

First report on the occurrence of a single species cyanobacterial bloom in a lake in Cyprus: Monitoring and treatment with hydrogen peroxide releasing granules

Eleni Keliri

Cyprus University of Technology <https://orcid.org/0000-0002-6537-5570>

Christia Paraskeva

Cyprus University of Technology

Angelos Sofokleous

Cyprus University of Technology

Assaf Sukenik

Israel Oceanographic and Limnological Research

Dariusz Dziga

Jagiellonian University: Uniwersytet Jagiellonski w Krakowie

Ekaterina Chernova

Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences

Luc Brient

Rennes 1 University: Universite de Rennes 1



Maria G Antoniou (✉ maria.antoniou@cut.ac.cy)

Cyprus University of Technology <https://orcid.org/0000-0003-0738-6068>

Research

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Abstract

Background: Cyanobacteria are phytoplankton microorganisms, also known as blue-green algae, and an essential component of the food web in all aquatic ecosystems. Excess loads of nutrients into waterbodies can cause their rapid and excessive growth which leads to the formation of cyanobacterial harmful algal blooms (cyano-HABs). Toxic species of cyanobacteria genera excrete into the water a broad range of bioactive metabolites, some of which are known as cyanotoxins. These metabolites can negatively affect the ecosystem, and human and animal health in various ways, thus their presence needs to be closely monitored. This study aimed to monitor a lake at the Athalassa National Forest Park in Cyprus, in order to correlate its trophic condition with its water quality characteristics and identify the key environmental variables driving cyanobacteria blooming and their toxicity. In addition, surface water during the blooming period was collected and used in bench-scale experiments in order to test novel hydrogen peroxide releasing granules as mitigation processes for cyano-HABs.

Results: The monitoring lasted throughout 2019 with ten sampling events taking place during this period. Samples were mainly analyzed for phytoplankton community, and various physicochemical parameters: pH, conductivity, salinity, total and dissolved nutrients. Obtained data indicated that cyanobacteria blooming lasted for four months (June – September), while microscopic observation of preserved samples showed that 99% of the phytoplankton biovolume was attributed to a single picocyanobacterial species, the *Merismopedia* sp. Select samples were analysed for the presence of toxins genes with positive results mainly for *mcvB* and *mcvE* genes. Further analysis with HPLC MS/MS, revealed that cyanotoxins' concentration was lower than the method detection limit - MDL (<2-6 ng/L).

Conclusion: The present study highlights the importance of monitoring several water characteristics to conclude on the main drivers of a bloom and its toxicity. The findings demonstrated that it is not enough to test cyanotoxin genes as indicator of their presence since, in case of mono-domination, cyanobacteria may not be active on producing the toxins. Treatment experiments of contaminated water indicated that slow releasing peroxide granules may be an alternative to hydrogen peroxide. Treatment with CaO_2 granules outperformed MgO_2 granules due to higher H_2O_2 releasing capacity.

Background

Cyanobacteria are phytoplankton microorganisms whose ability to oxygenate the atmosphere 3.5 billion years ago contributed to life formation [1]. It is a group of bacteria ranging from 1 to 100 μm in diameter, while some of them are even smaller having a diameter less than 2 μm . Those are described as picoplankton and includes species such as *Merismopedia* sp, *Aphanocapsa* sp., and *Synechococcus* [2]. Cyanobacteria gain energy from photosynthesis by capturing light through pigments which are chlorophyll-a and phycocyanin with excitation wavelength at $\lambda=450$ and $\lambda=620$ nm; respectively. The difference between the two excitation wavelengths of the pigments found in green algae and blue-green cyanobacteria, allows us to distinguish their presence in freshwater and marine environments [3].

Anthropogenic activities such as agricultural, urban, and industrial activities have intensely increased the load of nutrients in surface waters around the globe, making cyanobacterial blooming more persistent and prevalent [4]. Both nutrients and cyanobacteria are essential to maintain the balance in an aquatic ecosystem. Nutrients support fish and shellfish production [5] while cyanobacteria are an essential component of the food web in all aquatic environments. Disruption of this balance by excess load of nutrients causes the rapid and excessive

growth of cyanobacteria which leads to the formation of cyanobacterial harmful algal blooms (cyano-HABs). Blooming enhances water quality depletion by reduced light and oxygen penetration with serious consequences on biodiversity [6], while adding undesirable color, taste, and odor to the waterbodies. Toxic genera of cyanobacteria can excrete into the water a broad variety of bioactive metabolites, also known as cyanotoxins [7]. These metabolites can negatively impact the ecosystem and human health, making it an important environmental issue of concern [8]. Although their acute toxicity on humans is not extensively studied, mass mortalities of fishes, birds, mammals and many other animal taxa have been reported [9]. Recent studies have correlated liver-related deaths in U.S with several cyanotoxins [10]. Cyano-HABs presence in freshwaters used as drinking water reservoirs is not only a health issue, but it also raises the overall treatment and monitoring costs which are in the range of millions of euros annually [11]. Currently, there is no method for *in-situ* detection or a predictive model for the occurrence of these toxins since not all cyanobacterial species are active toxin producers under the same conditions. Therefore, it is imperative to find both predictive models and monitoring tools as well as efficient treatment methods to mitigate the problem to safeguard water quality and reduce water treatment costs at source and in the waterworks.

The concentration (total and dissolved fraction) of the main nutrients - nitrogen (N) and phosphorus (P), is a strong indicator of the eutrophic state of waterbodies thus, several models and relationships have been developed over the years to form correlations [12]. The most applied stoichiometric reference is the Redfield ratio which describes the nutrient limitation of planktonic production in coastal waters based on the TN:TP molar ratio [13,14]. Despite the fact that Redfield proposed this ratio for its use on oceanic studies, it was well adopted as a universal nutrient limitation threshold with multiple citations in different types of aquatic systems. Redfield referred to an average N:P molar ratio that when exceeds 16, phosphorus becomes the limiting element for phytoplankton growth while when the ratio is below 7, nitrogen is the limiting element. Over the years, different ratios and approaches have been developed for better understanding of the limiting element based on the specific conditions of each waterbody (e.g., nutrient sources, phytoplankton species present). Based on the cited literature, lakes with different characteristics showed different correlations of nutrients with their trophic status, and the efficiency of the Redfield ratio varied in each case [15]. Recent studies indicated that higher TN:TP molar ratios (>22, P-limitation) are more suitable for surface waters while DIN:TP and NO_3^- :TP mass ratios have been used more often during the past years for determining the limiting elements in lakes [16]. Studies on dissolved nutrients mass ratios (DIN:TP, NO_3^- :TP, NH_4^+ :TP) suggested that in freshwater systems the DIN:TP mass ratio is a better indicator than the TN:TP molar ratio. Also, the NO_3^- :TP mass ratio performed even better than DIN:TP as DIN includes ammonium which in some studies it showed a weak correlation with N-limitation, resulting in a weaker model [17]. The ratios and thresholds used in the present study for evaluating the trophic status of a lake in Cyprus and the limiting elements during the monitoring period are presented in Table 1.

Table 1. Nutrient limitation approaches and thresholds based on TN:TP, DIN:TP and NO_3^- :TP ratios in different aquatic environments.

Ratio	Units	Threshold	Nutrient Limitation	Reference	Aquatic environment studied
TN:TP	Molar	<7	N – limitation	[13,14]	Ocean
		>22	P – limitation		
TN:TP	Molar	<20	N – limitation	[16]	Lakes; Ocean
		20-50	N or P limitation		
		>50	P – limitation		
DIN:TP	Mass	<9	N – limitation	[18]	Lakes; ponds
		9-22	N or P co-limitation		
		>22	P – limitation		
NO₃:TP	Mass	<9	N – limitation	[18, 19]	Lakes
		9-22	N or P co-limitation		
		>22	P – limitation		

Monitoring the level of nutrients in waterbodies and estimating their corresponding ratios are critical for improving the applied management strategies based on its specific needs in order to better control the limiting factors and to prevent future blooming events.

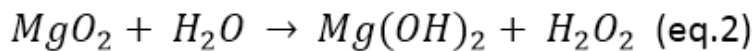
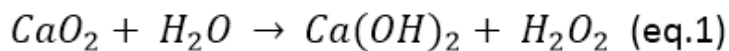
The main input of nutrients into the waterbodies comes from agriculture due to the misuse of fertilizers. The European Commission of Environment of the European Union has composed the Water Framework Directive (2000/60/EC) which aims to protect surface waters from chemical pollution [20]. Recently, it was proposed to include a group of cyanotoxins (microcystins) in a revised Drinking Water Directive on the Quality of Water Intended for Human Consumption [21]. If this comes through, it will enforce public authorities to include cyanobacteria and cyanotoxins into their monitoring and mitigation strategies in order to comply with the new directives and legislations of EU.

Additionally, the Organization for Economic Co-operation and Development (OECD) has actively participated in the development of frameworks and guidelines by reforming the surface water quality regulations in EECCA countries [22]. Based on those, OECD proposed a surface water classification (I – V class; excellent – bad) system based on different water quality characteristics. Its use has been widely adopted for monitoring water quality and apply restorative measures when needed. In the present study, water class was evaluated based on the limits set by OECD for acidification status and total and dissolved nutrients concentration as shown in Table 2.

Table 2. Proposed surface water quality standards by OECD based on the EU Directive (2000/60/EC).

Parameter	Unit	Class I	Class II	Class III	Class IV	Class V
pH	[-]	6.5 – 9.0				
Total Nitrogen	[mg N/L]	1.5	4	8	20	>20
Nitrate	[mg N/L]	1	3	5.6	11.3	>11.3
Nitrite	[mg N/L]	0.01	0.06	0.12	0.3	>0.3
Ammonium	[mg N/L]	0.2	0.4	0.8	3.1	>3.1
Total Phosphorus	[mg N/L]	0.1	0.2	0.4	1	>1
Orthophosphates	[mg N/L]	0.05	0.1	0.2	0.5	>0.5

Surface water monitoring is essential for maintaining a healthy status and protect the biodiversity of aquatic biotopes around EU. Unfortunate events of high nutrient loads that lead to the formation of cyano-HABs are mostly unpredictable and thus highly efficient methods are required to be applied *in-situ* for restoration. There are several physical, chemical, and biological methods that have been developed and applied over the years with the chemical ones to be more cost effective, rapid, and efficient [23]. The need to make chemical treatment “greener” has led to the application of hydrogen peroxide (H₂O₂) to different cyanobacterial species as an alternative to copper algicides, resulting in selective reduction of cyanobacterial species among other taxa of phytoplankton [24, 25, 26]. The hydroxyl radicals (·OH) formed by the oxidant inhibit the electron transport of photosystem II, causing reduction of its photosynthetic activity leading to cellular death [27,28, 29]. There are studies suggesting that its efficiency varies and depends on the nutrient load of the matrix, the species composition and abundance, the boom density, and light intensity. Usually high doses of H₂O₂ (>5 mg/L) are required for a complete destruction of cyanobacterial cells [30]. An alternative to liquid hydrogen peroxide is its granular form found as metallic peroxide granules which decompose slowly and release H₂O₂ [31]. Those are calcium and magnesium peroxide (CaO₂ and MgO₂ respectively) granules.



The goal of the present study was to examine the efficiency of treatment in a dense single-species bloom that occurred in St. George Lake of the Athalassa National Forest Park, in Cyprus with metallic peroxide granules and compare it with the traditionally used liquid hydrogen peroxide. We hypothesized that peroxide granules would have the ability to destroy cyanobacterial cells by inhibiting photosystem II electron transfer in the same way as H₂O₂ does, but in a more graduate and controlled manner, simulating multiple additions of hydrogen peroxide. The first objective was to determine the kinetic of the H₂O₂ release of two types of granules (CaO₂ and MgO₂) into an on-going bloom by monitoring its residual concentration during the treatment. The second objective was to compare the efficiency of granules in reducing the photosynthetic activity during a 48-hours treatment. This study also aimed to determine the most appropriate dose of oxidant for successful mitigation of the *Merismopedia sp.*

bloom in order to propose an efficient treatment method for *in-lake* application that will upgrade its water quality class, as proposed by EU.

Materials And Methods

Study Area

Saint George Lake is located at the Athalassa National Forest Park (ANFP) in Nicosia, the capital city of Cyprus. It is an artificial lake which covers an area of 68,000 m³ with an average depth of approximately 2 m. The ANFP covers an area of 8.5 km² and it is found between Aglatzia, Strovolos, Latsia, and Geri municipalities; four of the most densely populated locations in Nicosia. Among the most interesting parts of the forest park is Athalassa Lake and Saint-Gorge Lake (Scheme 1) that serve as aquatic life and bird habitats, making them an extremely important biotope for the island. The present study focuses on monitoring of St. George Lake and its treatment during its blooming period in 2019.

Scheme 1. Lakes in the National Forest Park of Athalassa: (A) St. George Lake and (B) Athalassas Lake located at the Athalassa National Forest Park.

Sampling and monitoring

Sampling was performed at a central part of the lake and water was collected from a depth of 0.1-0.2 m below the surface with the use of a 5 L bucket and a rope. Water samples were collected and stored in acid washed polyethylene (PE) bottles for the physicochemical water characterization and treatment purposes, and in glass containers for cyanobacterial genes and cyanotoxins analyses. All samples were stored at 4 – 6 °C in the dark, brought to the laboratory, and processed within 6 hours after sampling to ensure high accuracy and prevent decomposition of the water characteristics.

The monitoring in St. George Lake occurred between February to December 2019 and 10 samples were collected overall (Table 3). The main physico-chemical parameters (pH, conductivity, salinity, total and dissolved nutrients), the content of cyanobacteria and green algae, the presence of genes for main cyanotoxins synthesis, and the cyanotoxins concentration were determined. Samples were also collected during the blooming period for treatment experiments with liquid hydrogen peroxide and hydrogen peroxide releasing granules.

Table 3. Sample number and date of sampling event in St. George Lake.

Sample n°	Date
1	25/02/2019
2	04/03/2019
3	18/04/2019
4	12/07/2019
5	06/08/2019
6	22/08/2019
7	09/09/2019
8	15/10/2019
9	12/11/2019
10	09/12/2019

Physico-chemical water characteristics analyses

Raw samples were analyzed for total nitrogen (TN) and total phosphorus (TP) while samples filtered through cellulose nitrate membrane filter were analyzed for the dissolved nutrients content (ammonium - NH_4^+ , nitrates - NO_3^- , nitrites NO_2^- , and phosphates - PO_4^{3-}). Nutrients were determined by using Spectroquant® cell test kits (Merck Millipore) equivalent to EPA and APHA standard analytical methods and the Spectroquant® Pharo 300 spectrophotometer (Merck) with method standard deviations ± 0.15 mg/L-N, 0.027 mg/L PO_4 -P, 0.043 mg/L NH_4 -N, 0.13 mg/L NO_3 -N, 0.0027 mg/L NO_2 -N; respectively. Dissolved inorganic nitrogen was calculated as the sum of dissolved nitrogen ions (NH_4^+ , NO_3^- , NO_2^-). Temperature, pH, conductivity, and salinity were measured at the sampling site using the ExStik® portable pH Meter (EXTECH, FLIR Systems).

Algal content and Instantaneous Chlorophyll Fluorescence (FT) and Quantum Yield (QY)

Instantaneous Chlorophyll Fluorescence (FT) and Quantum Yield (QY) were determined using AquaPen AP 110/C (Photon Systems Instruments, Czech Republic) equipped with blue and red LED light emitters to monitor the growth of algae and cyanobacteria in St. George Lake and for evaluating the efficiency of the applied oxidants in the reduction of the photosynthetic activity of treated bloom.

For the characterization of cyanobacterial species in water samples, raw sample was placed directly or after filtration on a microscopy slide and tested under ECLIPSE Ci-L microscope (Nikon) equipped with OPTIKAM Wi-Fi camera (OPTIKA®, Italy). Phytoplankton samples were preserved with Lugol's iodine solution (2 % final concentration), stored in 4-6 °C under dark conditions and used within 3 weeks.

DNA isolation and PCR amplification

DNA isolation from the biomass collected on cellulose nitrate filters was performed as described by Rogers and Bendich (1994) with minor modifications [32]. Briefly, filters were placed in 2 mL Eppendorf tubes, frozen in liquid

nitrogen and grinded. Glass beads were added in ratio 1:1 and the content was dissolved in 700 µL of the extraction buffer I (100 mM Tris, 1.3 M NaCl, 20 mM EDTA, 4% cetrimonium bromide, 1% polyvinylpyrrolidone, 0.1% 2-mercaptoethanol). The mixture was beaten for 10 min using vortex shaker. After 45 min of incubation in 65 °C with 0.5% RNase A, 600 µL of the chloroform-isoamyl alcohol mixture (24:1) was added and the content was shaken and centrifuged at 14000 g for 10 min. The upper phase was transferred into a new tube and mixed with 50 µL of buffer II (10% cetrimonium bromide, 0.7 M NaCl). The chloroform washing step was repeated. After the addition of cold isopropanol in ratio 1:1 the mixture was centrifuged at 14000 g for 10 min. The pellet was washed in 500 µL of 70 % ethanol and the samples were centrifuged at 14000 g for 10 min. The supernatant was discarded, and the pellet was dried on air and resuspended in 50 µL of nuclease-free water.

PCRs for the identification of main genes of cyanotoxins were conducted using Dream Taq DNA polymerase (Thermo Fisher Scientific). Approximately 80 ng of isolated DNA was added to the reaction mixture (20 µl total volume) with 0.2 µM of each primer. PCR was performed with the following parameters: initial denaturation for 3 min at 95 °C, 30 cycles at 95 °C for 30 s, a primer-pair specific temperature for 30 s and 72 °C for 60 s; a final extension at 72 °C for 10 min. The electrophoresis of PCR products was conducted on 1% agarose gels at 100 V for 25-40 min. Gels were stained with Midori Green Advance DNA Stain (ABO).

Table 4. Primers used in the detection of cyanotoxin producing genes in St. George samples and amplification parameters used at PCR.

Targeting gene	Primer name	Sequence 5'-3'	Amplicon size (bp)	Annealing temperature (°C)	Reference or source of sequence for primers design
cyrJ	cyrJ_F	AGTAATCCCGCCTGTCATAGA	109	60	This study; KY550407.1
	cyrJ_R	ACTGAGCATTGTCTCGGTAAAC			
cyrB	cyrB_F	GCCTGAGTACCTATCTGCTTAAC	95	60	This study; EU140798.1
	cyrB_R	AGCCTGAAACTGCTCCATATC			
sxtA	sxtA_F	GCGTACATCCAAGCTGGACTCG	683	55	DOI: 10.1128/AEM.02285-09
	sxtA_R	GTAGTCCAGCTAAGGCACTTGC			
anaC	anaC_F	TCTGGTATTCAGTCCCCTCTAT	366	58	DOI: 10.1128/AEM.06022-11
	anaC_R	CCCAATAGCCTGTCATCAA			
mcyB	mcyB_F	CCTCAGACAATCAACGGTTAGT	119	60	This study; MPM020771.1
	mcyB_R	AAAGGCAGAAGGCACCATATAA			
mcyE	mcyE_F	CTGGTGGGAAAGGACTGATTTA	95	60	This study; CP020771.1
	mcyE_R	CGCCCTCAAGTCAAGAAAGA			

HPLC-HRMS

The high-performance liquid chromatography – high-resolution mass-spectrometry (HPLC-HRMS) method was used to check the presence of intra-cellular cyanotoxins in biomass stored on GF/C filters at -20°C until extraction. Sample preparation included extraction of cyanotoxins with 1 mL of 75% methanol in an ultrasonic bath [33]. All

chemicals used for analytical procedures were the analytical grades. Acetonitrile (HPLC-grade) and methanol (LiChrosolv hypergrade for LC-MS) were purchased from Merck (Darmstadt, Germany); formic acid (98–100%) was obtained from Fluka Chemika (Buchs, Switzerland). High quality water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) was produced by the Millipore Direct-Q water purification system (Bedford, MA, USA). The MC-LR, MC-RR, MC-YR standards were purchased from Sigma Aldrich. Sample preparation procedures were run according to Chernova et al. (2016). Analyses of extracts were performed using the LC-20 Prominence HPLC system (Shimadzu, Japan) coupled with LTQ Orbitrap XL Hybrid Ion Trap – Orbitrap Mass Spectrometer (Thermo Fisher Scientific, San Jose, USA). Separation of the toxins was achieved on a Thermo Hypersil Gold RP C18 column ($100 \text{ mm} \times 3 \text{ mm}$, $3 \mu\text{m}$) with a Hypersil Gold drop-in guard column (Thermo Fisher Scientific) by gradient elution (0.2 mL min^{-1}) with a mixture of water and acetonitrile, both containing 0.05% formic acid. Mass-spectrometric analysis was carried out under conditions of electrospray ionization in the positive ion detection mode. The identification of target compounds was based on the accurate mass measurement of $[\text{M}+\text{H}]^+$ or $[\text{M}+2\text{H}]^{2+}$ ions (resolution of 30000, accuracy within 5 ppm), the collected fragmentation spectrum of the ions and the retention times. Limits of the detection for different microcystin congeners ($2\text{--}6 \text{ ng L}^{-1}$) were evaluated in model experiments using standard compounds, natural water and biomass as matrixes.

Experimental set-up for treating cyano-HABs

Experiments on the treatment of *Mersmopedia sp.* bloom in St. George Lake were performed in 250 mL borosilicate glass containers and the oxidants used for this purpose were liquid hydrogen peroxide and metallic peroxide granules. Hydrogen Peroxide (30%) was purchased from Sigma-Aldrich and diluted to 1000 mg/L for the stock solution. Calcium peroxide (CaO_2) and magnesium peroxide (MgO_2) granules were provided in the form of IXPÉR® 70CG and IXPÉR® Magnesium Peroxide Granules 30MG by Solvay Chimika S.A. (free samples). H_2O_2 stock solution was added to 250 mL of raw sample from St. George Lake to reach a final concentration of 1, 2, 3, 5 mg/L H_2O_2 ; and a quantity of 1, 2, 3 g calcium peroxide and magnesium peroxide granules for treating cyano-HABs. The oxidant concentration was monitored by a colorimetric method as introduced by Sellers et. al (1980) [34]. In brief, 5 mL of sample was filtered through a PVDF syringe filter and immediately reacted with 0.5 mL of titanium oxalate ($[\text{C}]=50 \text{ g/L}$) and 0.5 mL sulfuric acid (1+17 v/v) (both reagents purchased from Sigma – Aldrich). The absorbance at 400 nm was measured by the Spectroquant® Pharo 300 spectrophotometer in a quartz cuvette and the concentration of H_2O_2 was quantified based on a calibration curve ranged between 0.5 and 20 mg/L. For determining the efficiency of oxidants on mitigating naturally occurred cyanobacterial bloom (*Merismopedia sp.*); the FT and QY values in both wavelengths (450, 620 nm) were recorded at 1, 2, 3, 4, 6, 24, 48 h with AQUAPEN as described previously. Physicochemical characteristics such as pH, conductivity, TDS and salinity were measured before and after treatment with the use of ExStick probe (EXTECH).

Data processing and statistical analysis were performed with the use of PRISM®-GraphPad software.

Results

1. Monitoring

1.1 Physicochemical water characteristics and nutrients

Water characteristics of St. George Lake varied during the monitoring period (February – December 2019). The recorded values of physicochemical characteristics and nutrients concentrations during the period of study are

illustrated in Figures 1 – 4. During the summer months air temperature was as high as 34 °C while during Spring, Fall and Winter the temperature varied between 15 – 25 °C. St. George Lake had a stable pH with small variations between 8.3 – 8.9 as shown in Figure 1. Conductivity ranged between 1200 and 2000 µS/cm while salinity found to be from 700 to 1000 ppm. Conductivity and salinity followed the same trend showing a noticeable increase during the summer period, both having their peak in August when the bloom occurred.

Nutrient concentrations, depicted in Table 5, also varied with time. Phosphorus was in most of the samples higher than 0.1 mg/L making St. George a hypertrophic lake [22]. The water class of St. George Lake determined based on OECD standards was found to be between II and III with respect to its nutrients' concentration. High nitrogen loads were detected in the early months of the year when the status of the lake was oligotrophic with low cyanobacterial content, while high phosphorus concentrations were recorded during the blooming period and declined afterwards (Figure 3). Soluble reactive phosphorus (SRP) was below the MDL before blooming period, stable during the blooming period and had a small drop which followed by a sharp increase during winter. This may be due to heavy rainfalls that caused nutrient run-offs. Dissolved inorganic nitrogen (DIN) remained high at the beginning of the year, radically decreased during summer and increased again in winter (Figure 4).

Table 5. Total and dissolved nutrients concentration (mg/L) in St. George Lake during monitoring period.

Sample	TN	TP	N-NO ₃	N-NO ₂	N-NH ₄	Water Quality Class	DIN – N	SRP – P
1	6.0	0.06	4.6	0.10	0.10	II-III	4.8	0.00
2	4.7	0.11	3.4	0.09	0.10	II	3.6	0.00
3	6.9	0.06	5.8	0.11	0.10	III	6.0	0.00
4	6.6	0.20	2.4	0.08	0.20	II-III	2.7	0.03
5	7.9	0.36	0.0	0.08	0.29	III	0.4	0.03
6	2.6	0.28	1.9	0.06	0.13	II	2.1	0.03
7	5.3	0.19	0.0	0.14	0.94	III	1.1	0.03
8	4.1	0.14	1.8	0.08	0.10	II	2.0	0.02
10	6.4	0.28	4.9	0.09	0.10	III	5.1	0.05

1.2 Photosynthetic activity

The blooming in St. George Lake was a seasonal phenomenon that peaked during the summer period. More specifically, July and August 2019, cyanobacterial density of the water was extremely high but after a light rainfall at the beginning of September the density of the bloom began dropping (Figure 5A). Until the end of September *Merismopedia* were present in the samples, but their density rapidly declined by the beginning of October.

1.3 Cyanobacteria species and cyanotoxins analyses

Microscopic observation of preserved samples (n° 5, 6) showed that 99% of the phytoplankton biovolume was attributed to a single picocyanobacterial species, *Merismopedia* sp. (Figure 6). These species are reported in the literature as microcystin and nodularin producers [35,36] which are both among the most detected cyanotoxin

groups in surface waters. Therefore, cyanotoxins genes analyses and cyanotoxins concentrations analysis were performed to examine the toxicity of this bloom.

After isolating the DNA of biomass collected on filter samples, the targeted genes were amplified and injected into gel electrophoresis wells for identification. The presence of *cyrB* and *cyrJ* was recorded only in sample 1. The presence of MC genes was recorded in samples 1, 3, 8 (*mcyB*) and 1, 3-8 (*mcyE*). *AnaC* and *sxtA* were not found in any sample (Figure 7).

Cyanotoxins genes analyses showed positive results in several samples especially for microcystins genes (*mcyB* & *mcyE*), therefore the samples were analyzed for a variety of microcystins analogues concentrations. Microcystins were not detected above the MDL in any of the samples. However, matrix compounds with *m/z* very close (2-4 ppm) to the one of microcystins were detected in a very low concentration, but the fragmentation patterns of their parent ions differ from ones of microcystins. In Figure 8 the fragmentation pattern of an microcystin-LR standard with the one found in the extract is compared. Lack of characteristics fragments for MCs as the *m/z* = 599.42 (Arg – Adda – Glu) confirmed the absence of microcystins in the *Merismopedia sp.* bloom.

2. Treatments

The oxidants used for cyano-HAB mitigation exhibited different efficiencies and impact on the *Merismopedia sp.* bloom. Hydrogen peroxide treatment was not effective for treating the dense bloom in concentrations of 1 - 5 mg/L. The average initial instantaneous fluorescence and quantum yield at $\lambda=620$ nm representing the cyanobacterial photosynthetic activity and wellness of the photosystem II, were 8500 and 0.37, respectively. Treatments with lower H_2O_2 doses (1 and 2 mg/L) were inefficient to treat cyanobacteria that continued to grow steadily. Treatment with H_2O_2 concentration 3 and 5 mg/L showed only a minor drop of the corresponding FT values compared with the control (Figure 9A). All treated samples showed a stable average of QY around 0.37, meaning that the bloom remained unaffected during the treatment with H_2O_2 (Figure 9B).

FT and QY at 450 nm excitation wavelength were also monitored during the 48 hour treatment, to determine the photosynthetic activity of green algae and plant suspensions, as illustrated in Figure 9 (C – D). There was a drop of photosynthetic activity in samples treated with 3 and 5 mg/L of H_2O_2 , giving also visual changes in the color of the treated water (as depicted in graphical abstract. Even though photosynthetic activity dropped at high H_2O_2 concentrations, QY was stable in all samples during the treatment meaning that the phytoplankton was not affected during the treatment.

Treatment with CaO_2 granules (2 and 3 g/L) effectively decreased the photosynthetic activity of cyanobacteria (Figure 10A). Even though 2 g/L of CaO_2 reduced the value of FT, QY was restored after 6 h of treatment, making it less efficient than 3 g/L which maintained a lower QY value for the duration of the 48 hour treatment (Figure 10B). Photosynthetic activity, measured at 450 nm of samples treated with 2 and 3 g/L CaO_2 , showed a drop of about 50% but the quantum yield of the same samples was not affected, meaning that FT could be restored after days of treatment, making it appropriate for *in-situ* applications (Figure 10C-D).

Magnesium peroxide treatment was inefficient for concentrations up to 3 g/L. Both FT and QY values at $\lambda=620$ and $\lambda=450$ nm were stable during the treatment period (Figure 11). The first 4 h of treatment, FT values decreased but then it was recovered within 6 h of treatment. In general, magnesium peroxide was not able to influence the bloom, and had no effect on phytoplankton.

It is apparent that MgO_2 had a much lower H_2O_2 releasing capacity than CaO_2 , making CaO_2 a much more efficient treatment method. Release curves showed that maximum accumulative hydrogen peroxide concentration from 1, 2 and 3 g/L of CaO_2 was 3.5, 8.0 and 11 mg/L respectively; while for 1, 2 and 3 g/L of MgO_2 it was 0.7, 1.2, and 1.8 mg/L of H_2O_2 ; respectively (Figure 12).

Physicochemical parameters (pH, conductivity, Salinity and TDS) variations during the treatments were monitored since the treatment with oxidants may negatively affect water matrixes. Hydrogen peroxide did not affect the water quality characteristics while magnesium peroxide granules slightly increased all the tested parameters. MgO_2 and CaO_2 granules made the solution more alkaline while H_2O_2 had the least effect on the pH of the water matrix (Table 6). The initial water characteristics for comparison can be found on Figures 1-2, 6th sample. Effects on the physicochemical characterization need to be accounted as well when deciding on a type oxidant and dosing to secure possible side-effects in the lake during treatment.

Table 6. Average pH, Conductivity, Salinity and TDS of St. George Lake water (pH= 8.88, conductivity= 1999 $\mu\text{S}/\text{cm}$, Salinity= 1015 ppm) after 48 hours of treatment with liquid H_2O_2 , CaO_2 and MgO_2 granules.

Oxidant	Concentration	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Salinity (ppm)	TDS (mg/L)
H_2O_2	1 mg/L	9.21	1707	843	1191
	2 mg/L	9.27	1664	816	1161
	3 mg/L	9.30	1674	838	1178
	5 mg/L	9.20	1783	876	1169
CaO_2	1 g/L	9.55	1794	898	1252
	2 g/L	10.2	1742	869	1214
	3 g/L	10.5	1733	862	1210
MgO_2	1 g/L	9.36	1850	921	1281
	2 g/L	9.52	1970	973	1351
	3 g/L	9.61	2020	1015	1396

Discussion

1. Monitoring

Monitoring of St. George Lake showed that the blooming period lasted for 4 months during summer and early autumn period (June – September). The increase of the water temperature and the low turbidity during summer in combination with nutrient load and/or release from the sediments [37] may probably result in periodical blooming of cyanobacteria. In St. George Lake, high nutrient content recorded throughout the year, favored *Merismopedia* sp. to become the dominant species and to form a dense bloom. Annual average of nutrients classifies the Lake at class III meaning that water quality improvements are essential to be applied since EU Directive requested member countries to maintain surface waters at class I and II.

An almost linear correlation ($R^2 = 0.80$) between FT (at 620 nm, related to cyanobacteria) and the TP content was documented (Figure 13). Concentrations of TP higher than 0.2 mg/L favored *Merismopedia* sp. blooming as shown in Figure 13. Phosphorus concentration during the bloom was higher than 0.2 mg/L which means that in such a hypertrophic lake (TP > 0.1 mg/L) nitrogen became the limiting element. To support these findings, different approaches on estimating nutrient limitation were tested in order to investigate which one fits better to the studied environment (Table 7).

Application of the Redfield ratio in our study indicated that P was the limiting factor for the whole season which does not reflect the actual trophic condition of St. George Lake. Guildford and Hecky (2000) proposed that lake systems tend to have higher than Redfield thresholds for P-limitation [16]. Those thresholds showed a better fit than TN:TP molar ratios, shifting from P-limitation to co-limitation during the bloom, but it does not still represent well the trophic status. The best adjustment was found when the ratio proposed by Levine (2001) and Symons (2012) applied. Both DIN:TP and NO_3^- :TP mass ratios showed relatively similar results, suggesting an N-Limitation during the bloom, co-limitation before and after the bloom and P-limitation in the remaining period. Overall, approaches that were based on ocean dynamics were found to have poor fitting on the trophic status of the lake in contrast with more recent approaches that were intended for fresh waterbodies. This stresses the need of better understanding the nutrient dynamics in lakes and the development of holistic approaches based on the different physicochemical characteristics of each waterbody, taking into consideration also other limiting factors that may affect trophic status such as light and temperature [38].

Table 7. Calculation of different ratios proposed in the literature for nutrient limitation

Sample n°	TN:TP	Limiting element (Redfield)	Limiting element (Guildford and Hecky)	DIN:TP	Limiting element (Symons)	NO_3^- :TP	Limiting element (Lavine, Symons)
1	221	P	P	80	P	77	P
2	95	P	P	33	P	31	P
3	254	P	P	100	P	97	P
4	73	P	P	13	N or P	12	Co-limitation
5	49	P	N or P	1	N	0	N
6	21	P	N or P	7	N	7	N
7	62	P	P	6	N	0	N
8	65	P	P	14	N or P	13	Co-limitation
10	23	P	N or P	18	N or P	18	Co-limitation

Nutrients and trophic status of each waterbody are highly correlated, indicating that when a high nutrient load is documented, algal and cyanobacterial monitoring is essential for the early detection of (toxic) blooms. High FT values at 620 nm (>3000) recorded in St. George Lake early in summer indicated an on-going bloom which was

expected based on its trophic condition. Since the photosynthetic activity was high in samples 5 - 7, they were observed under the microscope. A mono-domination of a picocyanobacterial species, *Merismopedia*, a known MC and NOD producer, was found. However, even though genes involved in MC and NOD synthesis were present (*mcyB* and *mcyE*), no cyanotoxins were detected in any of the obtained samples. Several species have a potency to produce MC, but in given conditions the genes may not be expressed. Scientific reports related to the regulation of *mcy* gene expression in response to external biotic and abiotic factors have been published recently. For example, in mixed cultures of *M. aeruginosa* and *P. agardhii*, both suppressed growth and downregulation of *mcyE* expression were observed [39] which suggests that the competition between two toxic strains may results in a lower MC production. It is also possible that the dominant species observed in the investigated lake lost their capability of MC synthesis. The presence of *mcyB* only in 3 samples and *mcyE* in 8 samples suggest that the genetic machinery for MC synthesis may be deficient. Furthermore, an indirect downregulation of MC synthesis was observed in response to iron limitation [40], probably as a result of a lower photosynthetic activity, therefore it was suggested that this parameter (iron level) may be helpful in predicting bloom toxicity. The light intensity is also known as an important abiotic factor influencing *mcy* expression and MC production [41].

However, it should be underlined that any genetic method applied should be complemented and there accompanied with analytical confirmation. The level of *mcy* transcripts is often not correlated with the MC concentration. The toxicity based on the *mcy* levels might be both under- and overestimated [42,43,44]. Therefore, these assays should not be considered as good indicators of bloom toxicity, but rather as a complementary tool in risk assessments. Similarly, it can be assumed that the detection of *mcy* does not ensure MC presence which should be proven through advanced analytical methods (LC-MS/MS). Attention should be paid on the obtained *m/z* and the corresponding fragmentation patterns so that cyanotoxins concentration is not overestimated.

2. Treatment

Dense blooms of *Merismopedia sp.* in a hypertrophic lake suggested the requirement of extremely high doses of H_2O_2 for efficient treatment. Since in those cases, doses over 5 mg/L of H_2O_2 may be harmful for the other components of microbial communities (bacteria, phytoplankton and zooplankton), alternative solutions are needed. CaO_2 and MgO_2 granules studied herein are an alternative to traditionally used liquid hydrogen peroxide [45]. To determine the most efficient dose we took into consideration not only the ability of the oxidant to destroy cyanobacterial cells but also the wellness of remaining phytoplankton. The 2 g/L CaO_2 treatment was most effective in bloom elimination, but it caused a noticeable change in the remaining phytoplankton. Despite that, the wellness of the photosystem II, recorded as the quantum yield was not affected at all, which means that phytoplankton's photosynthetic activity may be restored shortly after treatment. CaO_2 outperformed MgO_2 because of its higher H_2O_2 release from the granules. This may be due to the fact that the dissolution product of MgO_2 , which is magnesium hydroxide, is less soluble than the dissolution product of CaO_2 , which is calcium hydroxide, at the same pH. This also affected the suspended solids content of the treated water. Calcium peroxide granules caused drastic changes of pH as while decomposing it releases highly basic $Ca(OH)_2$ (equation 1-2).

Both granules released H_2O_2 with a reaction that follows a pseudo-zero-order kinetics pattern and their kinetics are greatly affected by temperature and the pH of the solution as explained by Wang et. Al (2016) [31].

Combining treatment efficiency and their releasing capacity, treatment with MgO_2 is inefficient and would require high amount of granular oxidant to mitigate a contaminated site. CaO_2 has the ability to release H_2O_2 in a more

effective way and only 2 g/L of granules can gradually release up to 8 mg/L H_2O_2 which is sufficient for treating blooms without affecting the rest phytoplankton species.

Conclusions

This is the first report from Cyprus on the occurrence of a dense cyanobacterial bloom with the *Merismopedia* sp. being the dominant species. The blooming occurred in St. George Lake of ANFP, in Cyprus during the summer and early fall of 2019. Conventional monitoring tools such as microscopic enumeration of phytoplankton species and trophic condition determination were applied. However, limited information on the factors driving the cyanobacterial blooming is obtained through these tools. Therefore, additional characterization of the lake ecosystem including physicochemical characteristics; total and dissolved nutrients; temperature, air and light intensity; cyanobacterial and green algae content; cyanotoxins genes and cyanotoxins analyses were found to be essential. Monitoring is useful for building predictive models as early response tools to avoid cyanotoxins contamination of source waters used for recreational activities and drinking water. Correlations between nutrients and eutrophication have been developed recently [46] with the DIN:TP and NO_3^- :TP mass ratio to be the most promising ratios, as confirmed also in our case. While these ratios are proving to be promising for understanding the eutrophic status of surface waters, they should be applied with caution after careful examination of each waterbody's unique characteristics (depth, size, water temperature etc.). Customized monitoring strategies for each waterbody and treatment application at the early stages of a bloom, are essential for protecting water quality of surface waters.

With climate change being linked to global expansion and persistency of harmful cyanobacteria, it is imperative that we find efficient methods to mitigate harmful cyanobacteria blooms at source. Treating cyanobacteria effectively, without harming the rest of the ecosystem is vital for restoring and safeguarding surface water quality. Currently, hydrogen peroxide is widely used for mitigating cyano – HABs as an alternative to algicides and an eco-friendly method. But treating dense blooms, such as the *Merismopedia* bloom occurred in St. George Lake, requires high oxidant doses (>5 mg/L) at once which is known that the remaining ecosystem (e.g., zooplankton; phytoplankton) is also affected. Peroxide granules that are H_2O_2 slow releasing oxidants were tested herein as an alternative method for hydrogen peroxide treatment. Their hydrogen peroxide releasing properties and treatment efficiency varied. Calcium peroxide outperformed other peroxides which can be a potential treatment method worth to investigate further for its efficiency on different cyanobacterial species and matrixes. Despite that it releases high amount of H_2O_2 , it is acting gradually on the species by first reacting with matrix organic load and then by reaching the contaminant making it more efficient than liquid hydrogen peroxide. Further studies will provide a clearer view on its properties as a promising mitigation technique since pH and other physicochemical characteristics were found to affect the treatment.

Abbreviations

Cyano-HABs	Cyanobacteria Harmful Algal Blooms
TN	Total Nitrogen
TP	Total Phosphorus
DIN	Dissolved Inorganic Nitrogen
SRP	Soluble reactive phosphorus
TDS	Total Dissolved Solids
MDL	Method detection limit
OECD	Organization of Economic Co-operation and Development
EC	European Commission
EU	European Union
mg	milligram
µg	microgram
ng	nanograms
µS	Micro siemens
µm	micrometer
cm	centimeter
nm	nanometer
mL	milliliter
L	liter
g	grams
h	hours
ppm	parts per million
St. George	Saint George
ANFP	Athlassa National Forest Park
sp.	Species
H₂O₂	Hydrogen Peroxide
CaO₂	Calcium Peroxide
MgO₂	Magnesium Peroxide
MC	Microcystins
NOD	Nodularins
QY	Quantum yield

FT	Photosynthetic activity
λ	wavelength
P	phosphorus
N	Nitrogen

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

E. Keliri worked on the development of the experimental design for the monitoring and treatment under the guidance of M. G. Antoniou.; EK performed monitoring and cyano – HABs treatment and initiated the first draft of the manuscript; C. Paraskeva performed cyano – HABs treatment experiments under the supervision of MGA and EK; A. Sofokleous was the technician responsible for the water sampling events; A. Sukenik involved in initial sampling events and he trained the researchers on the handling of lake samples and photosynthetic activity measurements with AP-100C; D. Dziga was responsible for the cyanotoxins genes analyses; E. Chernova performed HPLC – HRMS for cyanotoxins analysis; L. Brient was contributed to the cyanobacteria species characterization; and MGA was leading the research project on monitoring and treatment of St. George Lake. All authors read and approved the final manuscript.

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Author details

¹Department of Chemical Engineering, Cyprus University of Technology, 3036 Lemesos, Cyprus. ²The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research, Migdal, Israel. ³Department of Microbiology, Faculty of Biochemistry, Biophysics and Biotechnology Gronostajowa 7, 30-387 Kraków, Poland. ⁴St. Petersburg Federal Research Center of the Russian Academy of Sciences (SPC RAS), Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences Korpusnaya Str., 18, Saint-Petersburg, 197110, Russia. ⁵UMR 6553 Centre National de la Recherche Scientifique ECOBIO/OSUR, University of Rennes 1, Rennes, France.

References

1. Altermann W, Kazmierczak J, Oren A, Wright DT (2006) Cyanobacterial calcification in Earth history
Cyanobacterial calcification and its rock-building potential during 3.5 billion years of Earth history. *Geobiology* 4:147–166.
2. Jakubowska N, Szeląg-Wasielewska E (2015) Toxic Picoplanktonic Cyanobacteria—Review. *Mar Drugs*. 13:1497-1518.
3. Thomas J (1972) Relationship between age of culture and occurrence of the pigments of photosystem II of photosynthesis in heterocysts of a blue-green alga. *J Bacteriol.* 110:92-95
4. Moss B, Jeppesen E, Sondergaard M, Lauridsen TL, Liu ZW (2013) Nitrogen, Macrophytes, shallow lakes and nutrient limitation: Resolution of a current controversy? *Hydrobiologia* 710(1):3-21.
5. Bachmann RW, Jones BL, Fox DD, Hoyer M, Bull LA, Canfield DE (1996) Relations between trophic state indicators and fish in Florida (USA) lakes. *Can J Fish Aquat. Sci.* 53(4):842-855.
6. Krztoń W, Kosiba J, Pocięcha A et al. (2019) The effect of cyanobacterial blooms on bio- and functional diversity of zooplankton communities. *Biodivers. Conserv.* 28:1815–1835.
7. Meriluoto J, Spoof L, Codd GA (eds) (2017) *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*, Wiley, Chichester.
8. Souza NR, Metcalf JS (2020) Cyanobacterial toxins and their effects on human and animal health. In: Konur O. (ed) *Handbook of Algal Science, Technology and Medicine*, Academic Press, Elsevier.
9. Stewart I, Seawright AA, Shaw GR (2008) Cyanobacterial poisoning in livestock, wild mammals and birds—an overview. In: Hudnell K. H (ed) *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*, Springer, New York.
10. Zhang F, Lee J, Liang S et al. (2015) Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. *Environ Health* 14:41.
11. Hamilton DP, Wood SA, Dietrich DR, Puddick J (2014) Costs of harmful blooms of freshwater cyanobacteria. In: Sharma NK, Rai AK, Stal LJ (eds) *Cyanobacteria: An Economic Perspective*, Wiley, Hoboken.
12. Verburg P, Horrox J, Chaney E, Rutherford JC, Quinn JM, Willock RJ, Howard-Williams CW (2013) Nutrient ratios, differential retention, and the effect on nutrient limitation in deep oligotrophic lake. *Hydrobiologia* 718:119-130.
13. Redfield AC (1934) On the proportions of organic derivatives in sea water and their relation to the composition of the plankton. In: James Johnstone memorial Volume, University of Liverpool.
14. Redfield AC (1958) The biological control of chemical factors in the environment. *Am Sci.* 46:205–221.

15. Ptacnik R, Andersen T, Tamminen T (2010) Performance of the Redfield Ratio and a Family of Nutrient Limitation Indicators as Thresholds for Phytoplankton N vs. P Limitation. *Ecosystems* 13:1201-1214
16. Guildford SJ, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol. Oceanogr.* 45(6):1213-1223.
17. Bergström A (2010) The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. *Aquat Sci.* 72:277–281.
18. Symons CC, Arnott SE, Sweetman JN (2012) Nutrient limitation of phytoplankton communities in Subarctic lakes and ponds in Wapusk National Park, Canada. *Polar Biol.* 35: 481–489.
19. Levine M, Whalen S (2001) Nutrient limitation of phytoplankton production in Alaskan Arctic foothill lakes. *Hydrobiologia* 455:189–201.
20. EC (2000) Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for Community action in the field of water policy. *OJ L* 327:1-72.
21. EC (2018) Proposal for a directive - COM (2017)753, RECAST2017 Revision of the Drinking Water Directive. <https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/1563-Revision-of-the-Drinking-Water-Directive-RECAST-2017->.
22. Caspers H (1984) Eutrophication of Waters. Monitoring, Assessment and Control by: Organisation for Economic Co-Operation and Development (OECD). *Int Rev Hydrobiol.* 69 (2).
23. Paerl HW, Gardner WS, Havens KE, Joyner AR, McCarthy MJ, Newell SE, Qin B, Scott JT (2016) Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. *Harmful Algae* 54:213-222.
24. Barroin G, Feuillade M (1986) Hydrogen peroxide as a potential algicide for *Oscillatoria-Rubescens* DC. *Water Res.* 20:619
25. Drábková M, Admiraal W, Marsálek B (2007) Combined exposure to hydrogen peroxide and light – Selective effects on cyanobacteria, green algae, and diatoms. *Environ Sci Technol.* 41:309.
26. Matthijs H, Visser P, Reeze B, Meeuse J, Slot PC, Wijn G, Talens R, Huisman J (2012) Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Res.* 46(5):1460-1472.
27. Samuilov VD., Timofeev KN, Sinitsyn SV et al. (2004) H₂O₂-Induced Inhibition of Photosynthetic O₂ Evolution by *Anabaena variabilis* Cells. *Biochemistry (Moscow)* 69:926–933.
28. Bouchard JN, Roy S, Campbell DA (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. *Photochem Photobiol.* 82: 936.
29. Piel T, Sandrini G, White E, Xu T, Schuurmans JM, Huisman J, Visser PM (2020) Suppressing Cyanobacteria with Hydrogen Peroxide Is More Effective at High Light Intensities. *Toxins* 12:18.
30. Sandrini G, Piel T, Xu T, White E, Qin H, Slot PC, Huisman J, Visser PM (2020) Sensitivity to hydrogen peroxide of the bloom-forming cyanobacterium *Microcystis* PCC 7806 depends on nutrient availability. *Harmful Algae* 99:101916.
31. Wang H, Y, Tianyi Li T, Zhen Chen Z, Yinan Wang Y, Qin C (2016) Properties of calcium peroxide for release of hydrogen peroxide and oxygen: A kinetics study. *Chem Eng J.* 303: 450-457.
32. Rogers SO, Bendich AJ (1994) Extraction of total cellular DNA from plants, algae and fungi. In: *Plant Molecular Biology Manual*, Springer, Netherlands, pp 183–190.

33. Chernova E, Russkikh I, Voyakina E, Zhakovskaya Z (2016) Occurrence of microcystins and anatoxin-a in eutrophic lakes of Saint Petersburg, Northwestern Russia. *Oceanol Hydrobiol Stud.* 45(4):466-484.
34. Sellers RM (1990) Spectrophotometric Determination of Hydrogen Peroxide Using Potassium Titanium (IV) Oxalate. *Analyst* 105:950-954.
35. Jakubowska N, Szelaż-Wasielewska E (2015) Toxic picoplanktonic cyanobacteria—review. *Mar Drugs.* 13(3):1497-1518.
36. Mazur-Marzec H, Sutryk K, Kobos J, Hebel A, Hohlfeld, Błaszczuk A, Toruńska A, Kaczowska MJ, Łysiak-Pastuszek E, Kraśniewski W et al. (2013) Occurrence of cyanobacteria and cyanotoxins in the Southern Baltic Proper. Filamentous cyanobacteria vs. single-celled picocyanobacteria. *Hydrobiologia* 701:235–252.
37. Ni Z, Shengrui Wang S, Jingjing Cai J, Hong Li H, Alan Jenkins A, Stephen C. Maberly SC, May L (2019) The potential role of sediment organic phosphorus in algal growth in a low nutrient lake. *Environ Pollut.* 255(2):113235.
38. Chorus I, Spijkerman E (2020) What Colin Reynolds could tell us about nutrient limitation, N:P ratios and eutrophication control. *Hydrobiologia*. doi: <https://doi.org/10.1007/s10750-020-04377-w>.
39. Ngwa FF, Madramootoo CA, Jabaji S (2014) Comparison of cyanobacterial microcystin synthetase (mcy) E gene transcript levels, mcy E gene copies, and biomass as indicators of microcystin risk under laboratory and field conditions. *MicrobiologyOpen* 3(4): 411– 425.
40. Wang X, Wang P, Wang C, Hu B, Ren L, Yang Y (2018) Microcystin biosynthesis in *Microcystis aeruginosa*: Indirect regulation by iron variation. *Ecotoxicol Environ Saf.* 148:942-952.
41. Salvador D, Churro C, Valério E (2016) Evaluating the influence of light intensity in mcyA gene expression and microcystin production in toxic strains of *Planktothrix agardhii* and *Microcystis aeruginosa*. *J Microbiol Methods.* 123:4-12
42. Zuo J, Chen L, Shan K et al. (2018) Assessment of different mcy genes for detecting the toxic to non-toxic *Microcystis* ratio in the field by multiplex qPCR. *J Ocean Limnol.* 36: 1132–1144.
43. Beversdorf LJ, Chaston SD, Miller TR, McMahon KD (2015) Microcystin mcyA and mcyE Gene Abundances Are Not Appropriate Indicators of Microcystin Concentrations in Lakes. *PLoS ONE* 10(5):e0125353.
44. Pacheco ABF, Guedes IA, Azevedo SM (2016) Is qPCR a Reliable Indicator of Cyanotoxin Risk in Freshwater? *Toxins* 8:172.
45. Lu S, Zhang X, Xue Y (2017) Application of calcium peroxide in water and soil treatment: A review. *J Hazard Mater.* 337:163-177.
46. es PDF (2020) The misuse of ratios in ecological stoichiometry. *Ecology* 00(00): e03153. doi: 10.1002/ecy.3153.

Scheme

scheme 1 can be found in the Supp Files

Figures

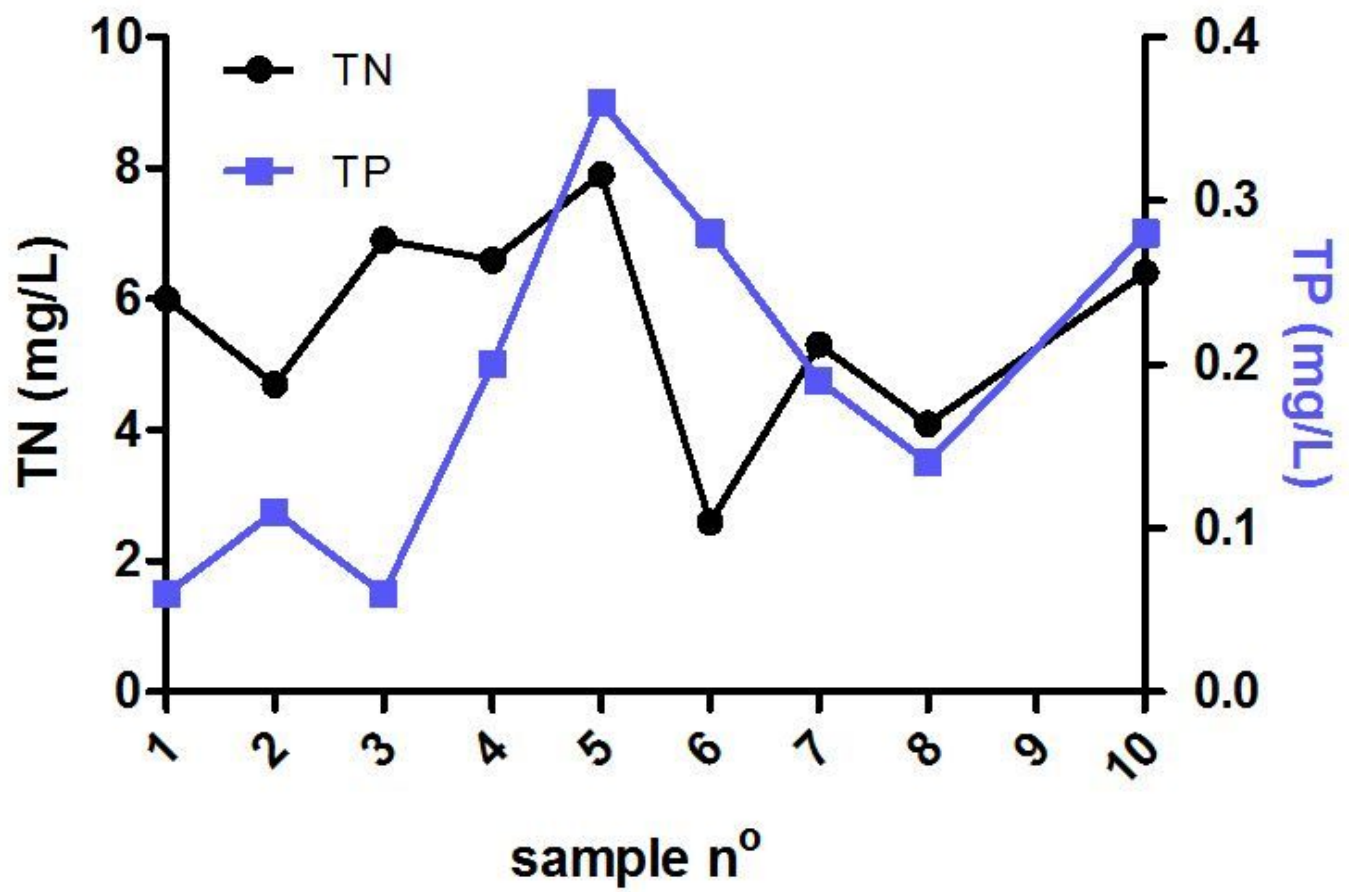


Figure 3

Total Nitrogen (left axis) and Total Phosphorus (right axis) in St. George Lake during monitoring period.

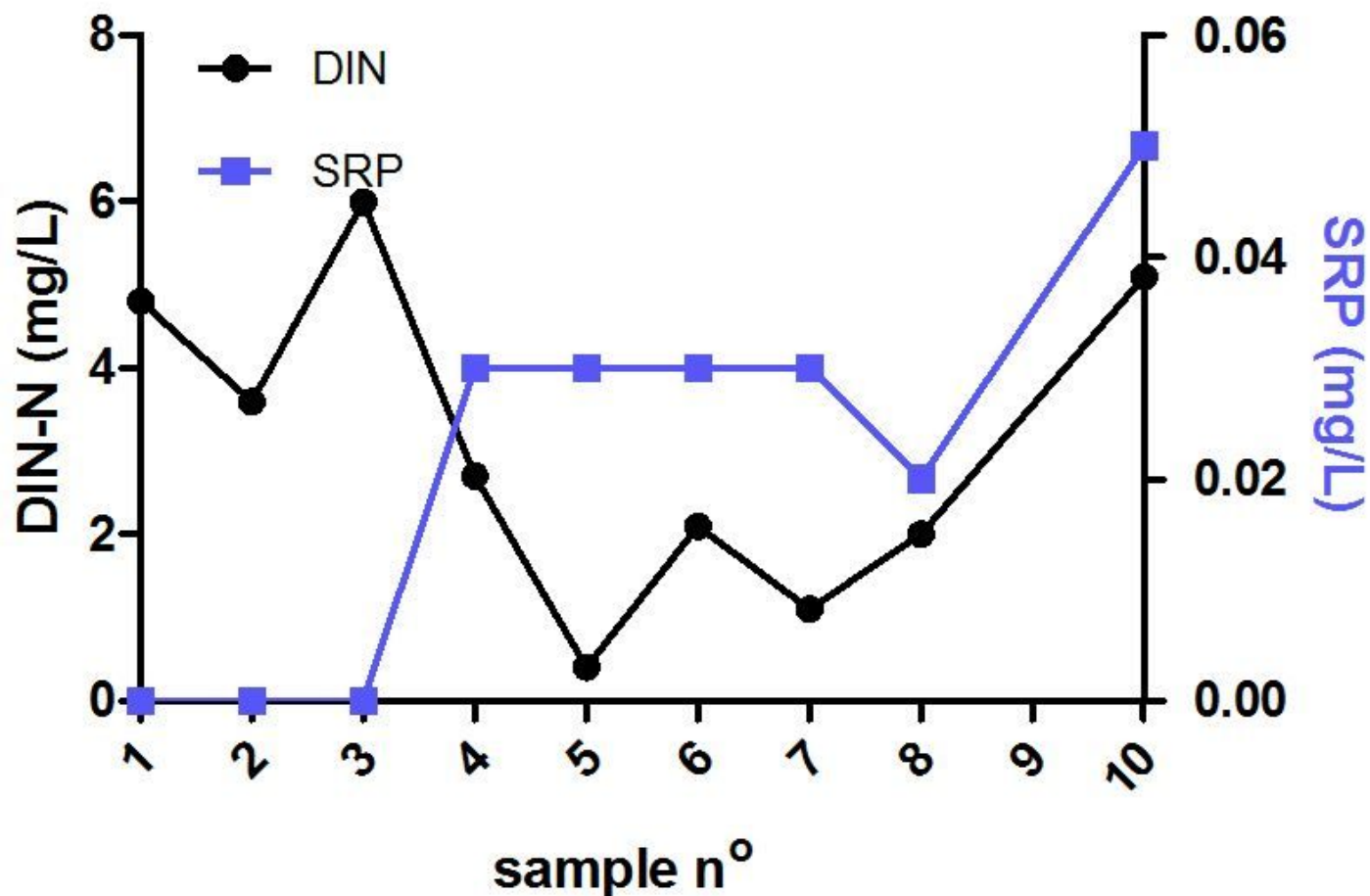


Figure 4

Dissolved nutrients content DIN-N (left axis) and SRP (right axis) in St. George Lake during the monitoring period.

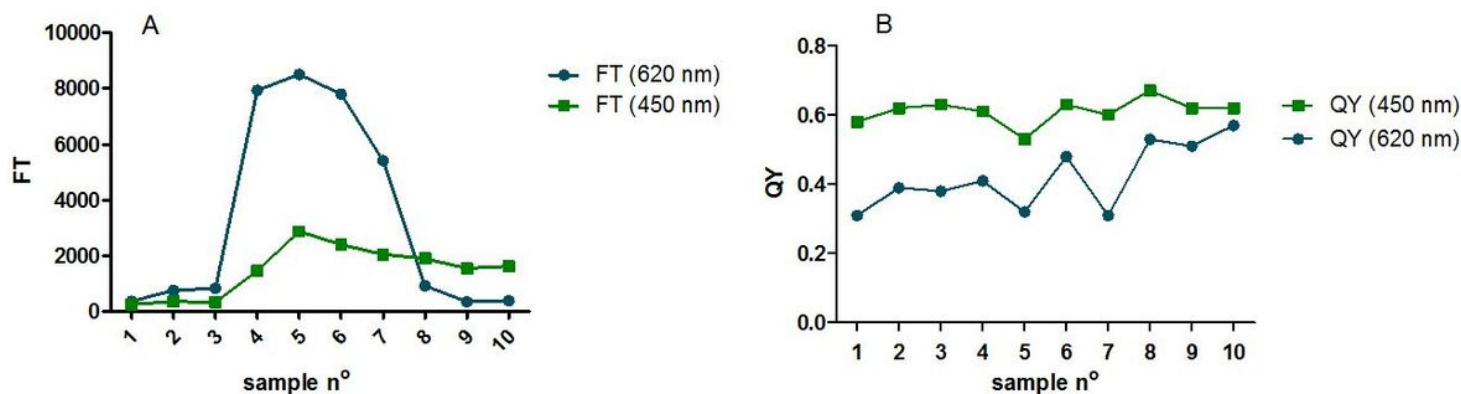


Figure 5

(A) Photosynthetic activity and (B) quantum yield measurements for cyanobacteria (620 nm, blue color) and green algae (450 nm, green color) in St. George Lake.

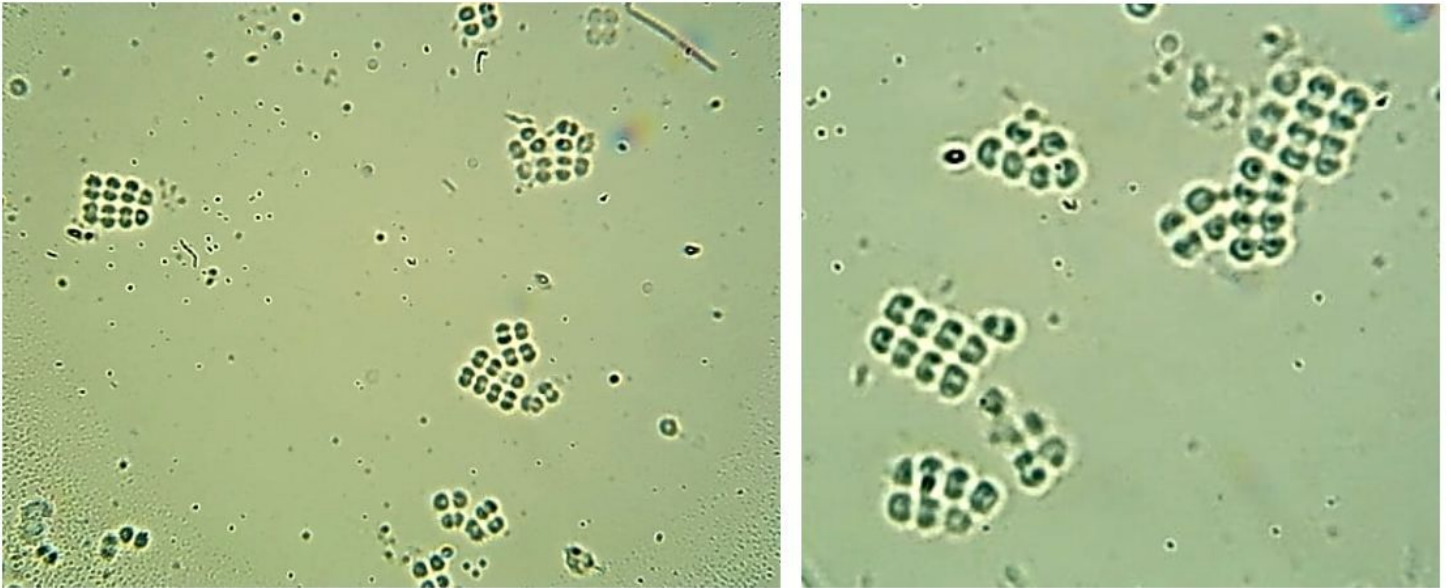


Figure 6

Merismopedia sp. captured under ECLIPSE Ci-L microscope (magnification 100x), equipped with OPTIKAM Wi-Fi camera.

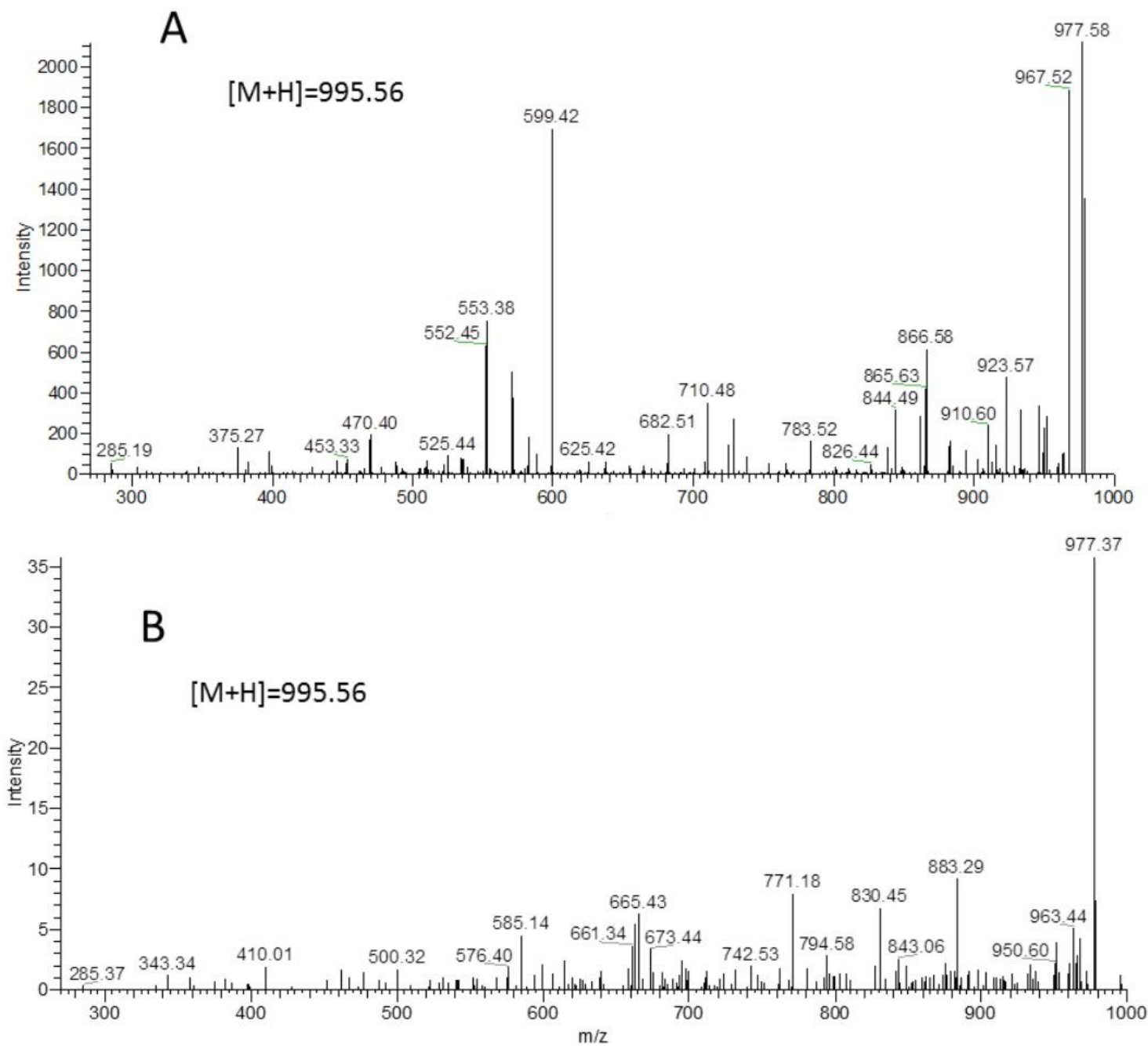


Figure 8

MS/MS spectra of the (A) MC-LR standard compound and (B) compound detected in samples with $[M+H] = 995.56$ but different fragmentation pattern.

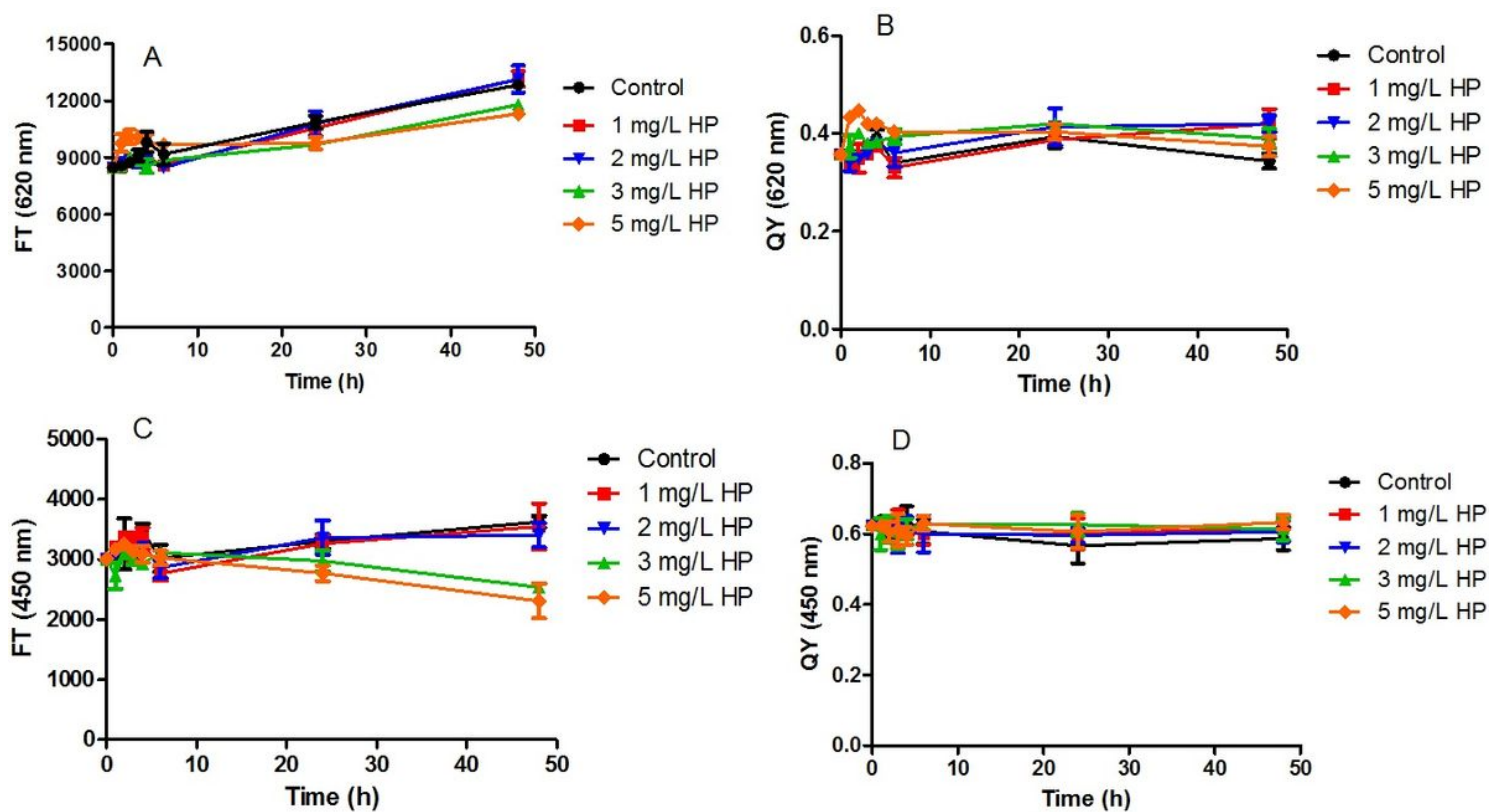


Figure 9

Instantaneous fluorescence (FT) and photosynthetic quantum yield (QY) at (A-B) 620 nm and (C-D) 450 nm excitation wavelength, for 48 hours of H₂O₂ 1-5 mg/L treatment.

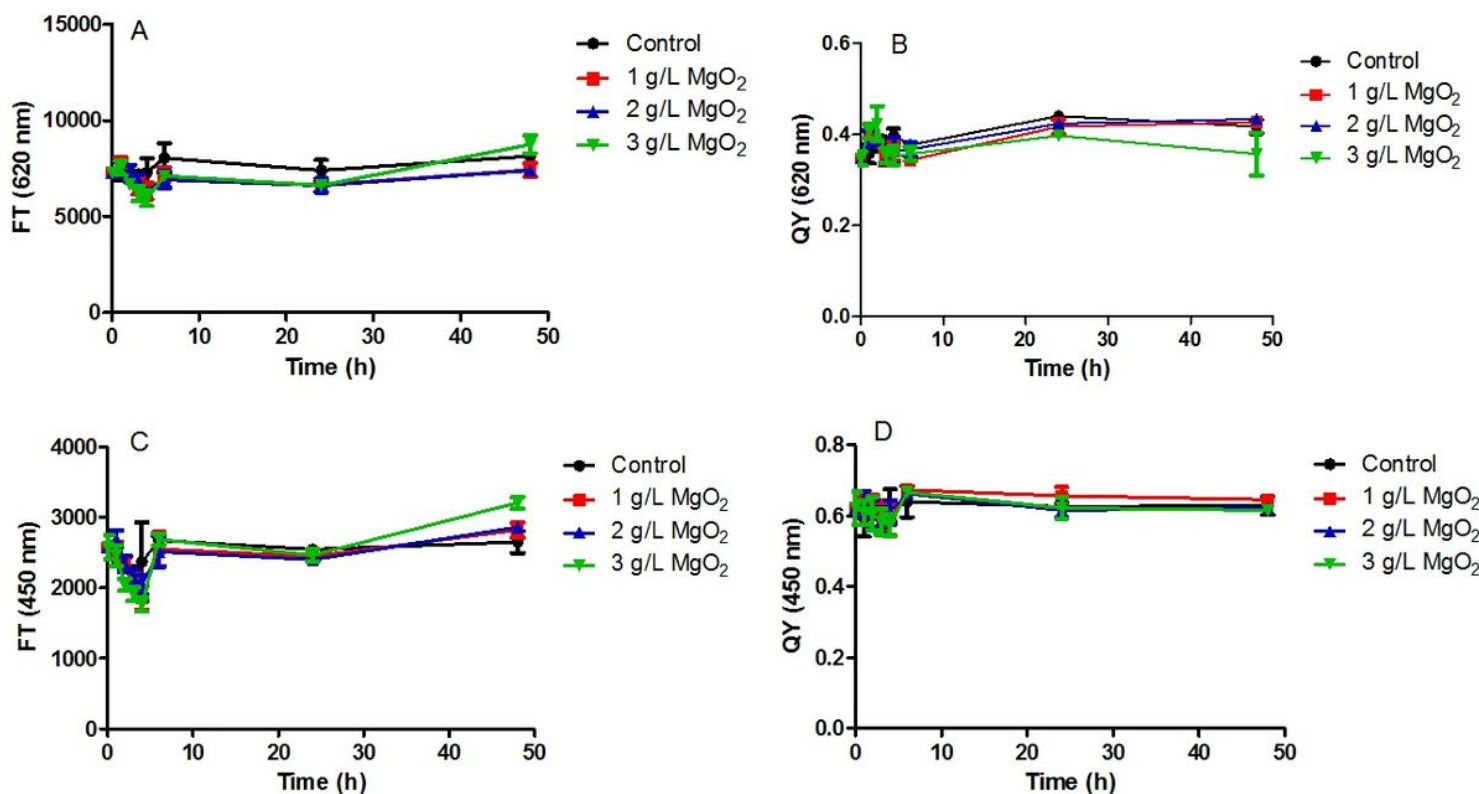


Figure 11

Instantaneous fluorescence (FT) and photosynthetic quantum yield (QY) at (A-B) 620 nm and (C-D) 450 nm excitation wavelength, for 48 hours of MgO₂ 1-3 g/L treatment.

Supplementary Files

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