bathing in waters containing cyanotoxins can seriously affect human and animal health: cyanotoxins may damage the liver, kidneys, central nervous system or may cause respiratory and gastrointestinal problems. Cyanotoxins are now recognized as an important emerging threat for water resources by WHO, USEPA and other authorities.

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"ARISTEIA" is implemented in the frame of the Operational Program "Education and Lifelong Learning" of the NSRF and funded by EU and Greek National Funds

# Cyanotoxins in Fresh Waters - Advances in Analysis, Occurrence and Treatment
# NCSR Demokritos, Athens, 5 May 2014

**Program**

08:30-09:00 **Registration**

09:00-09:15 **Welcome and Introductory Remarks**
Representatives of NCSR Demokritos and General Secretariat for Research and Technology of Greece
Dr. Anastasia Hiskia, Research Director at NCSR Demokritos, Coordinator of CyanoWater, GH of CYANOCOST

*Session 1, Chairs: A. Hiskia, D. Dionysiou*

09:15-09:45 **Treatment of cyanotoxins and contaminants of emerging concern in water using Advanced Oxidation Processes**
*Prof. Dionysios (Dion) Dionysiou, University of Cincinnati, USA*

09:45-10:15 **Rapid chromatography – mass spectrometry of microcystins and nodularins**
*Dr. Jussi Meriluoto, Åbo Akademi University, Finland*

10:15-10:45 **Cyanobacterial blooms in Serbia – Epidemiological studies and health risk assessment**
*Prof. Zorica Svirčev, University of Novi Sad, Serbia*

10:45-11:15 Coffee break

*Session 2, Chairs: J. Meriluoto, Z. Svircev*

11:15-11:45 **Integrated LC-HRMS workflow for target, suspect and non-target screening of polar emerging contaminants in the aquatic environment**
*Prof. Nikolaos Thomaidis, National and Kapodistrian University of Athens, Greece*

11:45-12:15 **Cyanotoxin research in NCSR "Demokritos" – Overview of projects and activities.**
*Dr. Anastasia Hiskia, NCSR Demokritos, Greece*

12:15-12:45 **CYANOCOST: An international network for cyanobacteria and cyanotoxins in water resources**
*Dr. Triantafyllos Kaloudis, EYDAP SA, Chair of CYANOCOST*

12:45-13:45 Lunch

*Session 3, Chairs: N. Thomaidis, T. Triantis*

13:45-14:15 **CyanoWater: From the analysis of cyanotoxins to advanced water treatment and purification**
*Dr. Theodoros Triantis, NCSR Demokritos, Greece*

14:15-14:30 **Method development for the analysis of cyanotoxins in water**
*Sevasti Zervou, NCSR Demokritos, Greece*

14:30-14:45 **Photocatalytic degradation of cyanotoxins and taste-odor compounds in water using modified $TiO_2$ and polyoxometalates**
*Theodora Fotiou, NCSR Demokritos, Greece*

14:45-15:00 **Preparation and characterization of novel POM-$TiO_2$ photocatalysts for water purification**
*Dr. Christophoros Christophoridis, NCSR Demokritos, Greece*

15:00-15:15 **Importance of cyanobacteria and cyanotoxins for loess formation in semi-arid environments**
*Tamara Dulic, University of Novi Sad, Serbia*

15:15-15:45 **Prospects and opportunities for young researchers in the field**
*Open discussion with students and early-stage researchers*

15:45-16:00 **Concluding remarks**
*Dr. Anastasia Hiskia, NCSR Demokritos, Greece*

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# CYANOWATER - Cyanotoxins in Fresh Waters, Advances in Analysis, Occurrence and Treatment”

**Co-funded by the European Social Fund and Greek national funds through The Operational Programme "Education and Lifelong Learning", Action ARISTEIA, 350 K€, Duration 36 months, Start date: 9/2012.**

**Coordinator: Dr Anastasia Hiskia, Researcher at NCSR DEMOKRITOS Rank A’**
**hiskia@chem.demokritos,gr**

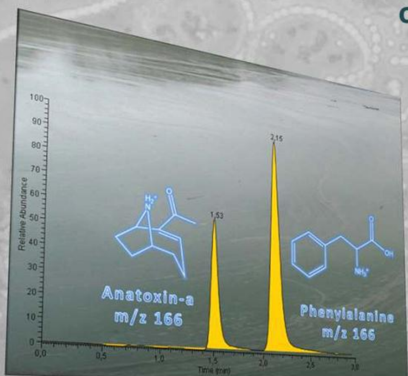
**WP1 : Development of new advanced analytical methods for the determination of cyanotoxins in environmental samples.**
Development of a multi-class cyanotoxin method of analysis, where cyanotoxins belonging to different chemical groups (microcystins, cylindrospermopsin, anatoxin-a, saxitoxins and BMAA) will be detected and quantified in a single analysis with use of LC-MS/MS.

**WP2 : Identification of the toxin-producing cyanobacteria species in freshwater bodies.**
Monitor the occurring and toxic bloom forming cyanobacteria in freshwaters which have high cyanotoxin diversity and/or density by combining the diversity by standard microscopic analysis and phylogenetic analysis after PCR amplification of the 16S rRNA gene with cyanobacterial-specific primers.

**WP3 : Use of Advanced Oxidation Processes (AOPs) for the detoxification of water containing cyanotoxins.**
Degradation/detoxification of cyanotoxins in water with use of AOPs based on the OH radical, to include toxins that are not yet studied (anatoxin-a, BMAA, cylindrospermopsin, microcystins) or AOPs that have not yet been applied to cyanotoxins. Synthesis and characterization of novel hybrid Titanium Dioxide-Polyoxometalate ($TiO_2$-POM) nanocatalysts for the photocatalytic degradation of cyanotoxins and intermediate products identification during the process.

**WP4 : Exploitation and dissemination of results.**
Exploitation and dissemination the project’s results to the research community, water authorities, policy makers and stakeholders in order to foster public health protection and better management of cyanobacteria and cyanotoxins.

## Detection

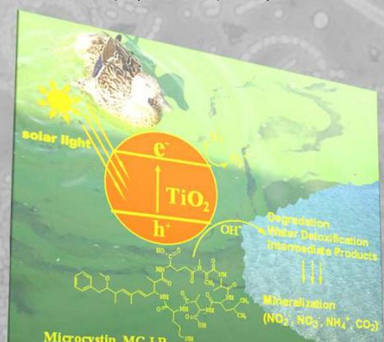



Novel Analytical Methods

## Identification of toxin-producing cyanobacteria species in lakes

Microscopic analysis and phylogenetic analysis

## Control




Advanced Water Treatment

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# Treatment of Cyanotoxins and Contaminants of Emerging Concern in Water Using Advanced Oxidation Processes

Prof. Dionysios (Dion) D. Dionysiou
Environmental Engineering and Science Program University of Cincinnati, Cincinnati, Ohio, USA, dionysios.d.dionysiou@uc.edu

Advanced Oxidation Processes (AOPs) are gaining popularity for the removal of contaminants of emerging concern, especially for applications in the purification of drinking water, treatment of wastewater effluent (i.e., for discharge or reuse applications), decontamination of industrial effluents, and remediation of groundwater. Homogeneous and heterogeneous AOPs are currently intensively explored, modified, and optimized for applications as stand-alone technologies or integrated with other processes in the overall treatment train. Several new AOPs demonstrating promising results have been developed in recent years. While most of these technologies are based on hydroxyl radical as oxidizing species, some AOPs involve other radical species such as sulfate radical and superoxide anion radical, which are also more selective. While less efficient for destruction of organic contaminants, superoxide anion radical can play a significant role in water disinfection by solar light. Recent interest has also spurred in reductive pathways of AOPs for application in reduction of metals, nitrate, bromate and other species of importance in water quality. More recent studies have also delved into mechanistic insights of AOPs, including identification of reaction intermediates, unveiling reaction pathways, determination of treated solution toxicity, and the role of water quality on the reaction mechanisms and reaction kinetics. Recent progress in nanotechnology also propelled new advances in the field of heterogeneous AOPs. The use of solar light by some of these processes (i.e., solar photocatalysis, solar photoelectrocatalysis, solar disinfection) has expanded potential application in parts of the world that have plenty of sunlight but also have critical needs in water quality and energy generation. In this presentation, Professor Dionysiou will overview mechanistic transformation pathways of treatment of cyanotoxins and other contaminants of emerging concern in water using AOPs. Emphasis will be given on UV and Solar-based Advanced Oxidation Processes such as $TiO_2$-based photocatalysis (UV and visible) (i.e., a heterogeneous AOP) and UV/$H_2O_2$ process (i.e., a homogeneous AOP). Details will be presented on the degradation of cyanotoxins and other selected contaminants. Most emphasis will be placed on the oxidative pathways for the degradation of microcystin-LR and cylindrospermopsin. Transformation kinetic rates and reaction intermediates formed by OH radical attack and other reactive oxygen species on specific sites of the target contaminants will be presented and the detailed reaction pathways will be discussed. Discussion will also be provided when oxidation takes place by other radicals such as sulfate radicals and superoxide anion radicals under certain modifications of the processes described above. The role of water quality parameters such as natural organic matter, alkalinity and pH will be discussed, considering also the chemistry of the target contaminants and, in the case of heterogeneous AOPs, the role of the catalyst nano-interface.

## Rapid chromatography – mass spectrometry of microcystins and nodularins

Jussi Meriluoto

Department of Biosciences, Åbo Akademi University, Turku, Finland, jussi.meriluoto@abo.fi

Microcystins and nodularins are cyanobacterial cyclic peptides which show strong mammalian hepatotoxicity and possible carcinogenic properties. Microcystins, in particular microcystin-LR, are regulated in drinking water, and the toxins can also be transferred in food webs and thus contaminate foodstuffs.

Microcystins and nodularins can be conveniently screened for by immunoassays or protein phosphatase inhibition assays but the verificationof positive samples necessitates the use of LC-MS/MS methods.The high number of microcystins and nodularins (altogether > 200 known variants)and subtle variationsin toxin structure make these analytes a great separation challenge for the chromatographer. Although MS detection does not necessitate baseline chromatographic separation, it is usuallyadvisable to aim at a high chromatographic resolution also in MS work. Efficient separations help to minimise ion suppression/enhancement caused by matrix components and co-eluting analytes, and the quantitation can thus be improved.

Recent developments in high-performance liquid chromatography include sub-2 micrometer particles in combination with ultra-high pressure instrumentation, miniatyrisation of column dimensions as well as novel stationary phase materials(e.g. various polar-embedded reversed-phase sorbents and monolithic columns). These features can be successfully utilised for the rapid chromatography (1-2 min) of microcystins and nodularins while still maintaining high resolution.The presentation explores the possibilities of modern chromatography combined with MS detection in the rapid and quantitative analysis of microcystins and nodularins in various matrices.

## Cyanobacterial blooms in Serbia – epidemiological studies and health risk assessment

Zorica Svirčev

Department of Biology and Ecology, Faculty of Natural Sciences, University of Novi Sad, zorica.svircev@dbe.uns.ac.rs

Over the past 130 years, research in Vojvodina has shown that the conditions in all investigated aquatic ecosystems enable blooming of cyanobacteria. In the Central Serbia more than 80% of sites bloomed in the same period. The population in Central Serbia may be exposed to these health risks through: oral intake, dermal contact, and inhalation during recreation. The toxin intake occurs through drinking the contaminated water, eating fish and agricultural crops with accumulated cyanotoxins, and also by the use of food supplements based on cyanobacteria. After acute contact with water from sources containing cyanobacterial bloom, the following health problems are possible: stomach discomfort (pain, vomiting, diarrhea), nausea, weakness, numbness of the lips and muscles, tingling in fingertips and toes, irritation of skin and mucous membrane of the eyes, nose and throat, asmatic attacks, blurred vision, headache, dizziness, fever, feeling a of lack of oxygen (or hypoxia), cyanosis, paralysis and respiratory or cardiac arrest resulting in death. Epidemiological studies show that unless ozonation is used in the process of water treatment, there was a statistically significant increase in incidence of primary liver cancer in the regions of Central Serbia in those individuals who used water prepared from the blooming reservoirs. On the other hand, no correlation was found between the incidence of liver cancer and other risk factors such as cirrhosis, HBV and HCV. This indicates that cyanotoxins might be the primary cause of increased incidence of liver cancer in those regions of Serbia. Increased incidence of some other cancers in these regions, such as melanoma, cancer of the colon and rectum, peritoneum and retroperitoneum, calls for a special caution and additional assessment of health risks that may be caused by exposure to cyanotoxins. Based on these epidemiological data, there is a reason to believe that long-term chronic exposure may lead to tumor initiation and promotion.

## Integrated LC-HRMS workflow for target, suspect and non-target screening of polar emerging contaminants in the aquatic environment

Anna A. Bletsou, Pablo Gago Ferrero, Aikaterini K. Psoma, Reza Aalizadeh, Nikolaos S. Thomaidis

Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zographou, 15771 Athens, Greece, ntho@chem.uoa.gr

Environmental waters contain a large number of organic contaminants. A limited fraction of these substances can be detected by target analysis. Advances in high resolution mass spectrometry offer the possibility of tentative identification of suspect and unknown compounds by a non-target approach. This talk focuses on the development of an integrated workflow for the target, suspect and non-target screening of environmental samples, using liquid chromatography quadrupole-time-of-flight mass spectrometry (LC–QToF-MS) and sophisticated software and approaches. More than 1500 contaminants of emerging concern (CECs) and transformation products (TPs) including, among others, pharmaceuticals, illicit drugs, personal care products, pesticides, industrial chemicals, and sweeteners were determined by target screening in wastewater and surface water samples. An in-house database of more than 7000 relevant organic pollutants and was also built including only the molecular formula for suspect screening, taking into account mass accuracy or isotopic pattern and retention time plausibility (using prediction models). For the tentative identification of unknown compounds, the peak peaking procedure was optimized, using molecular features algorithm. The most intense peaks, containing distinctive isotope patterns were studied. Thresholds of mass accuracy, isotopic pattern and commercial importance, among others, were applied to the selected masses, using the Seven Golden Rules. A deep evaluation of the MS spectra was performed for these peaks, using data bases and *in silico* fragmentation software (Bruker SmartFormula 3D and Metfrag) to find candidates. Chromatographic retention time prediction models were also applied to assess the plausibility of the candidates. This approach allows the obtaining of plausible candidates for most of the selected peaks and the tentative identification for some of them.

This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEIA 624 (TREMEPOL project).

# Cyanotoxin research in NCSR "DEMOKRITOS"
# Overview of projects and activities

Anastasia Hiskia

Nanomaterial-Based Catalytic-Photocatalytic Processes and Environmental Analysis, Institute of Nanoscience and Nanotechnology, NCSR "Demokritos", hiskia@chem.demokritos.gr

The Laboratory of Nanomaterial-based Catalytic-Photocatalytic Processes and Environmental Analysis, part of the Institute of Nanoscience and Nanotechnology of NCSR "DEMOKRITOS" is strongly activated on research activities related to environmental analytical chemistry, photo-catalytic processes for environmental detoxification (Advanced Oxidation Processes) and synthesis of nanostructured materials with/for environmentally friendly methods.
In this direction, our group was successfully developed analytical methods for the determination of toxic organic pollutants (PAHs, pesticides, PCBs, pharmaceuticals) in various matrices (water, waste water, soil, foodstuff). Recently, we are focused on the development of analytical methods for the determination of cyanotoxins using advanced analytical techniques, being the only Greek laboratory involved in this area of research. Cyanotoxins, a new class of emerging pollutants of biogenic origin are present in most countries around the world and they are considered a major concern in the European context. The recent purchase of a LC-MS/MS infrastructure by external financing was enabling us to detect and identify these toxins at very low concentrations. However, many research gaps should be overcome for the development of reliable and accredited analytical methods for all classes of cyanotoxins. Also, the analysis of small molecule - hydrophilic neurotoxins (such as BMAA) or the development of faster analytical methods that will detect as many toxins as possible in one analysis still present significant analytical challenges. In this frame, we have developed advanced analytical methods for the determination of different classes of cyanotoxins in water (e.g. microcystins, nodularin, cylindrospermopsin, anatoxin) using LC-MS/MS techniques. All these activities have been resulted in the accreditation of our Laboratory by the National Accreditation Body (ESYD) according to ISO 17025 (N. of Accreditation 580-2/ESYD) for the determination of microcystins and nodularin in water using LC-MS/MS technique and established it as the only accredited laboratory in Europe for the analysis of cyanotoxins.

In parallel, our group also studies the photocatalytic performance of semicontacting (TiO2 based) and polyoxometalate (POM) materials in terms of the overall mechanism of organic compounds photodegradation with pioneering contribution from our group in POM research. However, there are weak points regarding the elucidation of the photochemical mechanism in details. Understanding of the process is fundamentally important for further development of the research in this area, in terms of efficiency, selectivity and applicability of the catalysts.

By using various, of the wide-ranged POM on doping titania, the photoredox properties of titania surface can be precisely controlled in a molecular level. Hybrid nanostructured $TiO_2$-POM materials prepared by different methods show increased activity in organic pollutants photodegradation due to their synergistic action. The appropriate selection of POM can also increase the photofficiency of the $TiO_2$ to the visible light, since several POM are able to absorb light at wavelength >400nm. These nanostructured photocatalysts will be used by our group to innovative applications such as the development of advanced oxidation technologies for water detoxification from toxic organic pollutants. The long experience of our group on the photocatalytic processes enabling us to develop novel standardized procedures for the evaluation of the photocatalytic activity of new $TiO_2$-based nanostructured materials and participate as convenor of the Comité Européen de Normalisation CEN TC386 “Photocatalysis” / WG3 “Water purification” for the establishment of a European Standard for the evaluation of the photocatalytic activity of materials for water purification. The team leader (A.H.) together with colleagues Prof. D. Dionysiou (USA), Dr. T. Triantis (group member, DEMOKRITOS), Dr. T. Kaloudis (EYDAP S.A.) and Dr. M. Antoniou (Cyprus) are involved as Editors in a book entitled “Water treatment for purification from cyanobacteria and cyanotoxins”, to be published by Wiley Publ. at the end of 2014.

Research activities of our group is being carried in the frame of already funded projects and headed by our group such as CYANOWATER (Aristeia Action) as well as research projects in which our team participates as partner (THALIS and KRIPIS Actions). A significant interaction and knowledge transfer in the area of cyanotoxins research is implemented through the COST Action ES1105 “CYANOCOST – Cyanobacterial blooms and toxins in water resources: Occurrence, impacts and management” with NCSR “DEMOKRITOS” being the Grant Holder. With 60 partners (scientists, other professionals and companies), from 32 European countries as well as from USA and China, the main objective of this network 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. Significant knowledge transfer in POM research is also taking place in the frame of COST Acion CM1203 (Polyoxometalate Chemistry for Molecular Nanoscience (PoCheMoN)) were Dr. Hiskia participates as MC member.

# CYANOCOST : An international network for cyanobacteria and cyanotoxins in water resources

Triantafyllos Kaloudis

Chair of CYANOCOST – ES1105 COST Action
Athens Water Supply and Sewerage Company - EYDAP SA, kaloudis@eydap.gr

CYANOCOST (COST Action ES 1105) is an international 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 32 European countries in addition to USA and the Russian Federation. 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 and experts to be involved in its activities. Information about CYANOCOST can be found in www.cyanocost.com.

# CyanoWater: From the analysis of cyanotoxins to advanced water treatment and purification

Theodoros Triantis
National Centre for Science Research "Demokritos", triantis@chem.demokritos.gr

Cyanobacteria (CB) are ubiquitous organisms that are commonly found in freshwater bodies. Several CB genera produce toxins (cyanotoxins, CTs) that are released into water. CTs have been responsible for several incidents worldwide, of animal poisoning as well as human injury and death.

CTs comprise a large number of compounds with different chemical structures and toxic activities (e.g. hepatotoxic, neurotoxic). They can penetrate water utilities and even reach the consumers' taps but can also contaminate fish tissues and plants.

Research so far has focused on the group of hepatotoxic microcystins resulting in the adaptation by WHO of a limit (1 μg/L) for drinking water. Recently however, the risks by "emerging" CTs, e.g. alkaloids, have been stressed worldwide. Their presence is possibly linked to the invasive character of certain CB species as well as to climate change. In many countries, as also in Greece, possible toxin-producing CB have been found in lakes, including water reservoirs, however, studies on the presence of CTs are still very limited.

CYANOWATER project aims in filling research gaps and achieving breakthrough results in:

- Development of advanced analytical methods for emerging CTs and for simultaneous analysis of different groups of CTs. Analytical methods should be sensitive, accurate, and fast and also target many toxins of different groups in "one run" (multi-toxin method). This enhances the capabilities of researchers as well as water and public health authorities to better assess and control the associated risks.
- Identification of toxic bloom forming cyanobacteria species in Greek freshwater ecosystems that were never studied before. This contributes to a better understanding of the behavior of these microorganisms, their tendency to invade or adapt to new environments with the concomitant release of toxins, some of them being new and emerging in the European region. Furthermore, correlation between toxin-producing cyanobacteria and cyanotoxins concentration in Greek water bodies.
- Studies on advanced oxidation processes (AOPs) for the detoxification of water contaminated with CTs providing further effective and efficient tools for water utilities and authorities to eliminate public health risks associated with the consumption of cyanotoxins. In particular, development of novel advanced oxidation processes based on new $TiO_2$-based photocatalytic materials for the detoxification of water contaminated with CTs.

This research has direct impact on public health protection at an international level and significant benefits for researchers in the field, water utilities, public health authorities, aquaculture - agriculture sectors and policy makers.

## Method development for the analysis of cyanotoxins in water

Sevasti-Kiriaki Zervou
National Centre for Science Research "Demokritos", zervou@chem.demokritos.gr

Cyanotoxins are a large number of compounds with different chemical structures and physicochemical properties. Due to their widespread distribution and high toxicity they pose a threat to public health. Cyanotoxin concentration levels have become an important parameter in water quality control, environmental monitoring and toxicology. Several techniques (bioassays, ELISA, toxicity assays, HPLC-UV & LC-MS) have been developed for their determination in water. LC-MS techniques provide maximum sensitivity and selectivity. This study presents the optimization of an analytical method, utilizing solid phase extraction (SPE) and liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC-ESI-MS/MS), for the determination of 12 Microcystins (MCs), Nodularin (NOD), Cylindrospermopsin and Anatoxin-a in drinking water and surface water samples. The target compounds were MC-LR, [D-Asp3] MC-LR, MC-HilR, MC-RR, [D-Asp3] MC-RR, MC-YR, MC-HtyR, MC-WR, MC-LA, MC-LF, MC-LW, MC-LY, Nodularin-R, Cylindrospermopsin and Anatoxin-a. Phenylalanine d-5 was used as surrogate standard (SS) for Anatoxin-a.

During the development of the determination method Solid Phase Extraction (SPE) was employed. Different SPE cartridges and elution solvents were tested so as to achieve satisfactory recoveries for all targeted compounds. The effect of various sample pH on recoveries was also evaluated. Separation was achieved by HPLC using reversed phase chromatography and satisfactory resolution for all analytes was achieved after testing several chromatographic columns and gradient programs. Optimization of the mass spectrometer parameters was performed to maximize the sensitivity and reproducibility of the method. Detection of cyanotoxins was carried out using multiple reaction monitoring (MRMs). Two precursor – product ion transitions were monitored for each cyanotoxin, thus improving selectivity of the method. The method should be comprehensively validated according to ISO 17025. Assessed parameters should include linearity, precision, accuracy, robustness and limits of detection and quantification, so as to confirm its suitability for the proposed purpose.

**Keywords**: Cyanotoxins, LC-MS/MS, Solid Phase Extraction (SPE), method development, method validation

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# Photocatalytic degradation of cyanotoxins and taste and odor compounds in water using modified $TiO_2$ and polyoxometalates

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Titanium dioxide ($TiO_2$) photocatalysis has been considered a promising advanced oxidation process, which in combination with solar energy could effectively address the ever increasing concerns for pollution abatement and water purification. Although it appears to degrade a great variety of compounds in water, it generally requires UV irradiation for activation of the catalyst. Consequently, research on the development of new $TiO_2$ based catalysts has been receiving increased attention. $TiO_2$ photocatalysis can be effective for water purification from new classes of emerging pollutants such as cyanobacterial toxins (cyanotoxins) and metabolites, which can be produced by several genera of cyanobacteria. Cyanotoxins are considered an important risk for water quality since they are harmful to human and animal health. In this study, the photocatalytic degradation of cyanotoxins (microcystin-LR, MC-LR and Cylindrospermopsin, CYN) and water taste and odor compounds (Geosmin, GSM and 2-methylisoborneol, MIB) was presented. $TiO_2$ based photocatalysts (Degussa P25, Kronos vlp-7000, Ref-$TiO_2$, N-$TiO_2$, and GO$TiO_2$) were tested for their photocatalytic ability towards degradation and mineralization of target analytes in water using UV-A, solar and visible light. In order to evaluate the photocatalytic performance of the materials, several parameters such as light intensity, presence of oxygen, catalyst loading, initial concentration of substrate, adsorption, pH, irradiation wavelength, mineralization, intermediate products and toxicity, were investigated. Identification of the intermediate and final products was carried out and a complete degradation pathway is proposed were hydroxyl radicals ($^{\bullet}OH$) play a key role. Assessment of the residual toxicity in the case of MCLR using $TiO_2$ photocatalysis, complete detoxification of the solutions can be achieved. In addition, photocatalytic degradation of GSM and MIB using a polyoxometalate photocatalyst, $H_4SiW_{12}O_{40}$ was studied and compared with $TiO_2$. Elucidation of the mechanism using $^{\bullet}OH$ radical scavengers showed that process takes place via $^{\bullet}OH$ radicals for both catalysts. Overall results of this study show that $TiO_2$ based photocatalysis can be very effective in removing cyanobacterial toxins and taste and odor compounds from water and its applications can be extended to the visible-solar region of the spectrum by development of novel modified photocatalytic materials.

**Keywords**: Titanium Dioxide, Polyoxometalates, Cyanotoxins, Water Taste & Odor Compounds, Degradation & Mechanism Elucidation

# Preparation and characterization of novel POM-$TiO_2$ photocatalysts for water purification

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Polyoxometalates (POMs) have been employed in the past as electron scavengers in connection with photo-excited $TiO_2$ to decrease charge recombination, due to their similar electronic characteristics, although in a limited scale regarding the material preparative methods and characterization. The present study investigates the preparation and characterization of binary $TiO_2$ – $PW_{12}$ systems (Anatase and Degussa P25), through different preparation procedures and examines the possible enhancement of their photocatalytic performance on the degradation of model compound 2,4 DCP (2,4 Dichlorophenol) under UV-A irradiation.

Modified Anatase and Degussa P25, deriving from simple adsorption of $PW_{12}O_{40}$, did not exhibit any significant enhancement of the photocatalytic degradation of 2,4 DCP, regardless of the polyoxometalate loading. On the other hand, modified Anatase with thermal impregnation of $PW_{12}O_{40}^{3-}$, exhibits a significant improvement of 2,4 DCP photo-degradation, proportional to the POM loading of the material. The pseudo-first order kinetic constant almost doubled, while in 8 min of irradiation, removal was increased by 24%. Complete removal of the compound was achieved in 30 min. FT-IR analysis showed that for both thermally modified Degussa P25 and Anatase, the Keggin geometry of $PW_{12}O_{40}^{3-}$ is preserved in the material, retaining two of the characteristic stretching bands of P-O (1080 $cm^{-1}$) and W=O (990$cm^{-1}$). Energy dispersive X-Ray Spectroscopy (EDX) revealed a homogenous distribution of POM onto the surface of Degussa and Anatase, ranging 6.4-7.3%.

**Keywords**: polyoxometalates, $TiO_2$, photocatalytic degradation

# Importance of cyanobacteria and cyanotoxins for loess formation in semi-arid environments

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The essential abiotic components for loess formation are: material (dust), atmospheric circulation (wind), suitable surface conditions for the trapping of aeolian material, and for the subsequent development of typical loess sedimentary structures. In spite of the world-wide distribution of loess deposits, knowledge of the processes of dust accumulation and its transformation to mature loess sediment is still inadequate. Loess accumulation appears greatest during the most arid periods and in semi-arid regions. Biological crusted surfaces (BSC) are of great importance in loess formation, especially in semi-arid regions. BSC are highly specialized extremophile communities and apparently play and important role in atmospheric dust trapping and erosion prevention. Results indicate that cyanobacterial strains isolated from current Carpathian Basin loess exhibit specific morphological and eco-physiological characteristics that play a key role in loess formation, warranting adoption of the new term *biological loess crusts* (BLC). A model of the influence of cyanobacterial BLC life strategies on loess formation explains trapping, accumulation and preservation as well as loess texture and structure. This potential significance of BLC in the accumulation and preservation of loess sediments may be considered as a suitable model database for recognition of bioorganic modification of geological strata. Mycosporine and Scytonemin pigments can serve as BLC biomarkers, elucidating their role in soil evolution and in aiding palaeoclimatic, palaeoenvironmental and palaeovegetation reconstructions.

Current discovery of cyanotoxins in BLC raises an important question related to understanding of their influence to local environment during loess formation

**Keywords**: biological loess crusts, cyanobacteria, loess, cyanotoxins