1/4/2021

Monitoring, Detection, and Treatment of Surface Waters for toxic cyanobacteria

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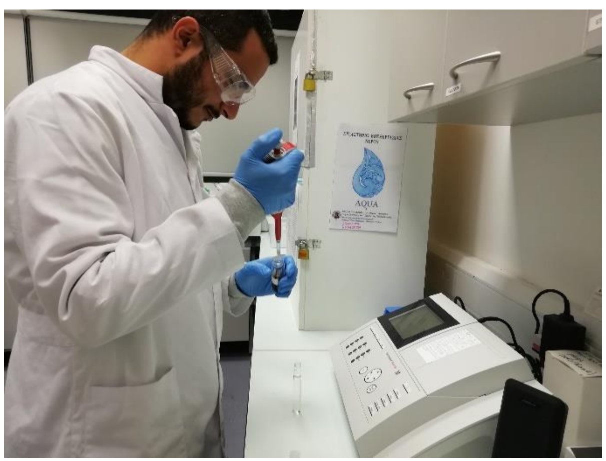
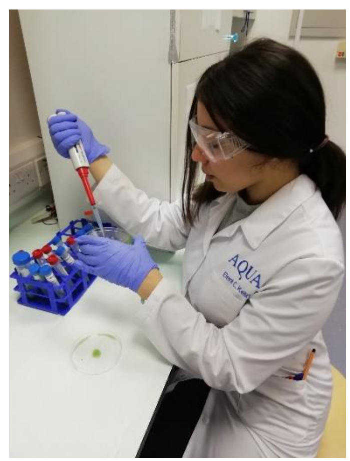
AQUA

WTL-AQUA

- Advanced Oxidation Processes for the removal of emerging contaminants from water and wastewater
- Toxic cyanobacteria
  - cyanotoxins



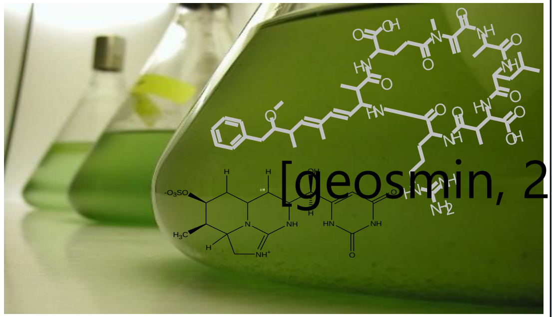




Collimated Beam


https://wtl-aqua.weebly.com/

Cyanobacteria and their toxins

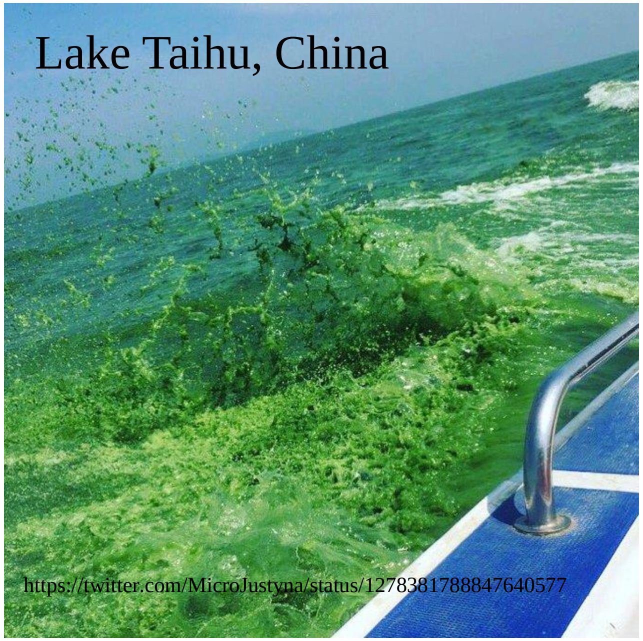
- Found in aquatic environments: surface water, drinking water, reclaimed water, distribution systems.
  - Warm water (T=15-30 °C) and pH=6-9, with reasonable nutrient level, mild winds, low turbulence
- The eutrophication of water resources, favors the formation cyanobacteria harmful algal blooms (cyano-HABs)

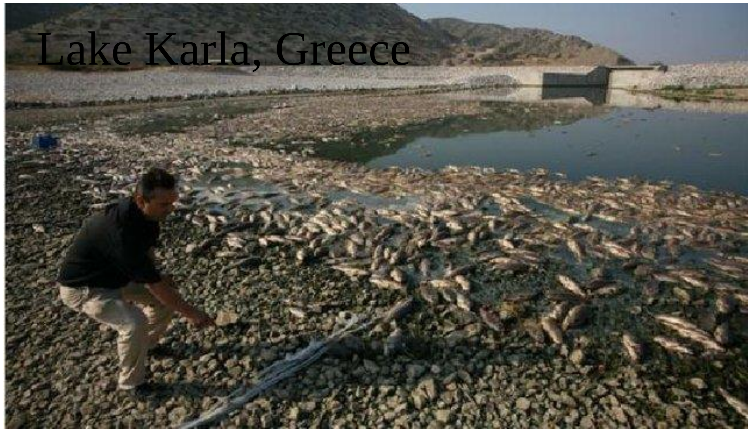


- Cyano-HABs cause:
- Green like bean soup color, taste and odor [geosmin, 2-methylisoborneol (MIB)]
- 50 out of the 150 different genera of cyanobacteria can production and release bioactive compounds, harmful to humans and the ecosystem: CYANOTOXINS

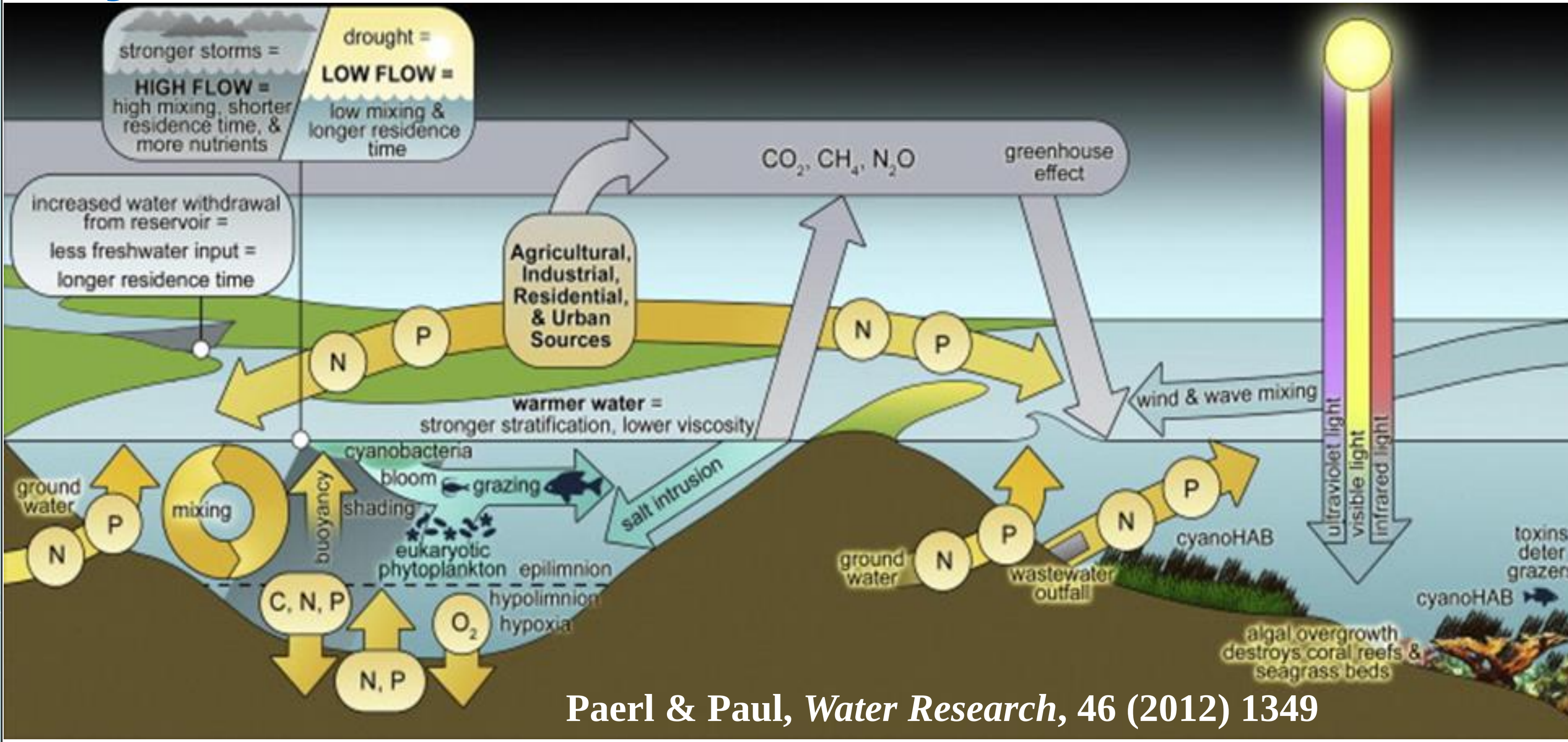
Antoniou *et al*., J. Environ. Eng. 131 (2005) 1239; Carmichael, Scient. Amer. 270 (1994) 78; Newcombe and Burch, *Opflow*, 5 (2003)

Cyanobacterial contaminated locations





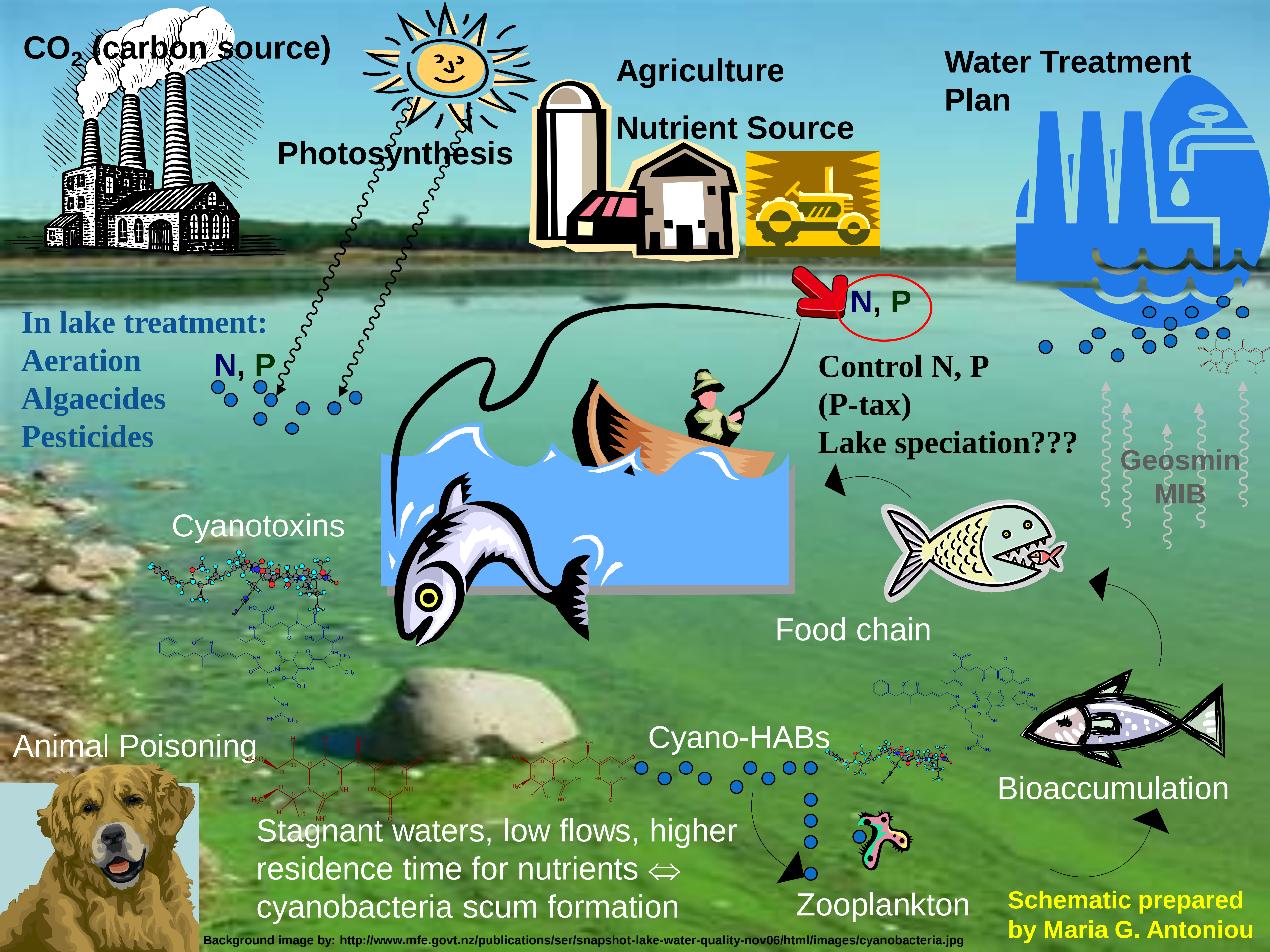
Cyano-HABs



Paerl & Paul, *Water Research*, 46 (2012) 1349

- Key factors controlling cyanobacterial growth and dominance of cyanobacteria:
- Nutrients and temperature major factors — Paerl & Paul, *Water Research*, 46 (2012) 1349
- Nutrients → eutrophication → hypoxia
- T → direct impact on mixing → stratification → hypoxia
- Rising CO_2 and global warming may stimulate harmful cyanobacterial blooms

Vissér et al., *Harmful Algae* 54 (2016) 145


CO2 (carbon source)
Photosynthesis
Agriculture
Nutrient Source
Water Treatment Plan
N, P
In lake treatment:
Aeration
Algaecides
Pesticides
N, P
Control N, P
(P-tax)
Lake speciation???
Geosmin
MIB
Cyanotoxins
Food chain
Animal Poisoning
Cyano-HABs
Bioaccumulation
Stagnant waters, low flows, higher residence time for nutrients ⇔ cyanobacteria scum formation
Zooplankton
Schematic prepared by Maria G. Antoniou
Background image by: http://www.mfe.govt.nz/publications/ser/snapshot-lake-water-quality-nov06/html/images/cyanobacteria.jpg

AQUA

Toxins produced by cyanobacteria

***Microcystis* spp.** → **Hepatotoxins - microcystins**

Planktothrix agardhi → **Hepatotoxins - microcystins**

Pseudanabaena limnetica → **Hepatotoxins - microcystins**

Woronichinia naegeliana → **Hepatotoxins - microcystins**

Hepatotoxins - microcystins
(> 90 analogues)
LD_{50}= 25-1000 µg/kg

- **$LD_{50,\ cobra}$ = 500 µg/Kg (white rats)**

Rogers, L. *Proceedings of the Royal Society of London*, 71 (1903) 485

***Dolichospermum* spp. / *Anabaena* spp.** →
- **Hepatotoxins** – **microcystins**
- **Neurotoxins** – anatoxin-a; anatoxin-a(s) saxitoxins, BMAA(?)

LD_{50}= 10-50 µg/kg

***Aphanizomenon* spp.** →
- **Neurotoxins** – anatoxin-a; anatoxin-a(s) saxitoxins, BMAA (?)
- **Cytotoxins** – cylindrospermopsins

Gloeotrichia echinulata →
- **Dermatotoxins**
- **Hepatotoxins - microcystins**

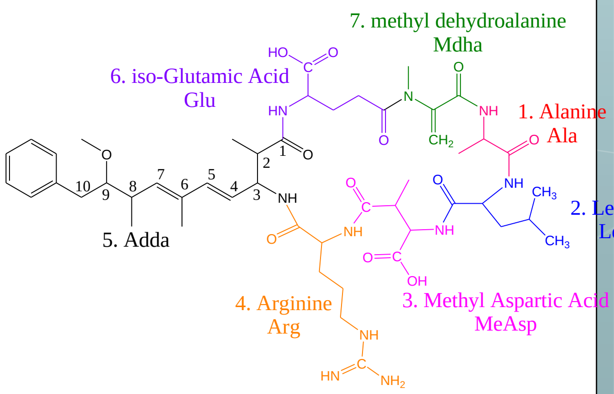
Cylindrospermopsis raciborski →
- **Cytotoxins** – cylindrospermopsins

LD_{50}= 200-2100 µg/kg

http://handboekhydrobiologie.stowa.nl/upload/handboek%20hydrobiologie/pdf/4_b24.pdf

Microcystin-LR (MC-LR)

- Hepatotoxin, Protein Phosphatase (PP) Inhibitor, Tumor Promoter
- Chemical Structure
- - First isolated from Botes et al., *Toxicon, 20* (1982) 945
- - Structural characterization Botes et al., *J. Chem. Soc. Perkin Trans. I, 20* (1984) 2311; (1985) 2747
- 5 invariant modified amino acids and
  - 2 variant amino acids (249 MCs isoforms)
  - - MC –LR (L= Leucine and R= Arginine)
- High chemical stability (cyclic structure)
- Very Soluble in water (functional groups)



- $LD_{50,\ MCLR}$ = 50 μg/Kg (mouse bioassay)
- $LD_{50,\ Enhydrina}$ = 70 μg/Kg (white rats)
- $LD_{50,\ cobra}$ = 500 μg/Kg (white rats)

Rogers, L. *Proceedings of the Royal Society of London*, 71 (1903) 485

Microcystin-LR

- WHO limit 1μg/L.
- The successful attachment of MC-LR in the receptor of the protein phosphatase is directly related to the 3-D configuration of the toxin.

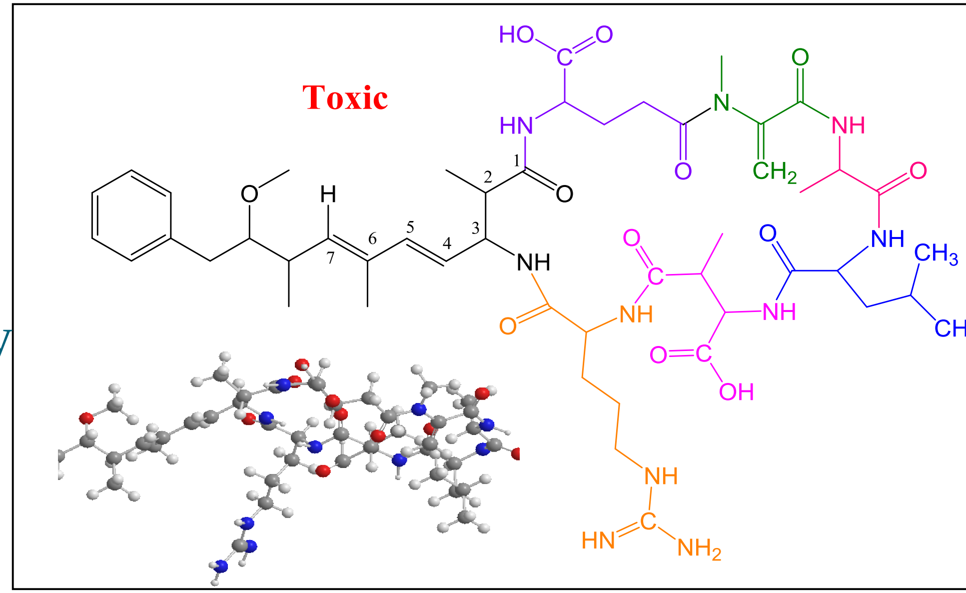
Goldberg *et al.*, <u>*Nature*</u> 376 (1995) 475

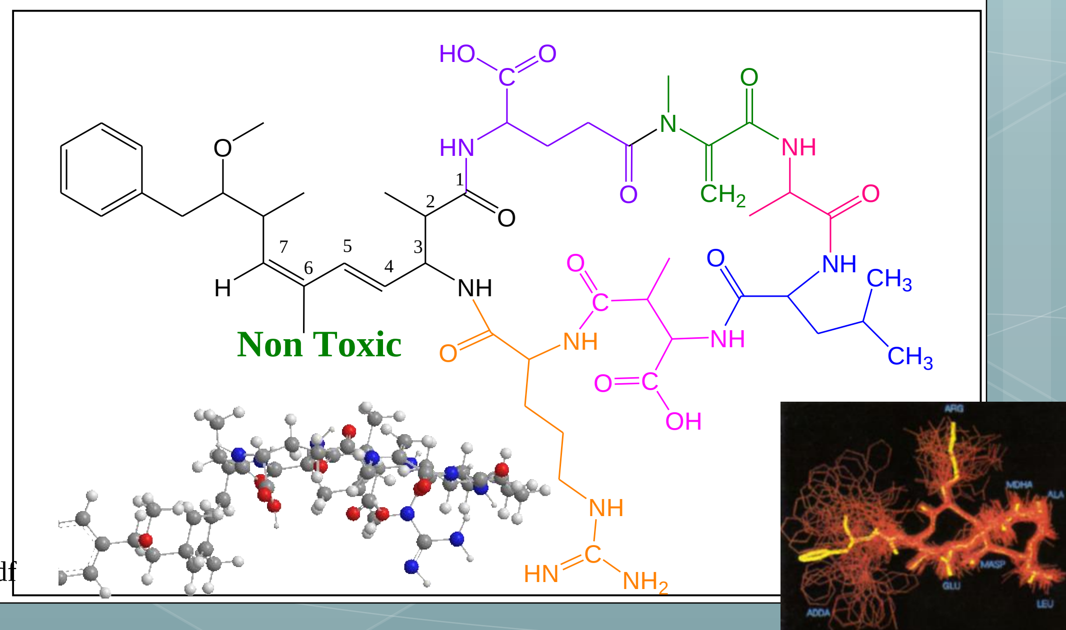
- So far, two derivatives of MCs (LR and RR) where the bond at C_6-methyl and C_7-hydrogen was in *cis* configuration have been found to be non-toxic.

Harada *et al.*, <u>*Chem. Res. Toxicol*</u>. 3 (1990) 473

- Proposed to be included in the new EU drinking water directive

https://www.consilium.europa.eu/media/42445/st05813-en20.pdf

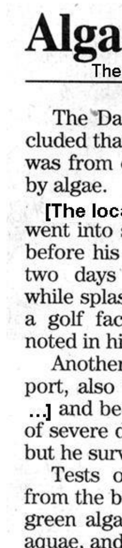


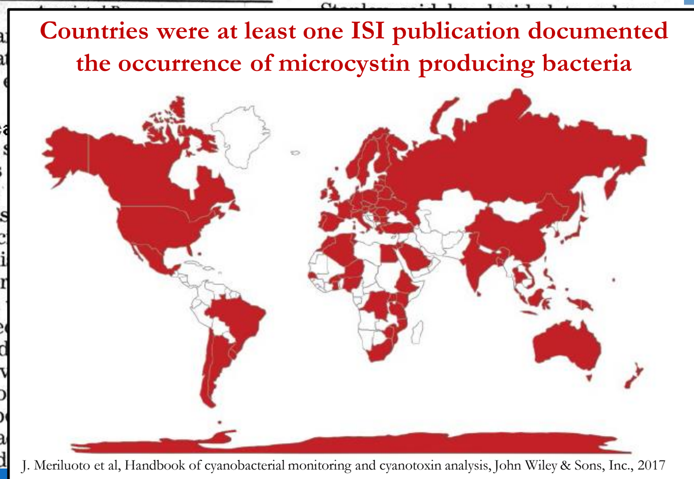


Bagu et. al., <u>*Nature Structural Biology*</u>, 2 (1995) 114

Health concerns for Cyano-HABs

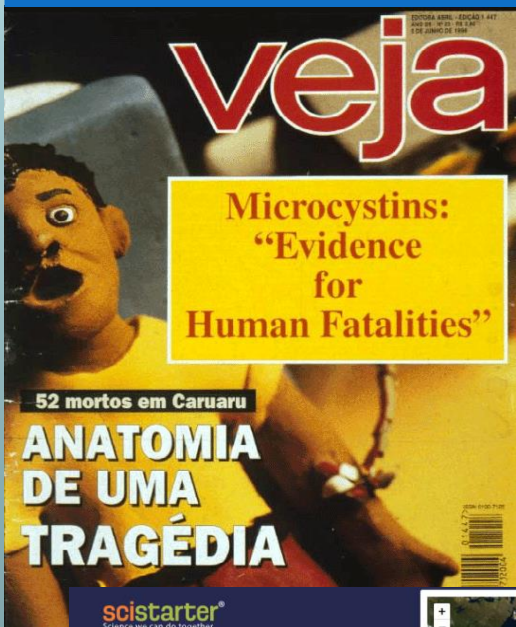
First recorded death in U.S. due to blue-green algal toxins!
Madison, Wisconsin – July 2002



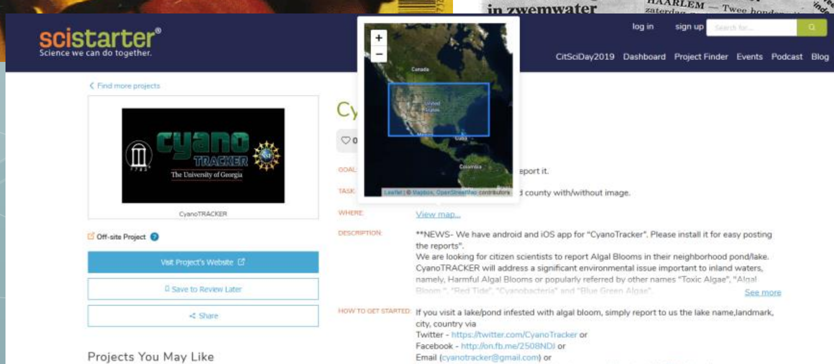


J. Meriluoto et al, Handbook of cyanobacterial monitoring and cyanotoxin analysis, John Wiley & Sons, Inc., 2017

"Caruaru: Death by dialysis", APRIL 22, 1996



Cyanobacteria blooms cause the enclosure of lakes in the NL



Algae in Griffy Lake may cause health problems, October 2018

Research activities related to Cyano-HABs

- AQUA's research activities

Affected Water Body

a) Monitoring-EMLS, CY

b) In-lake treatment

c) **CYANOS** https://cyanoscyfr.weebly.com/

Water Treatment Plan

Removal of cyanotoxins and cyanobacteria-RGU

Analytical Laboratory R&D

New analytical methods-WageningenUR

Agriculture/Hydroponics

Effects on crop growth, cyanotoxins uptake - CUT (Dr. N. Tzortzakis) & RGU

European Multi Survey Lake (EMLS) Evian-Les-Bains, Lac Leman, France 11-13 May 2015

Prof. Bastiaan Ibelings, Evanthia Mantzouki – Université de Genève, Institut F.A. Forel

Dr. Cayelan Carey –Virginia Tech, Department of Biological Sciences

Dr. Lisette de Senerpont Domis – NIOO, Netherlands Institute for Ecology.

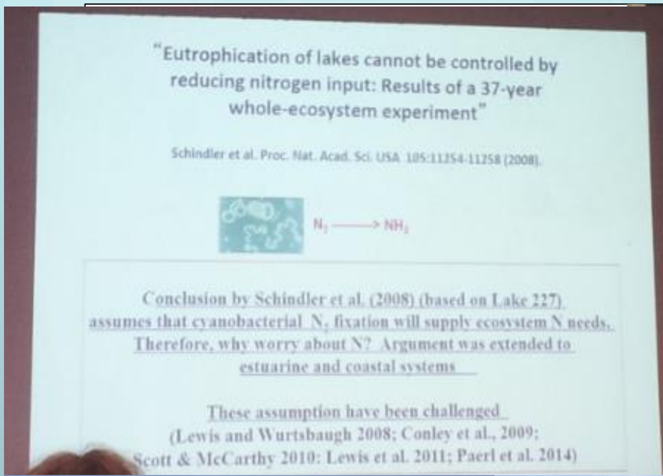
Objectives of EMLS

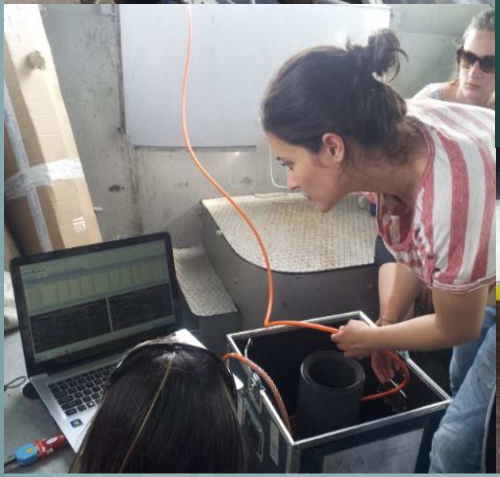
- Compensate for the lack of time data in terms of continues lake monitoring, with having sampling events at different geographical latitudes (time for space swap).
- Link bloom formation with the nutrients, temperature, altitudes (and therefore climate).

Deliverables of EMLS

- Collected data from the EMLS will benefit each country individually, e.g., for developing regional risk assessments of cyanobacterial blooms.
- European goal of improving the management of ecologically-resilient freshwater ecosystems.
- Peer-reviewed publications and reports.

AQUA

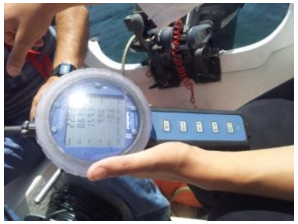

"Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment"
Schindler et al. Proc. Nat. Acad. Sci. USA 105:11254-11258 (2008).
N$_2$ ⟶ NH$_3$
Conclusion by Schindler et al. (2008) (based on Lake 227) assumes that cyanobacterial N$_2$ fixation will supply ecosystem N needs. Therefore, why worry about N? Argument was extended to estuarine and coastal systems
These assumption have been challenged
(Lewis and Wurtsbaugh 2008; Conley et al., 2009; Scott & McCarthy 2010; Lewis et al. 2011; Paerl et al. 2014)




CYANOS LIKE IT
HOT

AQUA's contribution in EMLS

- During June 2015, samples were taken from 5 different locations of Cyprus.
- Dams were chosen based on history, eutrophication state, and importance.



Measurements:

- pH = *f* (depth)
- DO = *f* (depth)
- T = *f* (depth)
- Secchi depth
- Total and dissolved nutrients
- Pigments
- Cyanotoxins
- Cyanobacteria

Published work

toxins MDPI

Article

Temperature Effects Explain Continental Scale Distribution of Cyanobacterial Toxins

Evanthia Mantzouki [1,*], Miquel Lürling [2,3], Jutta Fastner [4], Lisette de Senerpont Domis [2,3], Elżbieta Wilk-Woźniak [5], Judita Koreivienė [6], Laura Seelen [2,3], Sven Teurlincx [3], Yvon Verstijnen [2], Wojciech Krztoń [5], Edward Walusiak [5], Jūratė Karosienė [6], Jūratė Kasperovičienė [6], Ksenija Savadova [6], Irma Vitonytė [6], Carmen Cillero-Castro [7], Agnieszka Budzynska [8], Ryszard Goldyn [8], Anna Kozak [8], Joanna Rosińska [8], Elżbieta Szeląg-Wasielewska [8], Piotr Domek [8], Natalia Jakubowska-Krepska [8], Kinga Kwasizur [9], Beata Messyasz [9], Aleksandra Pełechata [9], Mariusz Pełechaty [9], Mikolaj Kokocinski [9], Ana García-Murcia [10], Monserrat Real [10], Elvira Romans [10], Jordi Noguero-Ribes [10], David Parreño Duque [10], Elísabeth Fernández-Morán [10], Nusret Karakaya [11], Kerstin Häggqvist [12], Nilsun Demir [13], Meryem Beklioğlu [14], Nur Filiz [14], Eti E. Levi [14], Uğur Iskin [14], Gizem Bezirci [14], Ülkü Nihan Tavşanoğlu [14], Koray Özhan [15], Spyros Gkelis [16], Manthos Panou [16], Özden Fakioglu [17], Christos Avagianos [18], Triantafyllos Kaloudis [18], Kemal Çelik [19], Mete Yilmaz [20], Rafael Marcé [21], Nuria Catalán [21,22], Andrea G. Bravo [22], Moritz Buck [22], William Colom-Montero [23], Kristiina Mustonen [23], Don Pierson [23], Yang Yang [23], Pedro M. Raposeiro [24], Vítor Gonçalves [24], Maria G. Antoniou [25], Nikoletta Tsiarta [25], Valerie McCarthy [26], Victor C. Perello [26], Tõnu Feldmann [27], Alo Laas [27], Kristel Panksep [27], Lea Tuvikene [27], Ilona Gagala [28], Joana Mankiewicz-Boczek [28], Meral Apaydın Yağcı [29], Şakir Çınar [29], Kadir Çapkın [29], Abdulkadir Yağcı [29], Mehmet Cesur [29], Fuat Bilgin [29], Cafer Bulut [29], Rahmi Uysal [29], Ulrike Obertegger [30], Adriano Boscaini [30], Giovanna Flaim [30], Nico Salmaso [30], Leonardo Cerasino [30], Jessica Richardson [31], Petra M. Visser [32], Jolanda M. H. Verspagen [32], Tünay Karan [33], Elif Neyran Soylu [34], Faruk Maraşlıoğlu [35], Agnieszka Napiórkowska-Krzebietke [36], Agnieszka Ochocka [37], Agnieszka Pasztaleniec [37], Ana M. Antão-Geraldes [38], Vitor Vasconcelos [39], João Morais [39], Micaela Vale [39], Latife Köker [40], Reyhan Akçaalan [40], Meriç Albay [40], Dubravka Špoljarić Maronić [41], Filip Stević [41], Tanja Žuna Pfeiffer [41], Jeremy Fonvielle [42], Dietmar Straile [43], Karl-Otto Rothhaupt [43], Lars-Anders Hansson [44], Pablo Urrutia-Cordero [22,44], Luděk Bláha [45], Rodan Geriš [46], Markéta Fránková [47], Mehmet Ali Turan Koçer [48], Mehmet Tahir Alp [49], Spela Remec-Rekar [50], Tina Elersek [51], Theodoros Triantis [52], Sevasti-Kiriaki Zervou [52], Anastasia Hiskia [52], Sigrid Haande [53], Birger Skjelbred [53], Beata Madrecka [54], Hana Nemova [55], Iveta Drastichova [55], Lucia Chomova [55], Christine Edwards [56], Tuğba Ongun Sevindik [57], Hatice Tunca [57], Burçin Önem [57], Boris Aleksovski [58], Svetislav Krstić [58], Itana Bokan Vucelić [59], Lidia Nawrocka [60], Pauliina Salmi [61], Danielle Machado-Vieira [62], Alinne Gurjão de Oliveira [62], Jordi Delgado-Martín [63], David García [63], Jose Luís Cereijo [63], Joan Gomà [64], Mari Carmen Trapote [64], Teresa Vegas-Vilarrúbia [64], Biel Obrador [64], Magdalena Grabowska [65], Maciej Karpowicz [65], Damian Chmura [66], Bárbara Úbeda [67], José Ángel Gálvez [67], Arda Özen [68], Kirsten Seestern Christoffersen [69], Trine Perlt Warming [69], Justyna Kobos [70], Hanna Mazur-Marzec [70], Carmen Pérez-Martínez [71], Eloísa Ramos-Rodríguez [71], Lauri Arvola [72], Pablo Alcaraz-Párraga [73], Magdalena Toporowska [74], Barbara Pawlik-Skowronska [74], Michał Niedźwiecki [74], Wojciech Pęczuła [74], Manel Leira [75], Armand Hernández [76], Enrique Moreno-Ostos [77], José María Blanco [77], Valeriano Rodríguez [77], Jorge Juan Montes-Pérez [77], Roberto L. Palomino [77], Estela Rodríguez-Pérez [77], Rafael Carballeira [78], Antonio Camacho [79], Antonio Picazo [79], Carlos Rochera [79], Anna C. Santamans [79], Carmen Ferriol [79], Susana Romo [80], Juan Miguel Soria [80]

Toxins **2018**, *10*, 156; doi:10.3390/toxins10040156 www.mdpi.com/journal/toxins

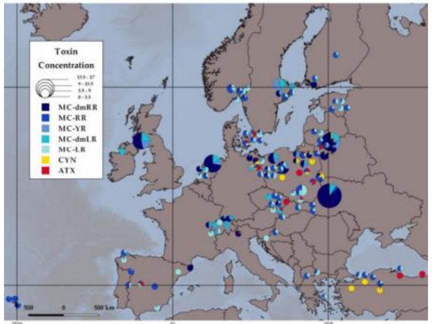
SCIENTIFIC DATA

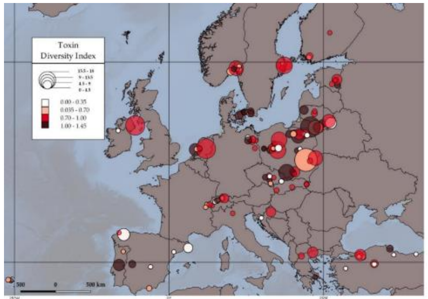
OPEN

Data Descriptor: A European Multi Lake Survey dataset of environmental variables, phytoplankton pigments and cyanotoxins

Received: 25 April 2018
Accepted: 3 July 2018
Published: 23 October 2018

Evanthia Mantzouki *et al.*#





RESULTS

- Direct and indirect effects of temperature were the main drivers of the spatial distribution in the toxins produced by the cyanobacterial community, the toxin concentrations and toxin quota.
- Generalized linear models showed that a Toxin Diversity Index (TDI) increased with latitude, while it decreased with water stability (**Figure 1**).
- The study concluded that while global warming continues, the direct and indirect effects of increased lake temperatures will drive changes in the distribution of cyanobacterial toxins in Europe, potentially promoting selection of a few highly toxic species or strains.

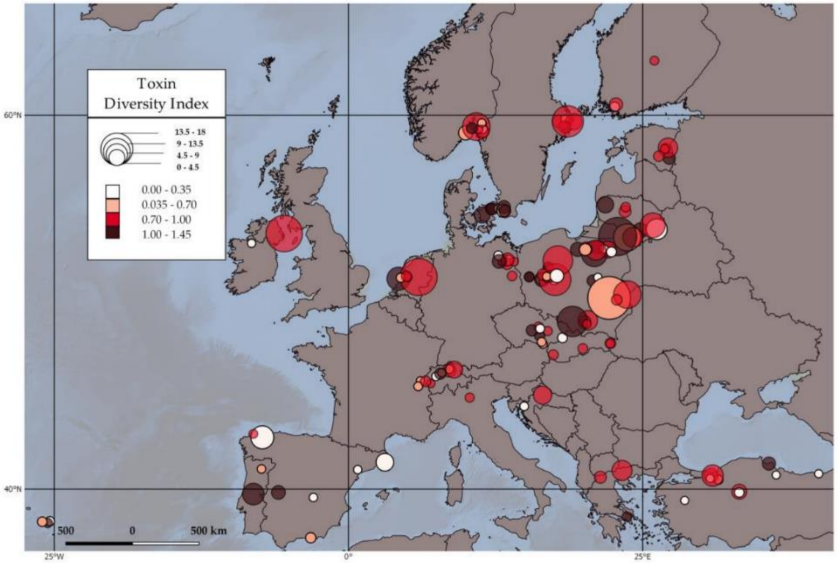
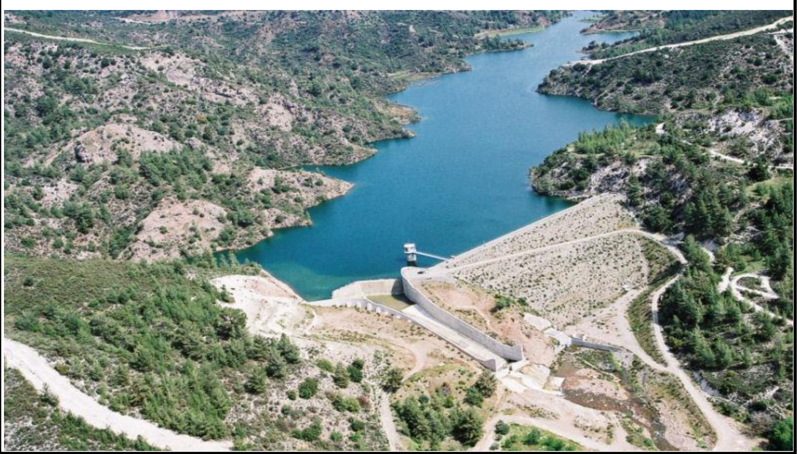


Figure 1: Map of the Toxin Diversity Index (TDI) of the 137 EMLS lakes, calculated using the Shannon equation. TDI is categorized in four classes with higher colour density (red) representing higher toxin diversity and lower colour density (white) lower toxin diversity. The radius of the markers corresponds to the total toxin concentration in µg/L. (Note: Cyprus lakes, total 5, were below the method detection limit for cyanotoxins and therefore were not included in the figure)



AQUA

Managing the risk of cyanobacteria through water quality characteristics analysis: A case study of two warm Mediterranean reservoirs

ANTONIOU M.G.[1], KAGALOU I.[2,3], HADJIOURANIOU G.[1], DASKALAKIS E.[1], TSIARTA N.[1], CHAMOGLOU M.[2] AND POLYKARPOY P.[4]

[1] Department of Environmental Science and Technology, Cyprus University of Technology, 3036 Lemesos, Cyprus

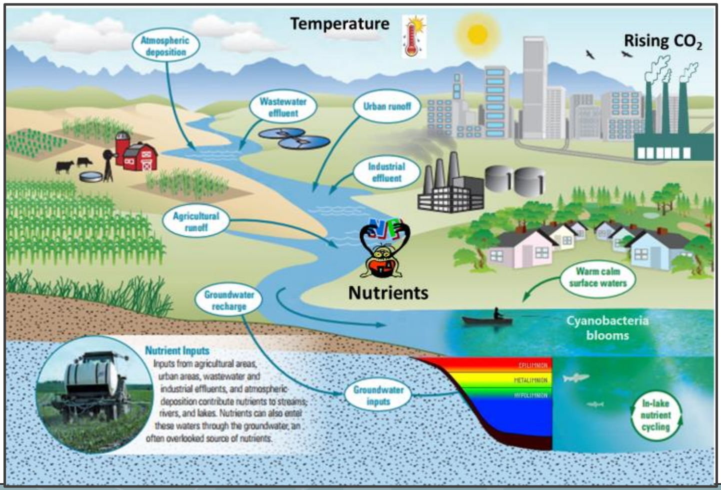
[2] Department of Civil Engineering, Democritus University of Thrace, 67100 Xanthi, Greece

[3] Management Body of Ecodevelopment Area of Karla - Mavrovouni – Kefalovriso – Velestino- Pinios delta, Kanalia, 38500, Greece

[4] Division of Hydrometry, Water Development Department, 1646 Nicosia, Cyprus

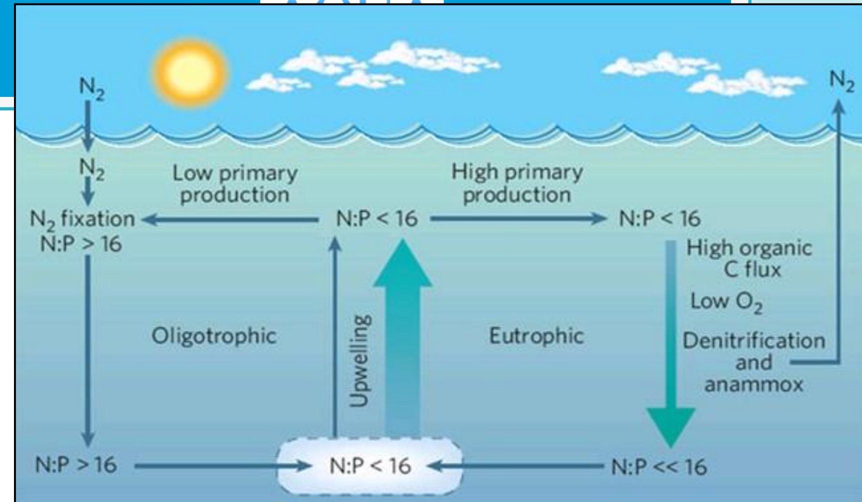
Main Objectives of Study

- Organize the collected data from the monitoring activities of the Water Development Department of Cyprus to Polemidia Dam.
- How nutrient fluctuations affect cyanobacteria blooming
- Use Multiple linear regression has been employed to identify the principle components of cyano-HAB formation in the mostly frequently affected surface water in Cyprus
- Identify the key- environmental variables driving eutrophication and cyanobacteria blooming in a re-established Greek reservoir (Lake Karla).



J. Meriluoto et al, Handbook of cyanobacterial monitoring and cyanotoxin analysis, John Wiley & Sons, Inc., 2017

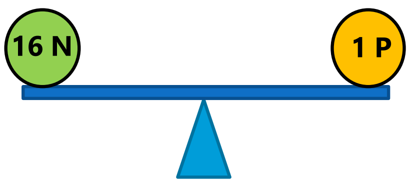
Redfield ratio concept



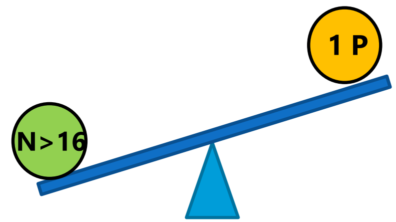
The 'average' phytoplankton consists of (in moles):

(Redfield, 1958)

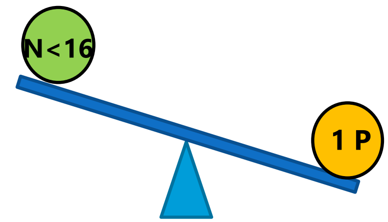
C	:	N	:	P
106	:	16	:	1



Redfield ratio



P-limitation

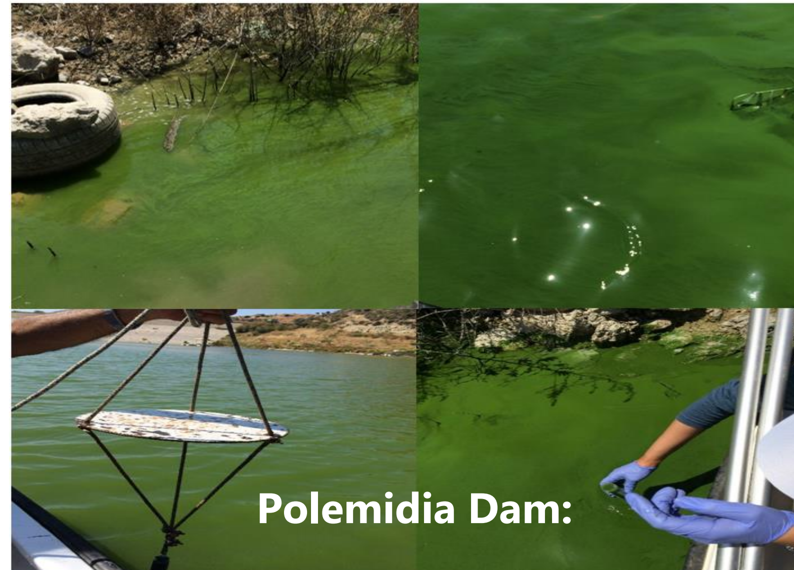


N-limitation

- TN : TP reflects the source of nutrients
  - **oligotrophic lakes:** TN/TP is high because they receive their N and P from natural, undisturbed watersheds which export much less P than N
  - **mesotrophic and eutrophic lakes** receive various mixtures of nutrient sources that have lower average N: P
  - **hypereutrophic lakes** have N: P that correspond very nearly to the N: P of sewage.
- TN/TP <22 N_2-fixing bacteria are favored (*Dolichosperum* (*Anabaena*), *Aphanizomenon*, *Microcystis*, *Oscillatoria*, and *Lyngbya*)

Case study 1 - Polemidia Dam, CY

Geological map of Polemidia.
Red: Vati Landfill
Blue: Garyllis River
Black: Polemidia Dam



- Built 1965
- Depth = 45m
- Capacity = 34000000 m^3
- Irrigation
- Quality state: Eutrophic

➢ **Eutrophic state** of the system due to:

i. Poor rainfall
ii. Influxes of untreated urban and industrial wastewater
iii. Fertilizers from near by farms

Case study 2 - Lake Karla, GR

The catchment

Lake Karla Watershed: 1076 Km²

- Closed basin
- The only way out to the sea → Karla tunnel
- Rural basin

Ecodevelopment Area: 1217 Km²

- 5 Special Protected Area Natura 2000
- 3 Sites of Community Importance Natura 2000
- 6 Wildlife refugees

Lake/Reservoir Karla: 38 Km²

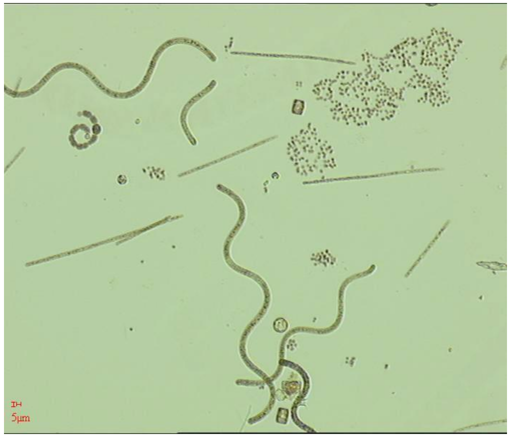
- Located in the lowest part of the basin

Water Stage	Absolute Height (m)	Area (Km²)	Water Volume (hm³)
Lowest	46.4	34.65	57.01
Highest Irrigation	48.8	35.45	141.14
Flood Highest	50	35.8	183.88

AQUA

Main goals

1. Polemidia Dam: correlate water quality characteristics of the Polemidia dam (TP, PO_4^{2-}, TN, NH_4^+, NO_2^-, NO_3^-, T_{water}) with its eutrophic state and the formation of cyanobacteria blooming, between the years 2007 and 2017, through multiple linear regression analysis.
2. Lake Karla: correlate water quality parameters with the eutrophication process through multivariate analysis



AQUA

Methodology

Case study 1: Multiple Regression Analysis (Matlab®)

- Used to explain the relationship between one **continuous dependent variable** and two or more **independent variables**.
- Find the relationship between two or more explanatory variables (TP, PO_4^{2-}, TN, NH_4^+, NO_2^-, NO_3^-, T_{water}) and a response variable (Cyanobacteria biovolume, mm^3/L; Chlorophyll-a mg/m^3; COD, mg/L; Phytoplankton Biovolume, mm^3/L;) by fitting a linear equation to the observed data.
- Dependent variables needed to be continuous.

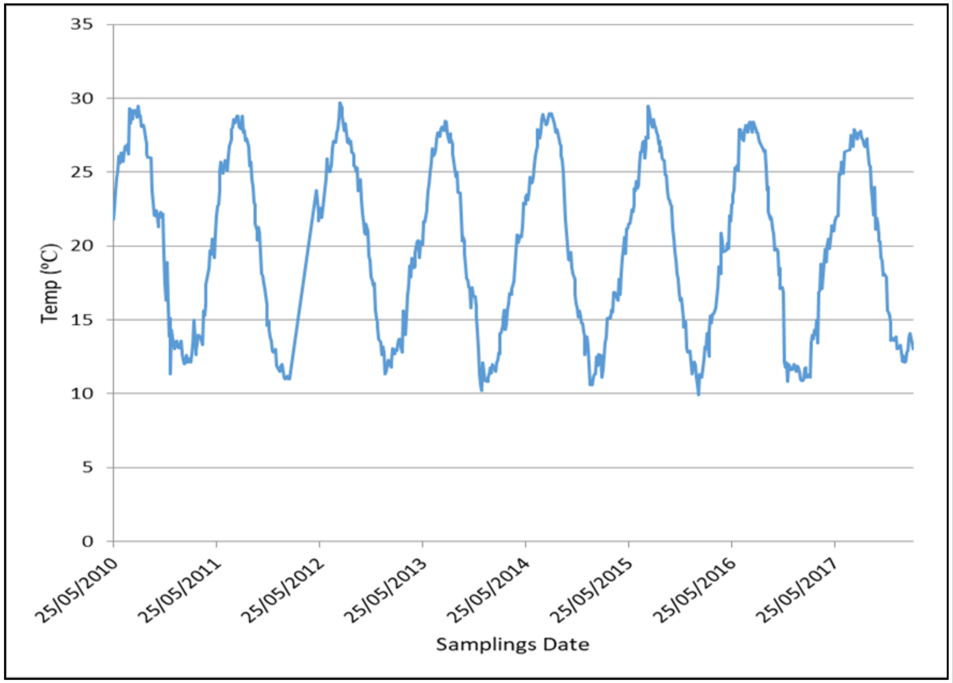
Case study 2: Multivariate Analysis

- The term "multivariate statistics" is appropriately used to include all statistics where there are more than two variables simultaneously analyzed.
- **Principle Component Analysis (PCA)** has been used to explore which **variables** (DO, pH, Secchi disk, T_{water}, Ammonia, Nitrate, TP, Phycocyanin, Chlorophyll-a, MCYST) **explain the best the variation** in the dataset **and bring out strong patterns.**
- Principal components are linear combinations of the original variables weighted by their contribution to explaining the variance in a particular orthogonal dimension

AQUA

Results 1-Temperature profile

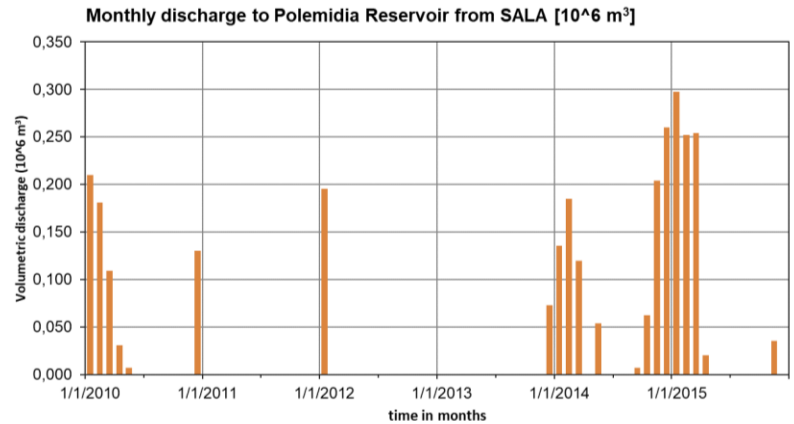
- Great variability of temperature within a year
- Seasonal trends remain the same
- Lowest recorded temperature: 9.9 °C on 29 January 2016
- Highest recorded temperature : 29.5 °C in August 2010, 2012, 2015
- Temperature does not comprise a limiting factor for cyanobacteria blooming throughout the year.
- Indirect estimation of light



Variation of surface water Temperature (°C) of Polemidia Dam (2010 – 2017).

Nutrient Ratio Correlations

- **Redfield Ratio** TN:TP = 16:1
- Alternative ratios DIN/SRP, και **DIN:TP = 22** Ptacnik et
- Based TN/TP ratio following 2010, Polemidia is P-limited but **years 2014, 2015!**
- For 2014 and 2015, TP concentrations are the highest of the
- DIN concentration for both summer periods, 2014 and 2015 was rich in N)
- Reason: High Photosynthetic activity in the lake. High concentrations of chl-a (222,5 and 236,5 mg/m^3).
  - Phytoplankton might use most of the DIN available in the water column (N requirements for pigments).



YEAR	TN (mg/L N)	TP (mg/L P)	TN/TP ratio (molar)	Nutrient Limitation	Chl-a_fresh (mg/m3)	NO3-N (mg/L N)	DIN (mg/L N)	SRP (mg/L P)	DIN /SRP ratio	DIN/TP ratio (mass)	Nutrient Limitation
2007	9,175	3,279	6,2	N	187,50	3,89	6,80	0,357	42	2,1	N
2008	3,057	1,080	6,3	N	-	0,53	1,17	1,213	2	1,1	N
2009	4,517	0,518	19,3	P	159,18	1,20	1,70	0,005	784	3,3	N
2010	4,786	0,237	44,7	P	155,88	1,99	2,35	0,005	1085	9,9	N
2011	3,250	0,032	222,4	P	134,13	1,25	1,54	0,025	136	47,6	P
2012	3,654	0,064	125,5	P	63,27	2,40	2,75	0,003	1900	42,7	P
2013	2,112	0,034	135,8	P	59,04	1,25	1,47	0,043	76	42,7	P
2014	4,525	0,200	50,0	P	156,43	0,41	0,99	0,233	9	5,0	N
2015	3,250	0,156	46,1	P	152,95	1,29	1,58	0,160	22	10,1	N
2016	2,175	0,036	133,7	P	-	0,54	0,88	0,015	129	24,3	P
2017	8,600	0,252	75,7	P	-	5,54	7,18	0,015	1058	28,5	P

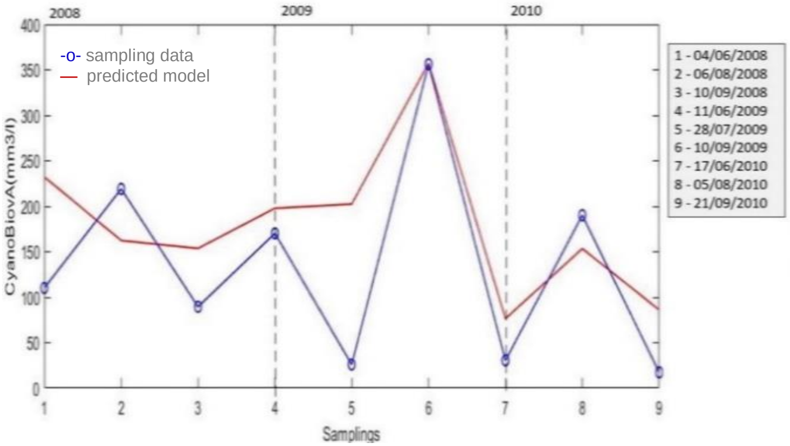
Effluent characteristics from the WWTP (SBLA)

YEAR	TN (mg/L)	TP (mg/L)
2007	14.1	4.01
2008	5.95	4.81
2009	5.15	2.59
2010	7.2	2.43
2011	6.92	2.03
2012	6.88	1.58
2013	7.25	1.74
2014	9.91	1.43
2015	15.97	1.57
2016	11.02	0.97
2017	12.91	1.54

Results 1 – Divided Multiple linear regression (2007 – 2010 and 2011 - 2015)

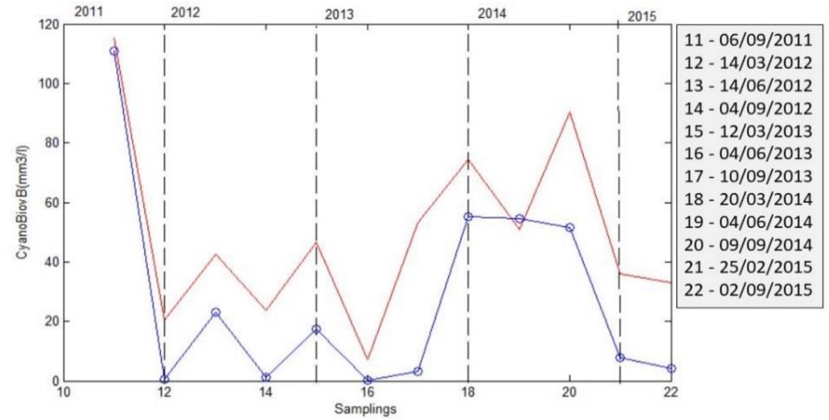
1. Cyanobacteria Biovolume [mm^3/L]

(A) from 2007 till 2010



CyanoBiovolumeA = 1 + 747.65*Ptotal – 53.96*PO4

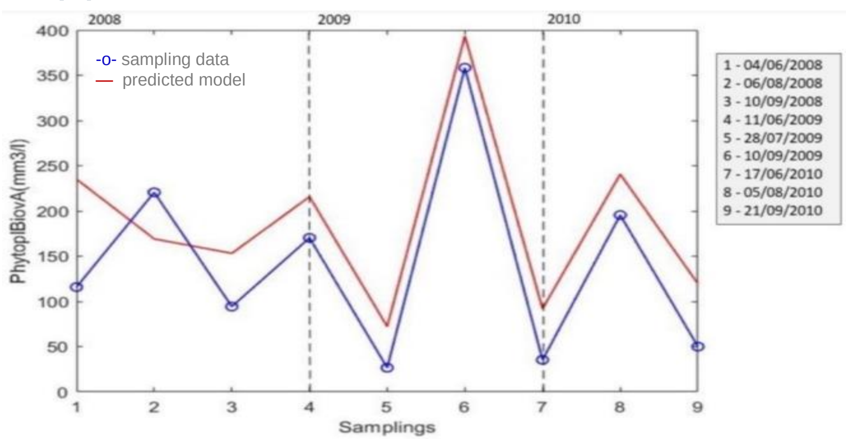
(B) from 2011 till 2015



CyanoBiovolumeB = 40 + 17.818*Ntotal + 88.333*NH4 – 888.33*NO2 - 47.669*NO3 – 282.12*Ptotal

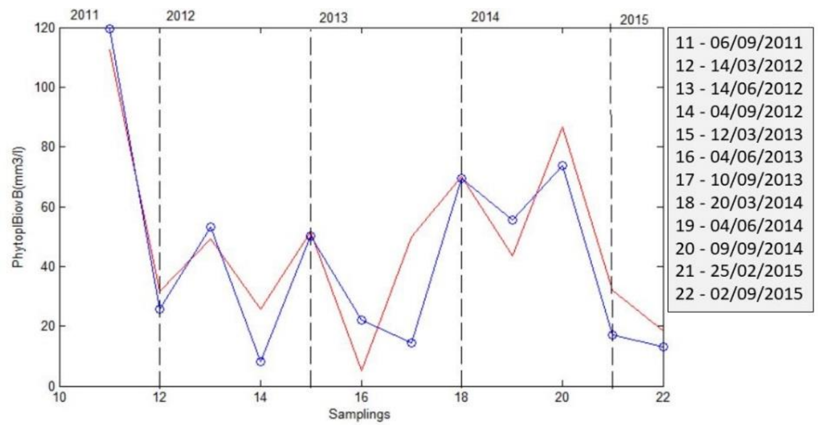
2. Phytoplankton Biovolume [mm^3/L]

(A) from 2007 till 2010



PhytoBiovolumeA = 1 – 1464.9*NO2 + 118.33*NO3 – 833.96*Ptotal – 669.37*PO4

(B) from 2011 till 2015

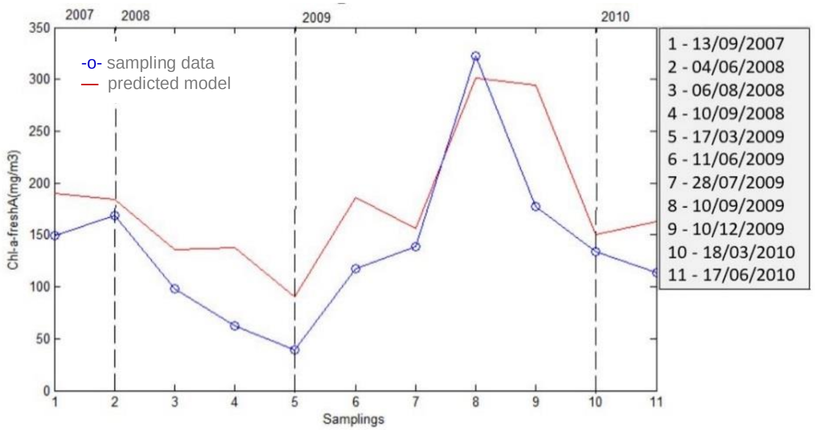


PhytoBiovolumeB = 40 + 19.367*Ntotal + 80.417*NH4 + 1037.1*NO2 – 47.371*NO3 – 370.41*Ptotal

Results 1 – Divided Multiple linear regression (2007 – 2010 and 2011 - 2015)

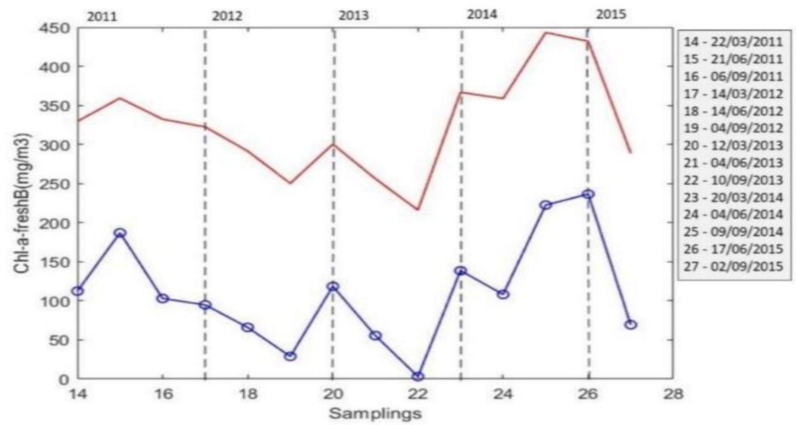
3. Chlorophyll - a [mg/m³]

(A) from 2007 till 2010



Chl-a A =500 + 3.693*NO2+35.106*NO3 – 0.7039*PO4 - 12.248*Temp

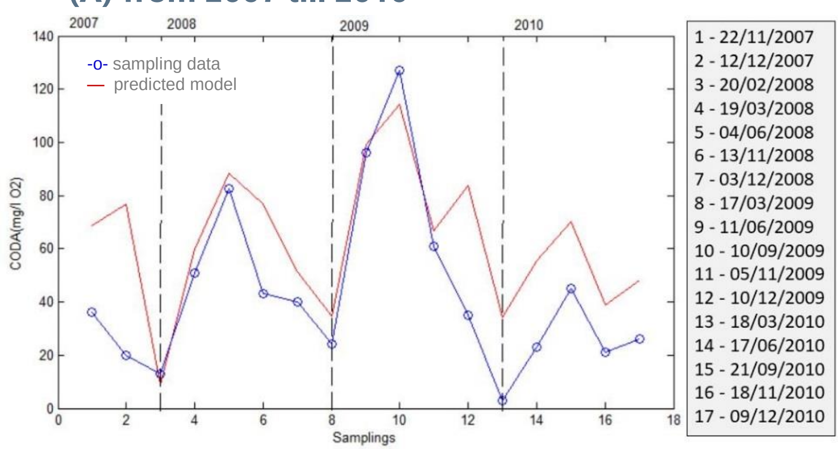
(B) from 2011 till 2015



Chl-a B= 1 + 58.654*Ntotal + 91.7*NH4 – 131.37*NO2 – 10.472*NO3 + 475.43*Ptotal – 44.961*PO4 – 5.357 *Temp

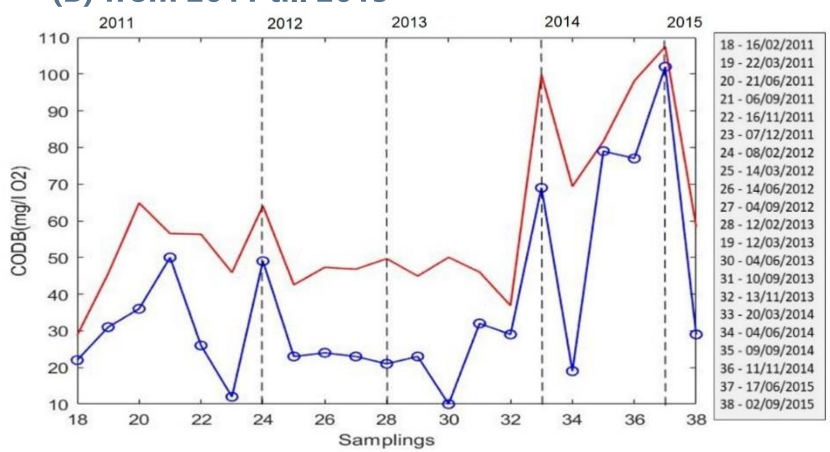
4. COD [mg/L]

(A) from 2007 till 2010



COD A = 30 – 115*NH4 – 30.137*NO2 – 5.714*NO3 + 123.75*PO4 + 2.0835*Temp

(B) from 2011 till 2015



COD B= 1 + 8.5119*Ntotal + 1.4008*NH4 – 115.27*NO2 – 3.551*NO3 + 96.219*Ptotal – 155.05*PO4 + 1.225*Temp

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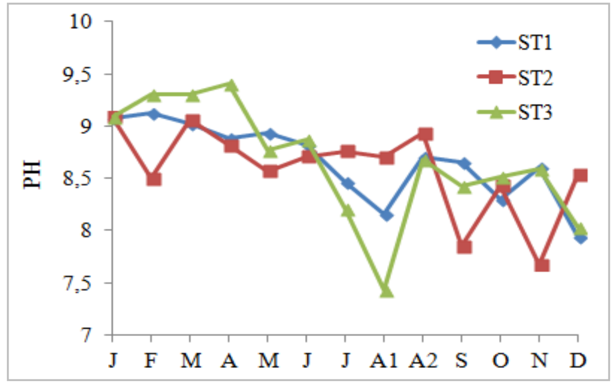
Results 2 – Lake Karla (monthly monitoring in 2012-13)

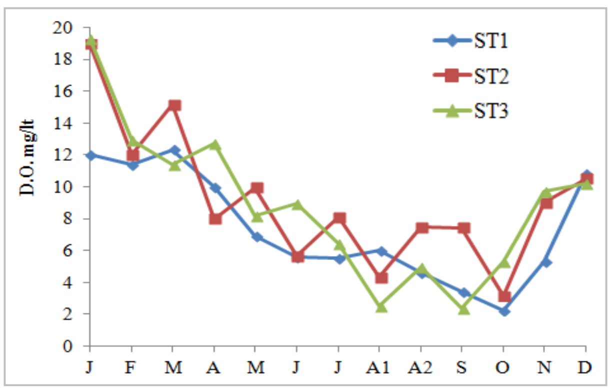
1. Temperature [°C]

2. pH

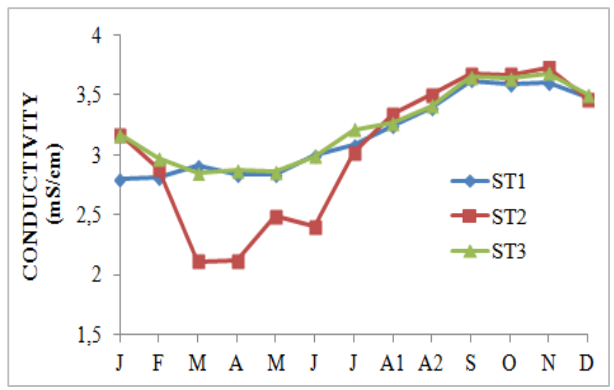


- It is a warm lake (special typology)
- Alkalic lake (high metabolic rates)

3. DO [mg/L]



4. Conductivity [mS/cm]



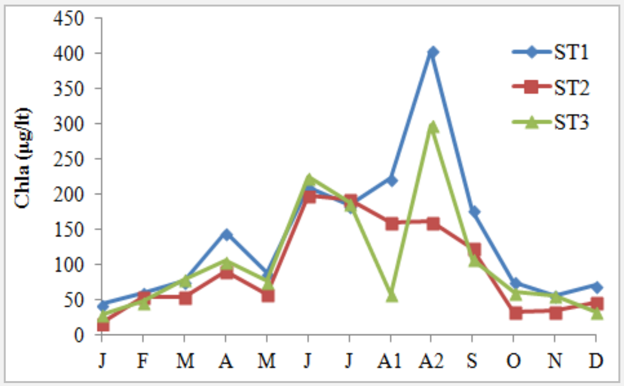
- Low DO during the warm dry period (depth<1m)
- High ions concentrations (geology, inputs, salinization)

Results 2 – Lake Karla (monthly monitoring in 2012-13)

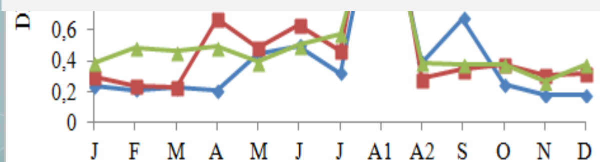
5. NO_3^- [mg/L]

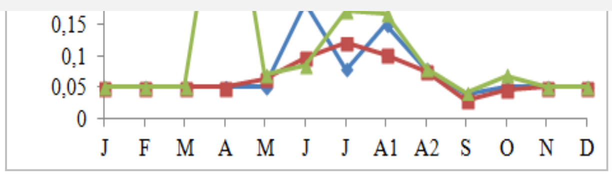
6. NH_4^+ [mg/L]

9. Chlorophyll-a [μg/L]



- Chl-a values indicate **eutrophication** conditions throughout all the year.

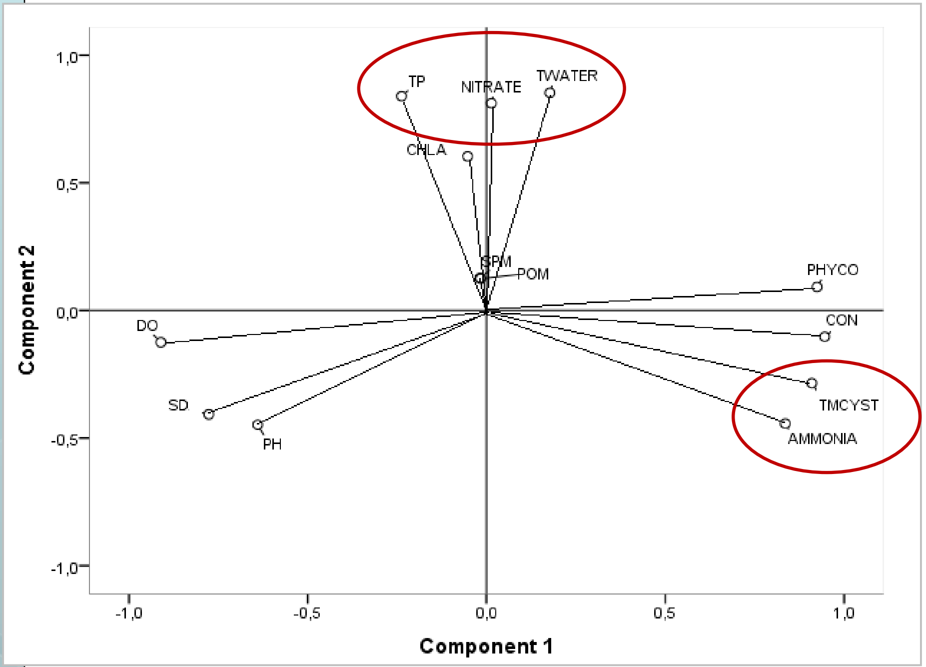


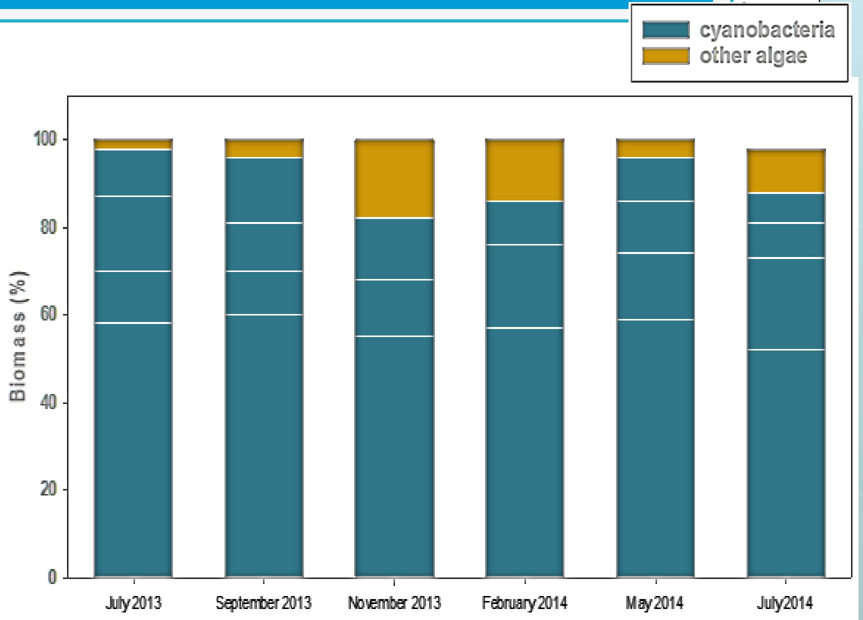


- High nitrification rates during warm period
- Ammonia - N higher than the limit of 0.2 mg/l (fish intoxication limit, EU 2006/44)
- DIN (~ 0.5 mg/L N)
- TP >> 0.05 mg/L (eutrophication limit for SMLs, Beklioglu et al., 2007)
- TP near the lower limit of hypereutrophy (0.1 mg/L, OECD, EEA guidelines)

Results 2 – Lake Karla (monthly monitoring in 2013)

AQUA





Cyanobacteria's contribution to Lake's Karla algae composition

(Gkelis et al. 2017)

Principal component analysis:

- first axis (PC1 component) explains the 40,37 % of the total variance highlighting the positive correlation between **MCYSTs** and **Ammonia**.
- second axis (PC2 component) explains the 25.3% of the variation showing the positive correlation with **Temperature**, **TP** and **Nitrates**.

Conclusions

- Organizing the collected data from the monitoring activities we were able to clearly see the annual changes of water characteristics in the Polemidia Dam.
- Correlate intake fluxes of tertiary treated wastewater streams in the dam with its eutrophic state
- The DIN:TP ratio better descript the system than the Redfield ratio (TN/TP)
- Multiple linear regression assisted into identifying the parameters controlling the blooming of cyanobacteria but in order to form predictive models a more systematic sampling is needed (dynamic system)
- Based on the above, a third phase is expected to begin since the restoration of Vati landfill began in 2017
- Lake Karla is a eutrophicated warm lake. Both nutrients are in excess. Temperature, nitrogen compounds (inorganic) and phosphorus seem to be the driving factors for the cyanotoxicity. Increasing warming amplifies the present conditions.
- There is also a need to correlate the findings on both reservoirs with the cyanobacterial species detected each time.
- Climate change will make cyano-HABs more prevalent and persistent. **Monitoring for cyanotoxins will no longer be optional!**

In-lake treatments

- **Aeration**
  - Pros: Disturbs stratification, minimizes blooming, reduces P release from sediments, odor control
  - Cons: effectiveness greatly varies (mixing rate, deep lakes, 80% of the water body is mixed)
- **Conventional Chemicals**
  - **Herbicides**: diuron, parquat, atrazine
    - Pros: Effective, affordable
    - Cons: Non-selective, toxin release from lysed cells, regulated substances (EQS, Directive 2013/39/EU).
  - **Copper algaecides**
    - Pros: Effective, affordable
    - Cons: Non-selective, toxin release from lysed cells, regulated substances, copper is easily oxidized and precipitates, repeated applications, human poisoning related to its application (Palm Island Disease, 1979)
  - **Phoslock**: rapidly binds and permanently removes free reactive phosphorus (FRP) from the water column (lanthanum permanently binds phosphorus)
- **Emerging Treatments**
  - Application of chemicals used in water treatment trains: Hydrogen peroxide, ozone (risk of lysing cells), permanganate (in combination with alum and copper sulfate).

EPA -810F11001, September 2014
https://www.epa.gov/sites/production/files/2014-08/documents/cyanobacteria_factsheet.pdf

AWWA_ A Water Utility Manager's Guide to Cyanotoxins_2015
http://www.waterrf.org/PublicReportLibrary/4548a.pdf

CYANOCOST

CyanoCOST Training School Peroxide (Amsterdam NL, September 3-7 2014)

In lake treatment with H_2O_2 for Cyano-HABs control

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

IBED

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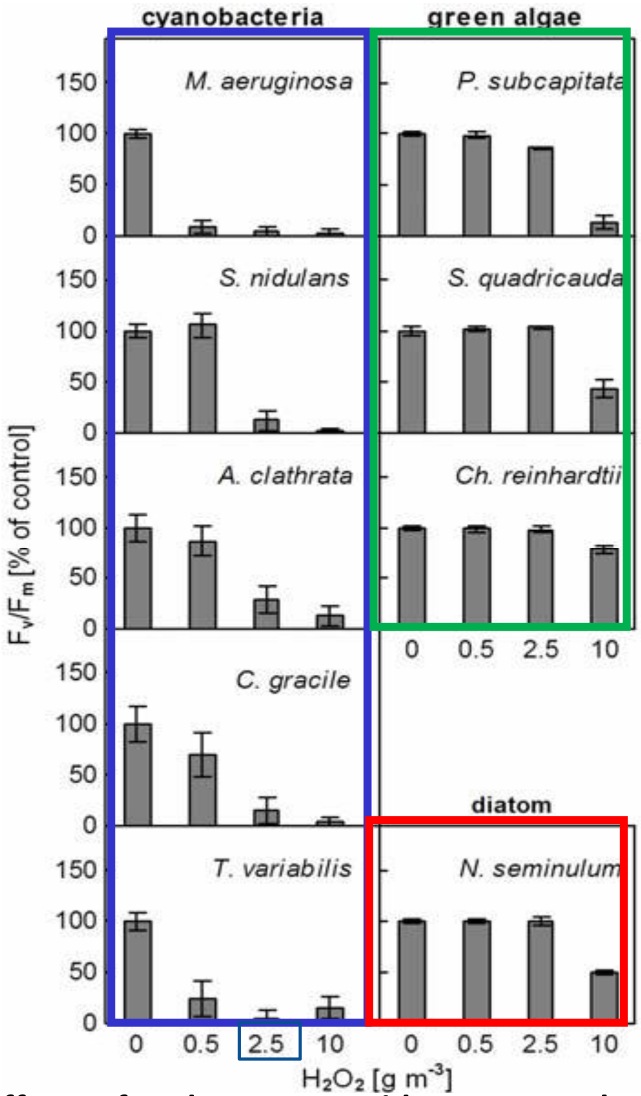
Main Goals

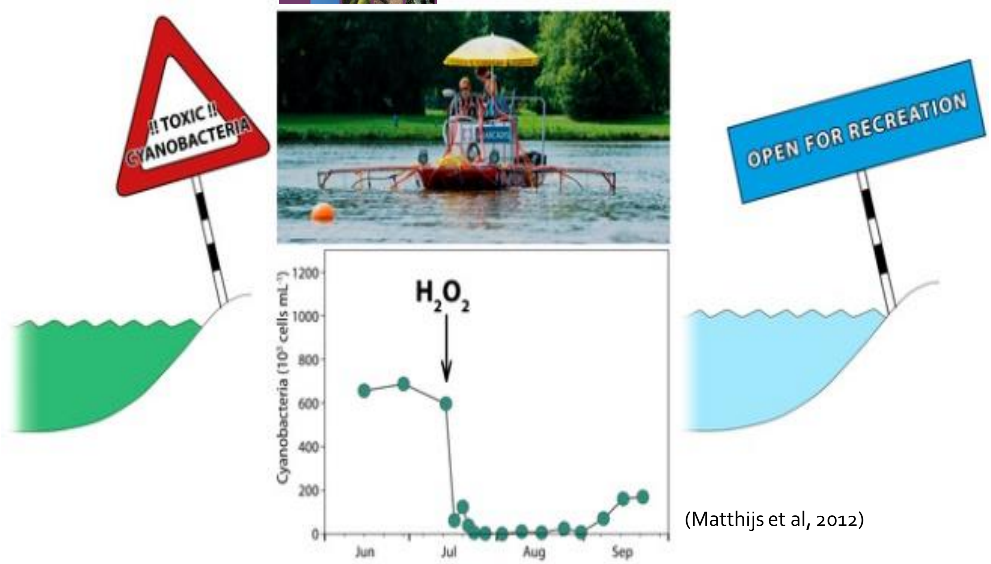
- HP dosing
- Photosynthetic vitality analysis before and after treatment (phyto-PAM)
- HP degradation (colorimetric assay p-NPBA)
- Dead and surviving species
  (phyto-PAM and microscopy species analysis)
- Fate of toxins (LC/MS/MS analysis, RGU, UK)

#	Lake, place	Country	Dominant species
1	**Polemidia Dam**	**Cyprus**	***Microcystis sp.***
2	Lake Köyliönjärvi	Finland	*Anabaena, Microcystis, Planktothrix*
3	Rennes	France	*Woronichinia naegeliana , Aphanizomenon flos aquae, Anabaena*
4	Fancsika	Hungary	*Cylindrospermopsis raciborskii*
5	Sirvys	Lithuania	*Nodularia*
7	Lubiaskie	Poland	*Planktothrix*
8	Cuiperca	Romania	*Planktothrix*
10	Lunha	Serbia	*Microcystis aeruginosa*
12	Kovada	Turkey	*Microcystis aeruginosa*
17	Szeged pond	Hungary	*Anabaena spiroides Planktothrix sp.*

Hydrogen Peroxide (H_2O_2)



Effects of Hydrogen Peroxide on green algae and cyanobacteria



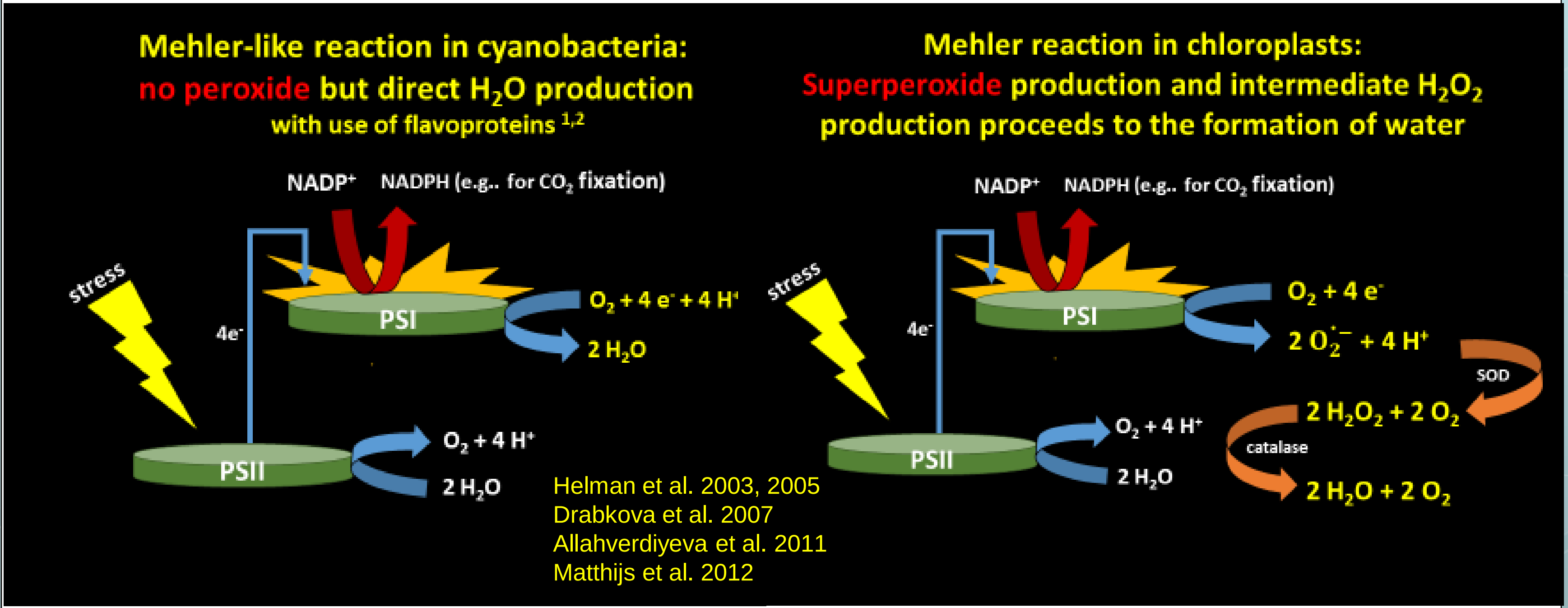
(Matthijs et al, 2012)

Recommendation: Residual H_2O_2 should be 2 mg/L after 5 hours to have enough exposure of H_2O_2 to the cyanobacteria.

Drabkova et al., *Photosynthetica*, 45 (3) (2007) 363-369
Matthijs et al., *Water Research*, 46 (5) (2011) 1460-1472

AQUA

How H_2O_2 selectively oxidizes cyanobacteria



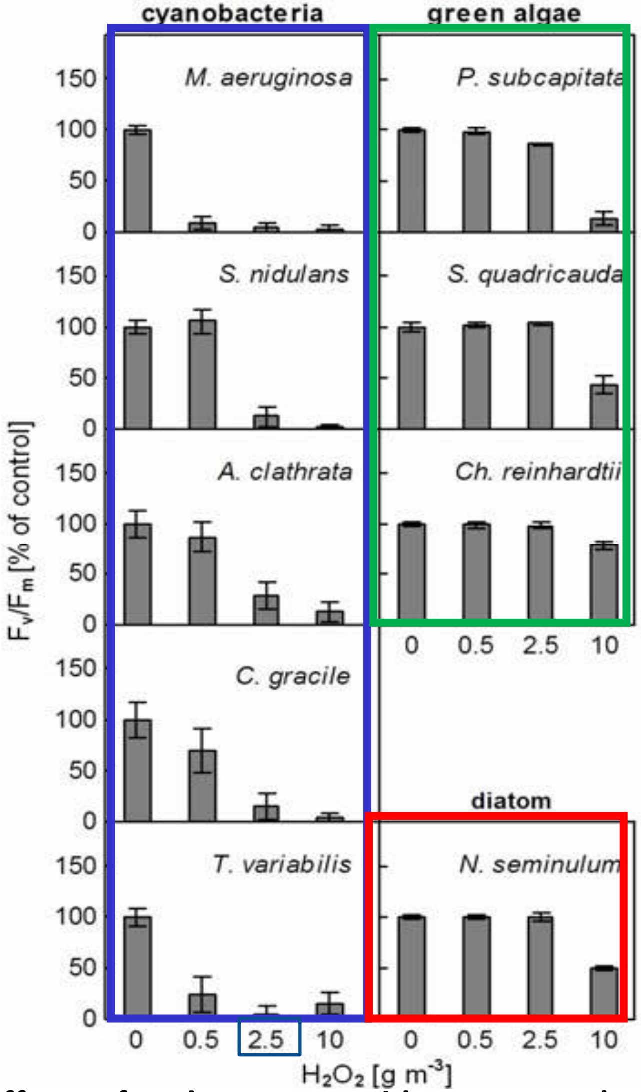
Prokaryotes

Eukaryotes

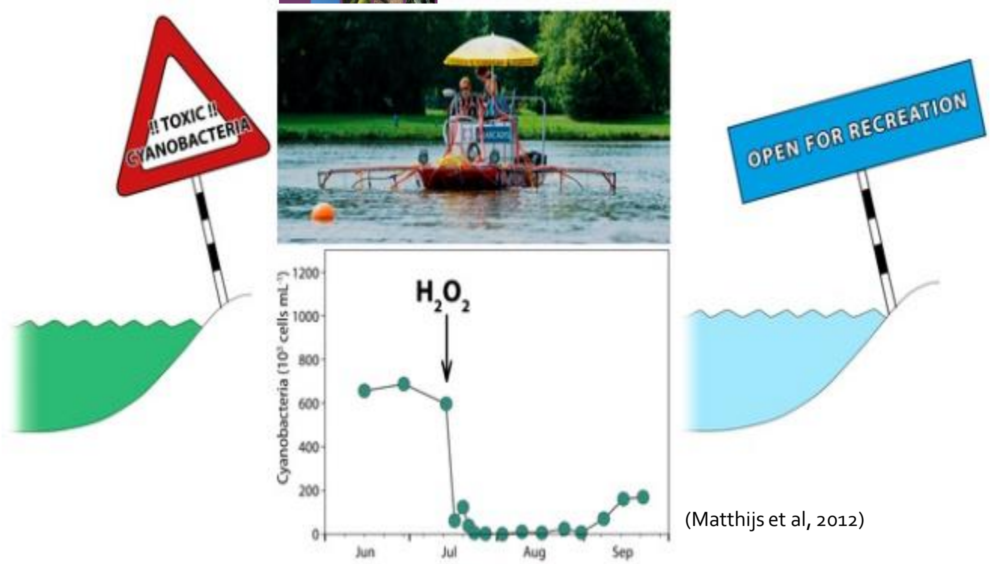
Mehler-like reaction in Cyanobacteria: Less ROS defense, Sensitive to peroxide.

True Mehler reaction in green algae: ROS defense, Protected to peroxide!

Hydrogen Peroxide (H_2O_2)



Effects of Hydrogen Peroxide on green algae and cyanobacteria



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Drabkova et al., *Photosynthetica*, 45 (3) (2007) 363-369
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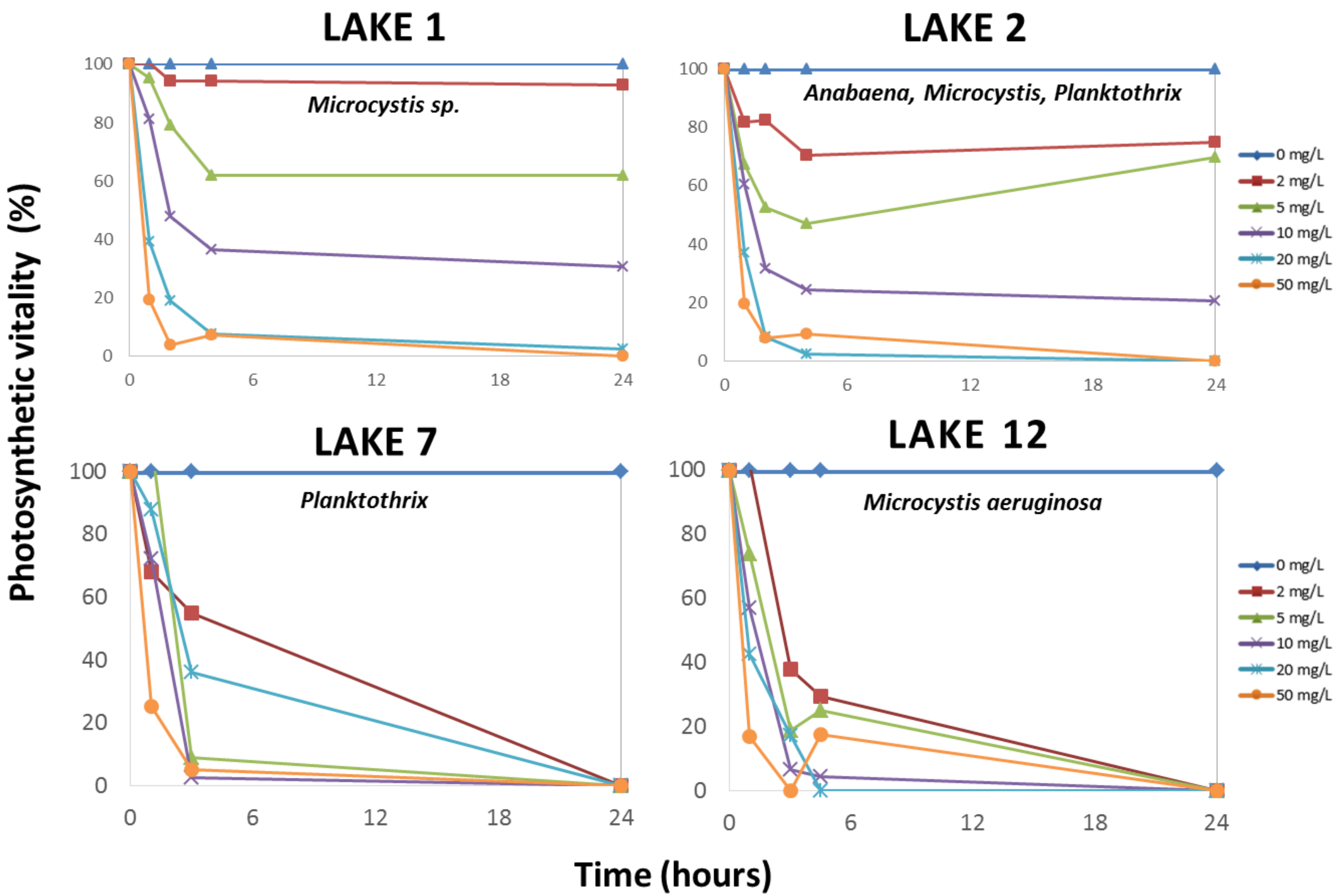
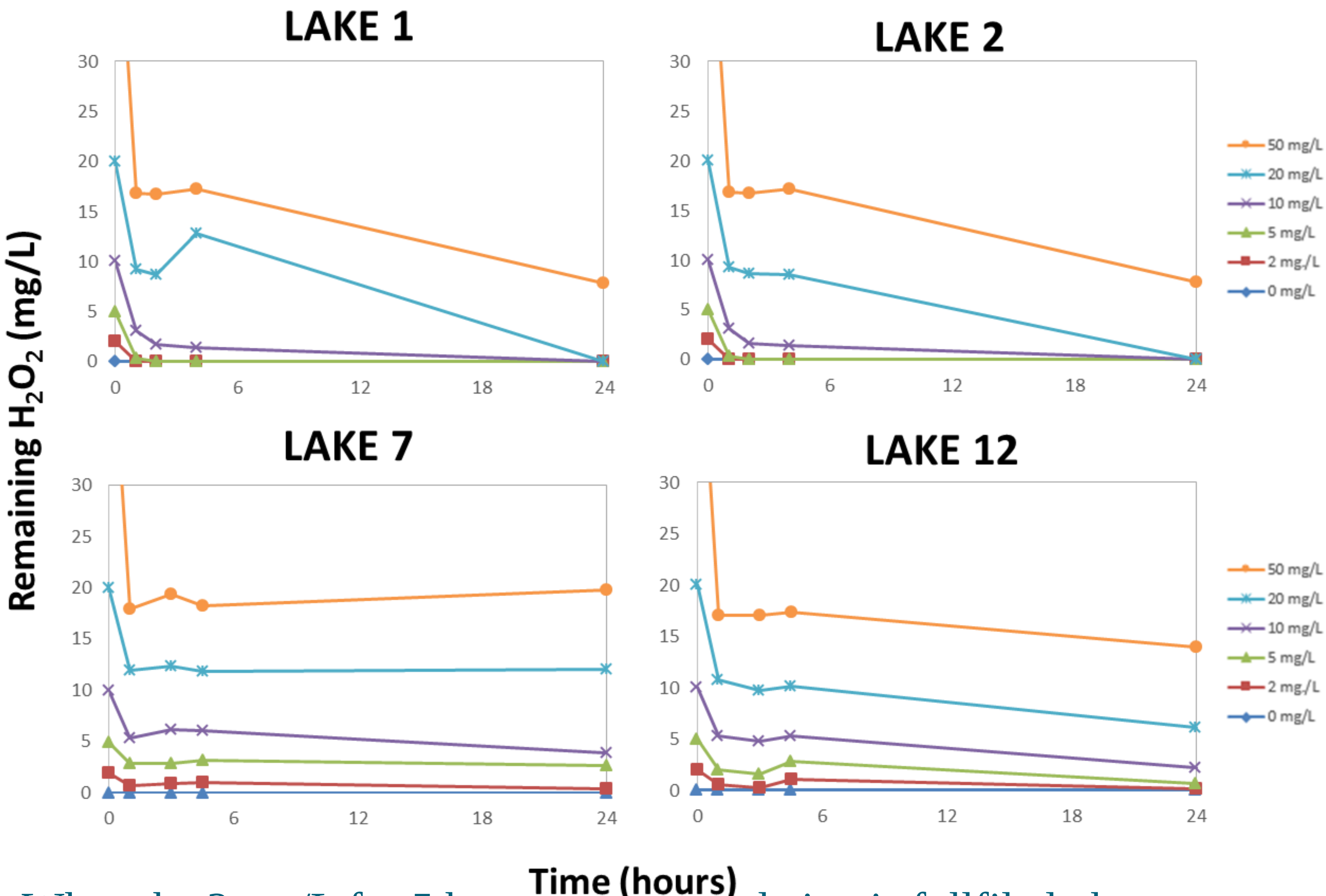
Effect of oxidant on photosynthetic vitality

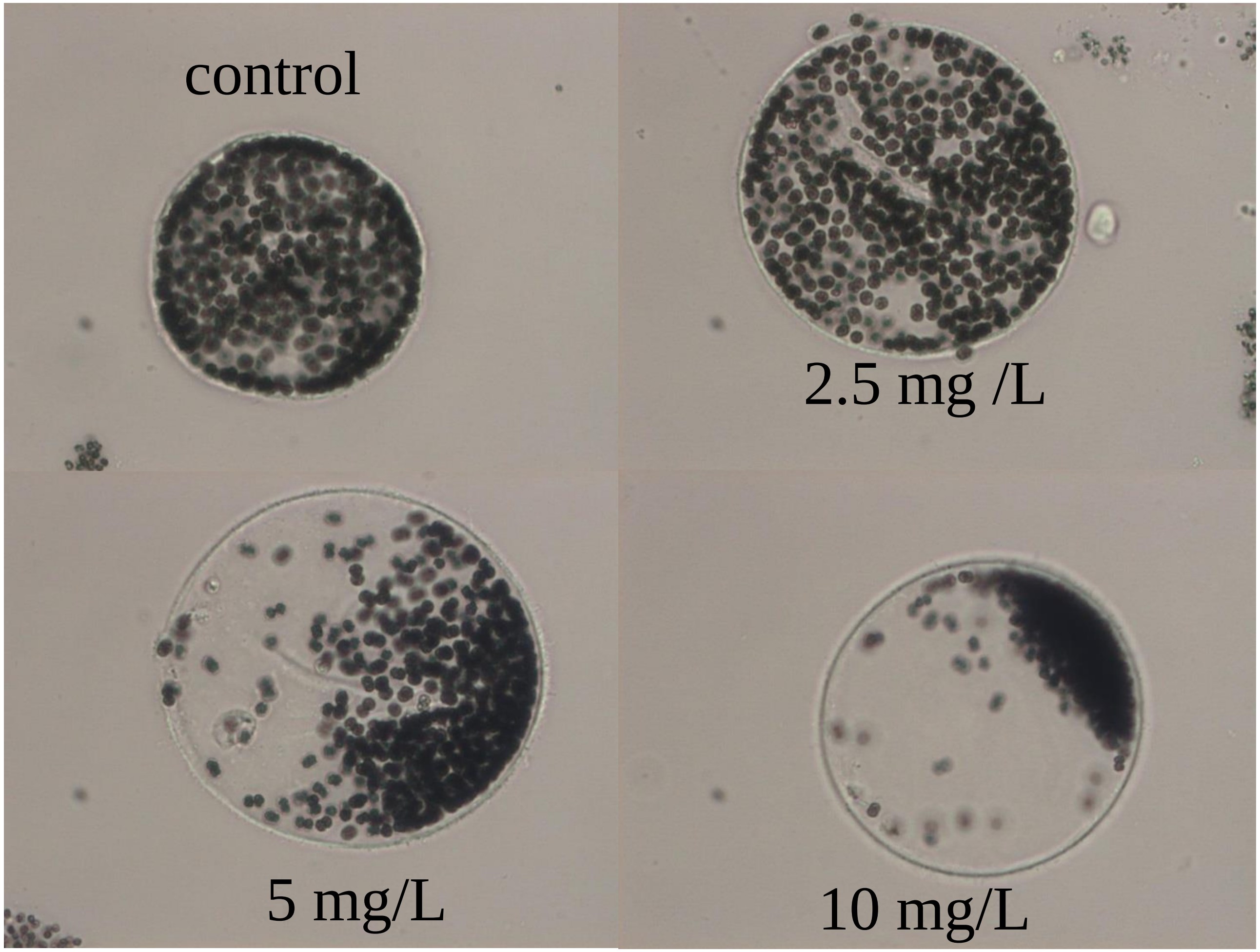


Remaining oxidant concentration



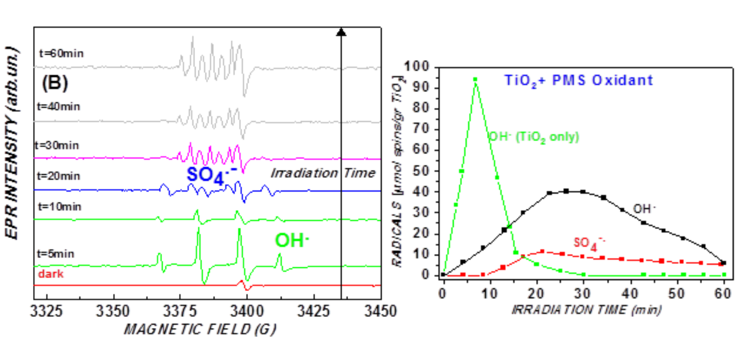
- When the 2 mg/L for 5 hours recommendation is fullfiled, then treatment is successful.

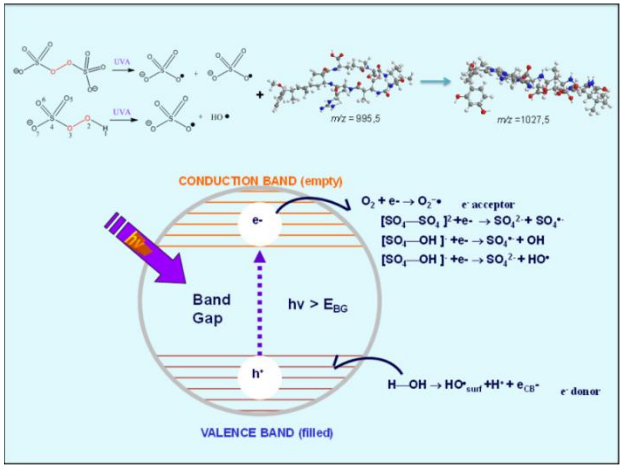
Morphological effects of treatment



- H_2O_2 induced morphological changes in *Microcystis wesenbergii*
- If H_2O_2 is less than 5 mg/L then zooplank is not affected.

AQUA









Application of Electron Paramagnetic Resonance (EPR) for radical identification during the photocatalytic degradation of cyanotoxins with enhanced photocatalysis

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ROBERT GORDON UNIVERSITY ABERDEEN

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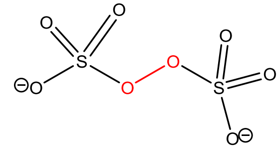
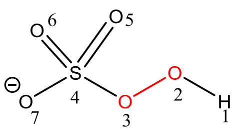
CYANOCOST
COST ES 1105 Action

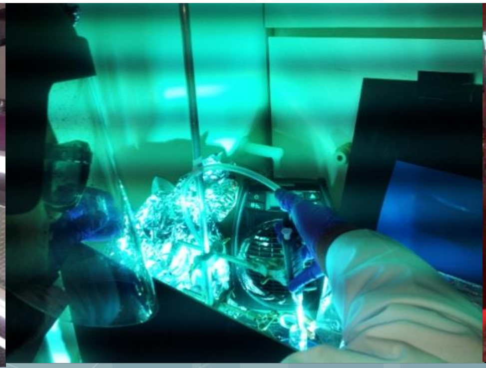
Cyprus University of Technology

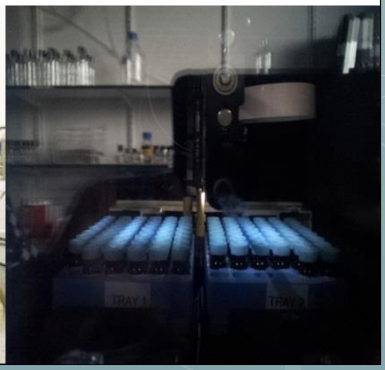
Main Goals

- Couple TiO_2 photocatalysis with sulfate radical oxidants.
  - UVA/TiO_2/PS
  - UVA/TiO_2/PMS

PS  PMS 

- Calculate the energy per order (E_{EO}) of each system.
- Identification of transformation products (LC/MS/MS).
- Inhibition studies.
- **RADICAL IDENTIFICATION & QUANTIFICATION**

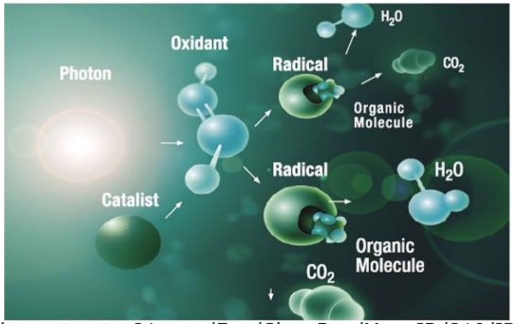



Advanced Oxidation Processes (AOPs)

- **Advanced Oxidation Processes (AOPs):** involve the generation of highly oxidizing species also known as **reactive oxygen species (ROS),** through the activation of oxidants with catalysts, radiation, heat, and ultrasounds as well as their combinations.
- Benefit: **synergistic effect** between the substances susceptibility to chemical and light/heat degradation which reduces the overall energy demand of the treatment (costs).
- Typical examples of AOPs include:
  - Photolysis with UVC radiation

  in the absence and presence of oxidants (H_2O_2)
  - Sonolysis
  - Semiconductor Photocatalysis (TiO_2) with UVA radiation
  - Sulfate Radical based AOPs (SR-AOPs)
  - Peroxone (O_3/ H_2O_2)



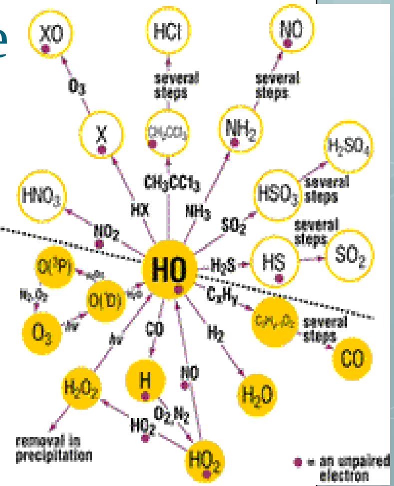
http://www.treatec21.com/Eng/ShowDoc/MenuID/310/ID/888/

http://www.triplepundit.com/2007/11/tio2-paint-at-war-with-pollution/

Reactive oxygen species (ROS)

- ROS are highly reactive species (radicals) that contain oxygen.
- Have unpaired e^- in the valence shell (orbit) which makes them very chemically unstable.
- In order to be stabilized, they obtain e^- from other compounds via oxidation.

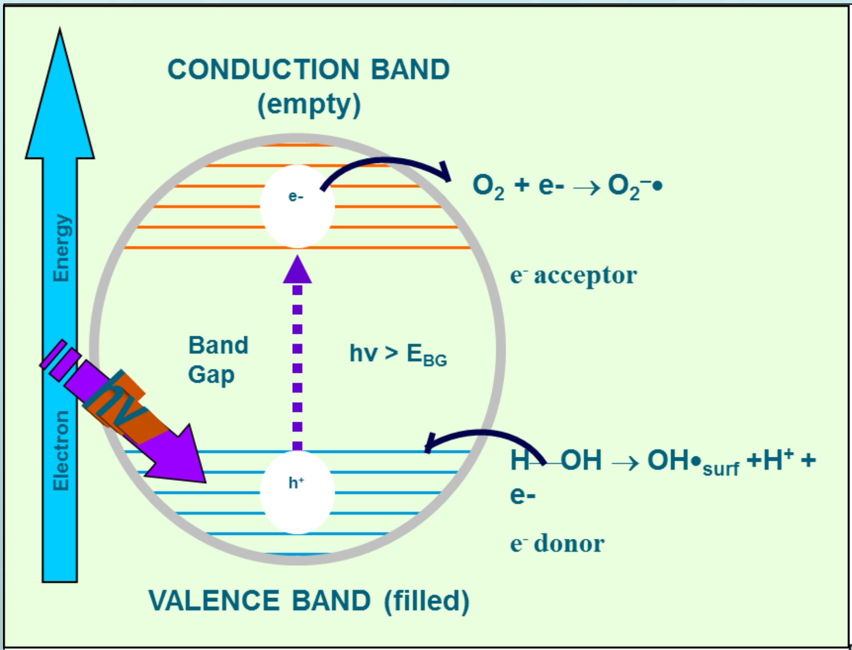
- **Hydroxyl radical ($HO^{\bullet}$)**, perhydroxyl radical ($H_2O^{\bullet}$), singlet oxygen ($O_2^{\bullet -}$), **sulfate radical ($SO_4^{\bullet -}$),** and the persulfate radical ($SO_5^{\bullet -}$).



http://www.dietisan.com/5Freeradicals.php

- Radical reactions are separated into three distinct steps:
  - Initiation: $H_2O_2 + hv \rightarrow 2HO^{\bullet}$
  - Propagation: $H_2C{=}CH_2 + HO^{\bullet} \rightarrow H_2C(OH)\text{-}\dot{C}H_2$
  - Termination: $H_2C(OH)\text{-}\dot{C}H_2 + HO^{\bullet} \rightarrow H_2C(OH)\text{-}CH_2OH)$

Cermenati et al., *J. Phy. Chem. B*, 101 (1997) 2650
Antoniou et al., *Appl. Cat. B*, 96 (2010), 290
Anipsitakis et al., *ES&T*, 40 (2006) 1000
Antoniou et al., *Water Research*, 43 (2009), 3956



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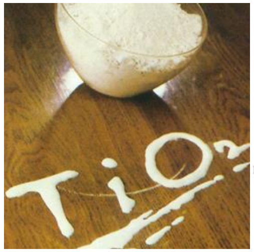
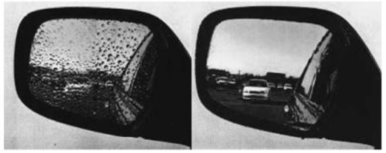


http://www.tayloreason.com/corksew/archives/eight-creative-last-minute-wine-gift-ideas/attachment/oreo-cookies/



Hashimoto et al, *Jpn J. Appl. Phys,* 44 (2005), 8269

http://faithfulprovisions.com/2011/06/07/sunscreen-deals-roundup/

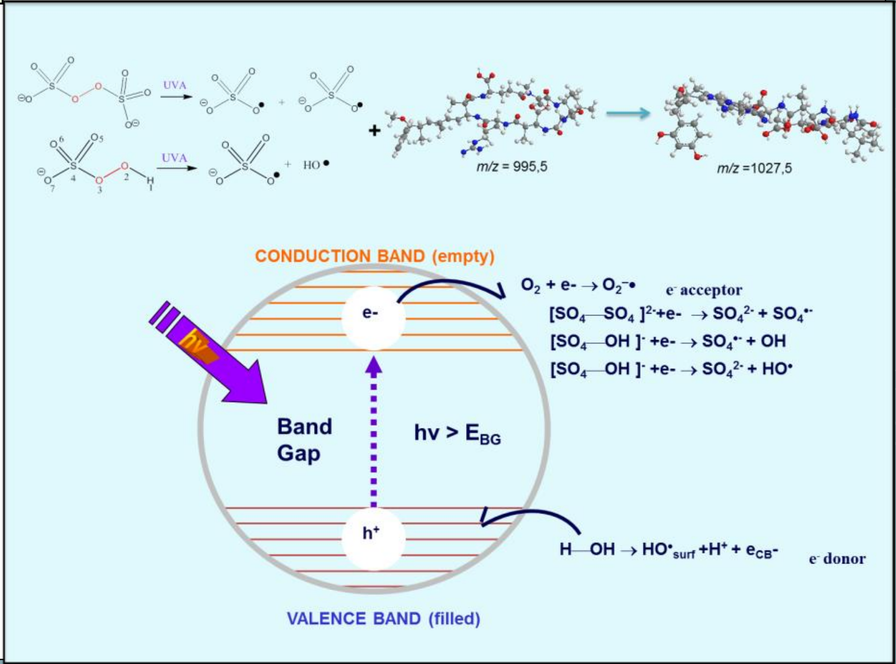
http://mrbarlow.wordpress.com/2011/04/05/better-toothpaste-with-added-bacteria/

http://www.cnchemicals.com/PressRoom/PressRoomDetailN.aspx?prNewsId=490

Enhanced Photocatalysis:

Three mechanisms for ROS formation:

1. Photocatalysis of TiO_2
2. Photolysis of Oxidants
3. e- transfer mechanism



Antoniou *et al.*, Toxicon, 51 (2008) 1103
Antoniou *et al.*, Environ. Sci. Technol, 42 (2008) 8877
Antoniou *et al.*, Appl. Cat. B: Environ., 91 (2009) 165

Enhanced Photocatalysis

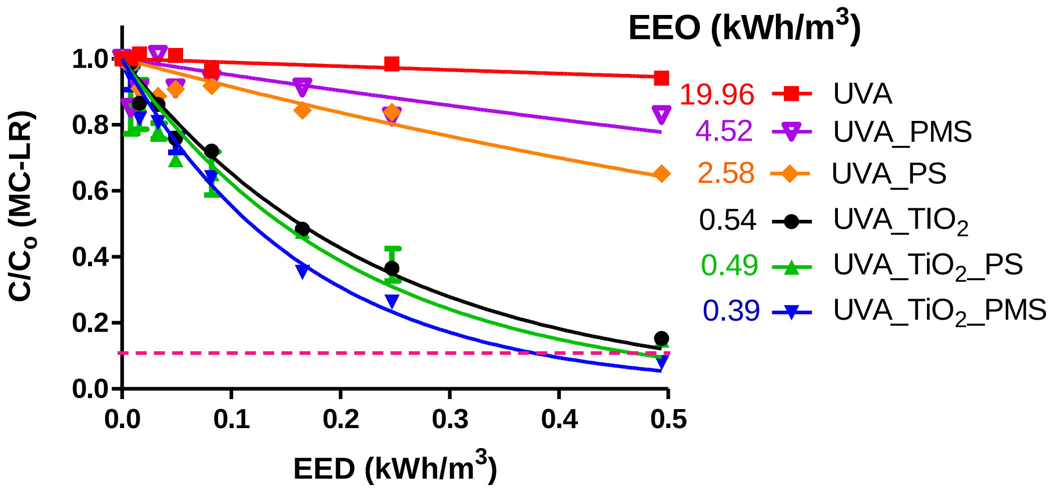
- Data where processed based on the Bolton 's Equations for estimating Electrical Energy Demand (EED) and Electrical Energy per Order (E_{EO}).
- E_{EO} is a measure of the electrical energy (kWh) needed to reduce the concentration of a contaminant by one order of magnitude in 1 m^3 of contaminated water.

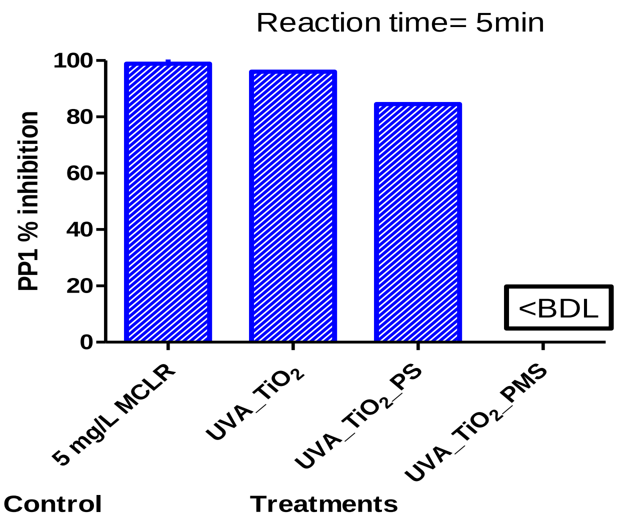
$$EED = \frac{Pt}{60V}\left(\frac{kW}{m^3} = \frac{W}{L}\right)$$

$$\log\left(\frac{C}{C_o}\right) = \frac{-EED}{E_{EO}} \Leftrightarrow C = C_o \cdot 10^{-\left(\frac{EED}{E_{EO}}\right)}$$

where: EED = electrical energy per order
P = total electrical power or flux entering the reactor (W)
t = treatment time (min)
V = volume of water treated (L)

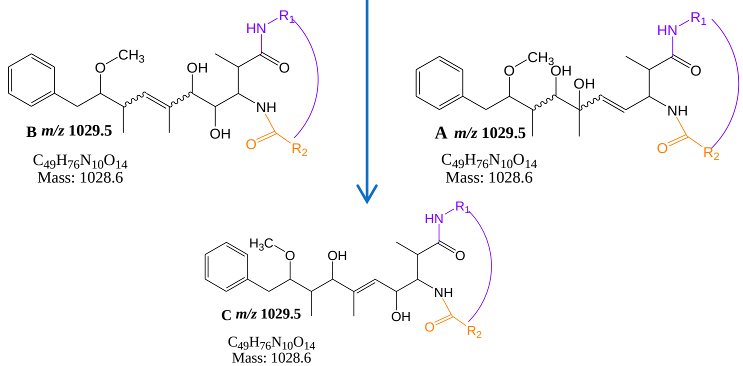
James R. Bolton, *Ultraviolet Applications Handbook* Third Edition, Canada, Bolton Photosciences Inc., 2010.

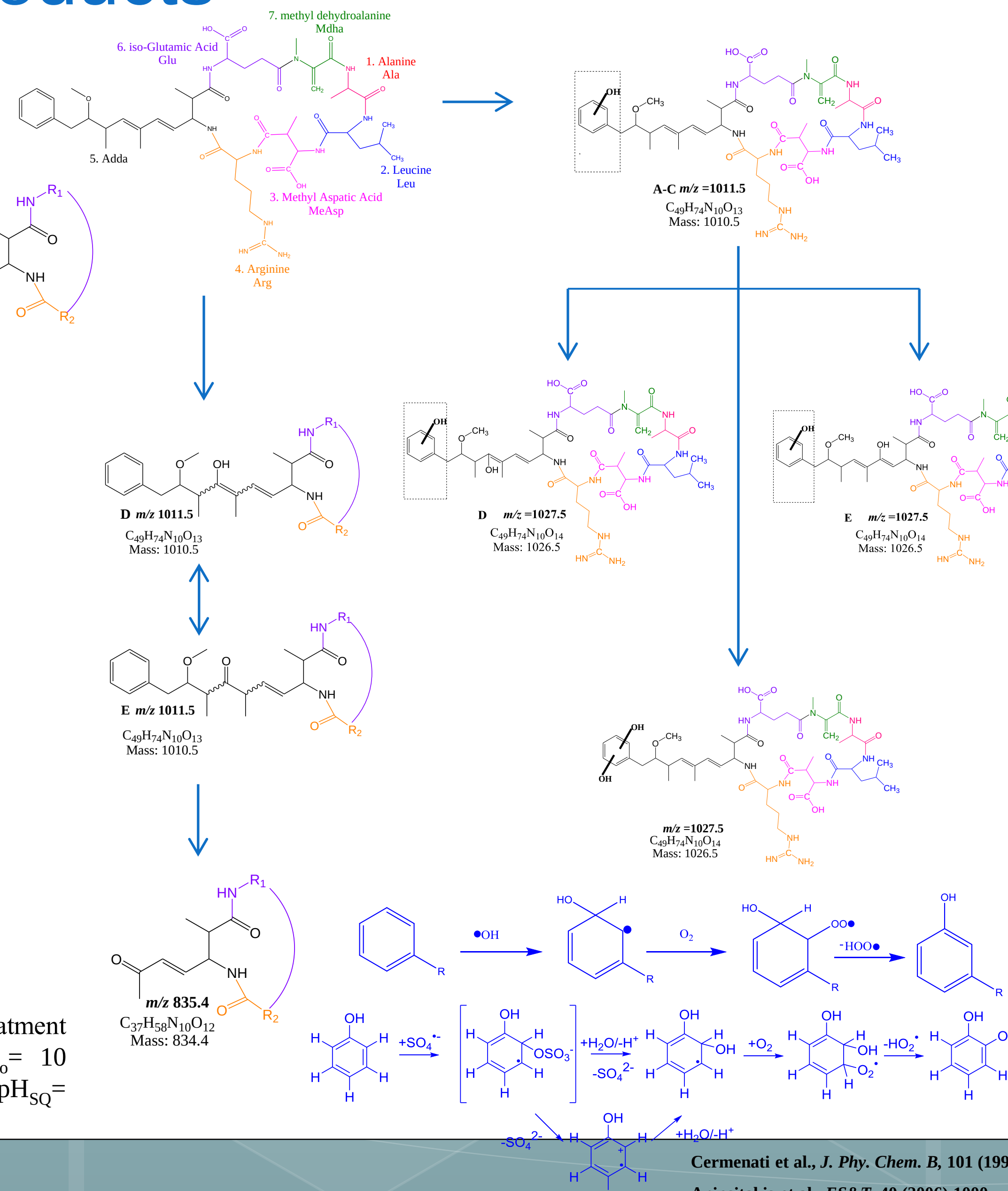


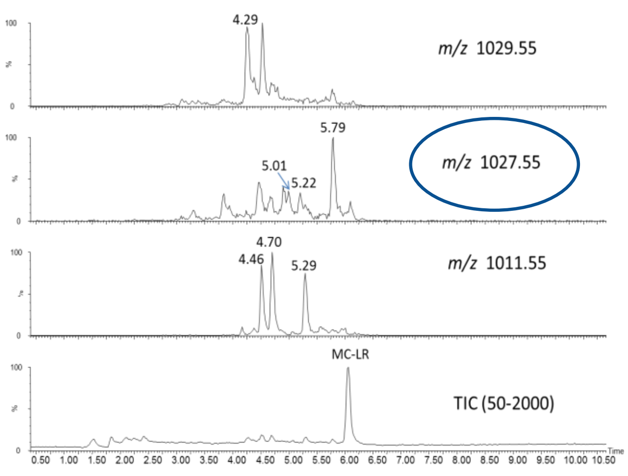


Maria. G. Antoniou, I. Boraei, M. Abhishek, C. Edwards, and L.A. Lawton. Enhancing photocatalytic degradation of the cyanotoxin microcystin-LR with the addition of sulfate-radical generating oxidants. *Chemical Engineering Journal, 2016 (to be resubmitted)*.

Transformation Products







LC/MS chromatograms of MC-LR after 3 min of treatment with UVA/TiO_2/PMS. (Experimental Conditions: C_o= 10 mg/L, P_{365}= 35.58 W, PMS 0.052 mM, $TiO_2$10ppm, pH_{SQ}= 5.6, treatment time= 3min)

Cermenati et al., *J. Phy. Chem. B*, 101 (1997) 2650
Anipsitakis et al., *ES&T*, 40 (2006) 1000

marine drugs MDPI

Article

A Collaborative Evaluation of LC-MS/MS Based Methods for BMAA Analysis: Soluble Bound BMAA Found to Be an Important Fraction

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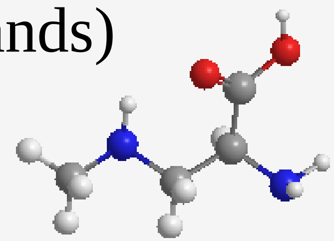
Academic Editors: Lucio G. Costa and Vítor Vasconcelos
Received: 15 October 2015; Accepted: 6 February 2016; Published: 29 February 2016

CYANOCOST

CyanoCOST Training School Workshop BMAA analysis (May 18th – 22nd 2015 Wageningen, The Netherlands)



Dr. Els Faassen

Prof. Miguel Lurling

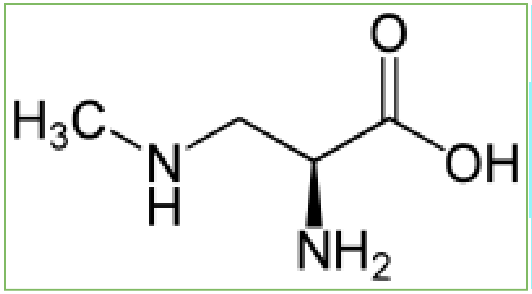
Aquatic Ecology and Water Quality Management Group

Wageningen University

WAGENINGEN UNIVERSITY
WAGENINGEN UR

Main Goals

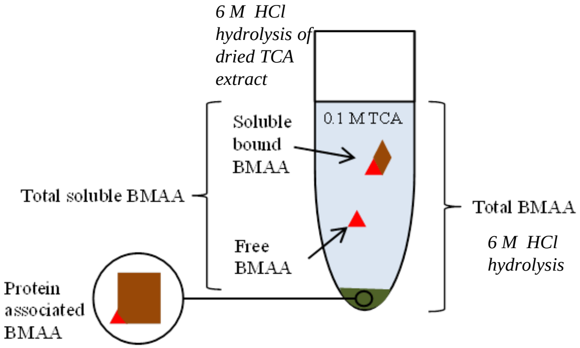
- Discuss the current literature on BMAA (β-N-methylamino-L-alanine) and current analytical methods (LC/MS/MS).
- Application of different protocols (derivatizated and underivatized samples) for animal, brain, cyanobacterial samples with LC/MS/MS.
- Distinguish between the different fractions of BMAA (Total BMAA, Total Soluble BMAA (Free BMMA+ Soluble bound BMAA), Precipitated bound BMAA)



$C_4H_{10}N_2O_2$, MW: 118

- No chromophore
- Polar (log P -0.85)

Sold as BMAA· HCl!







Results

Table 1. Trueness (mean D3BMAA recovery (%)) and intermediate precision (relative standard deviation of D3BMAA recovery, $n = 6$, results of both pairs combined), for samples prepared for underivatized analysis. Trueness outside the acceptable range is indicated with blue (<70%) and red (>120%). Precision exceeding the acceptable value (20) is indicated with red [40].

Protocol	Animal (A)			Brain (B)		Cyanobacteria (C)	
Fraction	Free	T.S. [1]	Total	Free	Total	Free	Total
Blank	85 (2.6)	65 (4.9)	81 (13.7)	78 (4.8)	72 (8.4)	100 (7.8)	59 (6.3)
Cycad	93 (7.8)	64 (11.4)	86 (2.1) *	69 (7.5)	73 (2.5)	103 (8.5)	65 (4.3)
Seafood	96 (6.6)	78 (7.9)	108 (6.7)	-	-	-	-
Daphnia magna	141 (2.5)	75 (1.0)	110 (8.0)	-	-	-	-
Brain unspiked	-	-	-	77 (11.1)	84 (15.7)	-	-
Brain spiked	-	-	-	80 (6.0)	82 (9.0)	-	-
Anabaena	-	-	-	-	-	103 (7.4)	78 (2.3)
Leptolyngbya	-	-	-	-	-	21 (61.0)	7 (41.5)

[1] Total Soluble, * $n = 5$.

Table 2. Intermediate precision expressed as relative standard deviation of the BMAA concentration (µg/g DW) determined in cycad seed by underivatized analysis, data with and without correction for D3BMAA recovery are shown ($n = 6$, results of both pairs combined). Results exceeding the acceptable value (20, [40]) are indicated with red.

Protocol	Animal (A)			Brain (B)		Cyanobacteria (C)	
Fraction	Free	T. S. [1]	Total	Free	Total	Free	Total
uncorrected for D3BMAA	10.3	8.4	22.9 *	13.5	31.4	18.5	20.5
corrected for D3BMAA	10.4	13.6	23.9 *	9.2	31.6	11.6	20.9

[1] Total Soluble, * $n = 5$.

- Derivatized protocol had poor recovery of D_3-BMAA (<10%).
- Trueness of protocols A, B and C, expressed as mean recovery of D_3BMAA.
- Added BMAA and D_3BMAA behave similarly in terms of stability and signal suppression during extraction and hydrolysis.
- Based on recovery of the internal standard D_3BMAA, the underivatized methods were accurate (mean recovery 80%) and precise (mean relative standard deviation 10%).
  - Exception: D_3BMAA recovery in *Leptolyngbya* was very low (7%–21%)
- Most BMAA in positive samples (seafood, cycads seeds, and *Daphnia*) was found in a trichloroacetic acid **soluble bound form** and it is recommended to include this fraction during analysis.

Elisabeth J. Faassen, Maria G. Antoniou, Wendy Beekman-Lukassen, et. al., *Marine Drugs*14 (2016), 45.

CURENT RESEARCH ACTIVITIES: CYANOBOX

Research Promotion Foundation

2016-2020 RESTART RESEARCH

THE RESEARCH PROMOTION FOUNDATION

PROGRAMMES

FOR RESEARCH, TECHNOLOGICAL DEVELOPMENT

AND INNOVATION

"RESTART 2016 – 2020"

PROPOSAL DETAILS	
PILLAR	I. SMART GROWTH
PROGRAMME	RESEARCH IN ENTERPRISES
RPF PROPOSAL NUMBER	ENTERPRISES/0618/157
PROPOSAL TITLE	Automated In-situ CYANOtoxin Assessment ToolBOX for Real-Time Surface Water Monitoring
PROPOSAL ACRONYM	CyanoBox

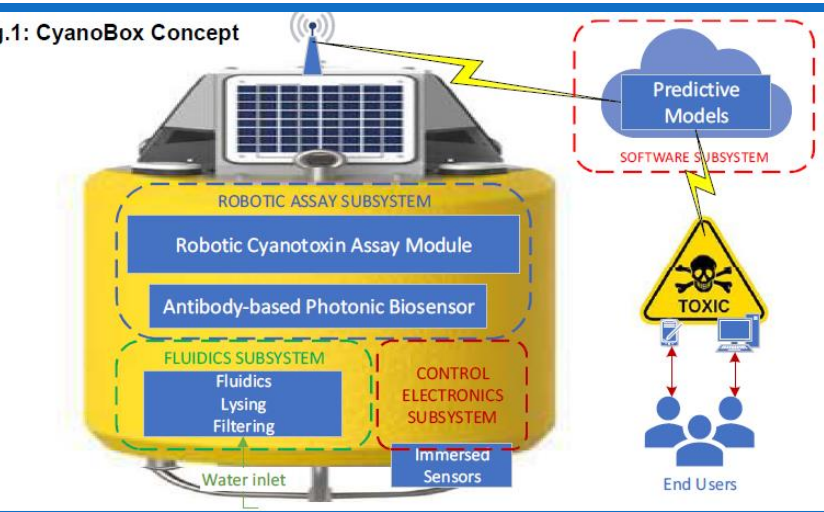
Ευρωπαϊκή Ένωση
Ευρωπαϊκό Ταμείο Περιφερειακής Ανάπτυξης

Κυπριακή Δημοκρατία

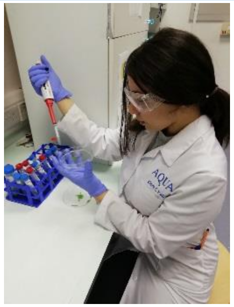
Διαρθρωτικά Ταμεία της Ευρωπαϊκής Ένωσης στην Κύπρο

Key Objective: CyanoBox is a 2 year project that aims to develop an innovative system that can perform continuous monitoring of the quality of cyanobacterial contaminated water remotely in urban, rural, and isolated sites.

Fig.1: CyanoBox Concept



CYRIC



Water Treatment Laboratory -AQUA

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  - **Dr. Theodora Fotiou**
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  - **Nomiki Kallikazarou**
  - **Eleni Keliri**
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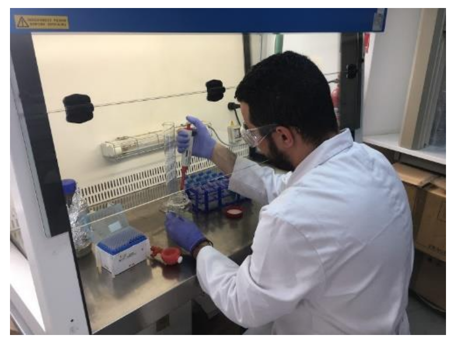
  - Iosif Konstantinou
  - Andreas Chrysanthou
- Undergraduate Students
  - Georgia Mikrommati
  - Elena Nikoalou
  - Constantina Kyriakou
  - Dimitris Dimitriou
  - Stella Komodromou (TUC, GR)
  - Nikoletta Tsiarta (UCM, ES)
  - Josef Boraei (RGU, UK)
  - Miltiadis Samanis (FLOW, CY)
  - Louis Juliet (Erasmus Visitor from RGU)
  - Danae Pantelidou (RGU, UK)
  - Georgia Hadjiouraniou
  - Manolis Christofi
  - Christia Paraskeva
  - **Nektarios Eystathiou**
- Guest Researchers: Isabella Aristidou, Marios Mavrogenis, Socratis Fotiou
- Honorable Members: Ioannis Vrachnou, Loukas Theodorou, Nikolaos Rotsidis, Marilena Louka, Stalo Dimosthenous, Sofia Georgiou, and Sotiria Nikolaou



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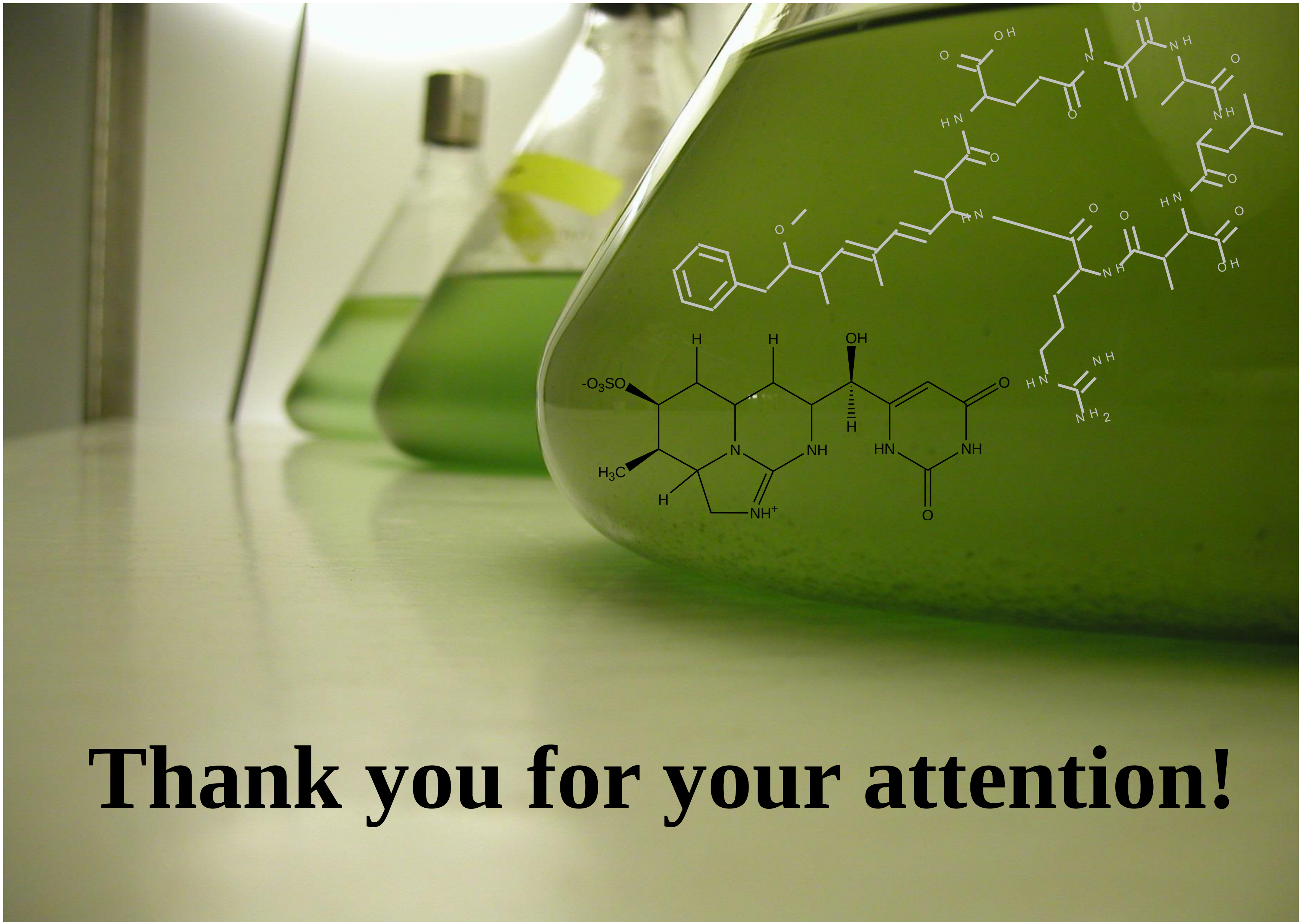

Thank you for your attention!