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# *Microcystis, Raphidiopsis raciborskii and Dolichospermum smithii*, toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar

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ABSTRACT

Yezin Dam is a man-made reservoir located close to Yezin village in Myanmar. Its water is used for irrigation, domestic purposes and as drinking water for many urban communities in the watershed area. In recent years, increased pollution due to the concurrent development around the dam has led to water quality deterioration. No detailed study on the distribution of cyanobacteria and toxin production has been conducted so far. In order to provide insight into the extent of cyanobacteria and cyanotoxins in the dam, water samples were collected once in January 2014 for the isolation of cyanobacterial strains and eight times between March 2017 and June 2018 for the investigation of physical, chemical and biological parameters. A total of 99 phytoplankton taxa belonging to 50 genera were recorded from Yezin Dam. Microscopic examination showed that a *Dolichospermum* sp. was the dominant cyanobacterium followed by small numbers of *Microcystis*, and *Raphidiopsis raciborskii* in all samples throughout the sampling period. 15 isolated cyanobacterial strains were classified morphologically and phylogenetically as *Dolichospermum smithii*, *R. raciborskii* and *Microcystis* and tested for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins (STXs) and anatoxins (ATXs) by liquid chromatography-tandem mass spectrometry (LC–MS/MS) and enzyme-linked immunosorbent assay (ELISA). The toxin analysis of all isolated *Dolichospermum* strains by ELISA and LC–MS did not indicate the presence of ATXs, STXs, CYNs nor MCs. Four of the five isolated *Raphidiopsis* strains produced CYN and deoxyCYN. One of the isolated *Microcystis* strains (AB2017/08) from Yezin Dam produced 22 MC congeners. Concentrations of 0.12 μg $L^{-1}$ CYNs and 0.34 μg $L^{-1}$ MCs were also found in an environmental sample from Yezin Dam by ELISA. The potential therefore exists for the use of untreated water from Yezin Dam to cause harmful effects on humans, domestic and wild animals.

## 1. Introduction

Like other countries in Asia, Myanmar's economy is based on agriculture. Myanmar has made vast investments in the construction of irrigation dams throughout the country to enhance the availability of water for irrigation during the dry season to increase agricultural production and socioeconomic development (Oo et al., 2017). In Myanmar, around 240 dams have been constructed so far (Oo et al., 2017). Most of these dams, especially those near urban areas, are subject to quick deterioration due to increasing population growth and anthropogenic activities. Sewage discharge and agricultural runoff are the main causes of eutrophication, and harmful algal and cyanobacterial blooms in these water bodies lead to deterioration in water quality and ecosystem health.

Cyanobacterial blooms are often accompanied by the production of cyanobacterial toxins and have been recognized to cause serious chronic human and acute animal health problems and even mortalities (Carmichael, 2001; Hilborn and Beasley, 2015; Paerl and Huisman, 2009). The knowledge about cyanobacteria and their toxins in lakes and reservoirs in Myanmar is, however, poor. Only one recent study has described in detail the presence of cyanobacteria and cyanobacterial toxins, in Meiktila Lake close to the city of Meiktila in Myanmar (Ballot

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et al., 2020). It showed that cylindrospermopsin (CYN)- and deoxy-cylindrospermopsin (deoxyCYN)-producing *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*) (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno and microcystin-producing *Microcystis aeruginosa* (Kützing) Kützing and *Microcystis novacekii* (Komárek) Compère are common cyanobacteria in Meiktila Lake (Ballot et al., 2020).

Yezin Dam, which is located near Yezin village in the Zeyar Thiri Township, Nay Pyi Taw, is a man-made earth embankment reservoir. It was completed in 1974 with a total capacity of 0.074 $km^3$ (FAO, 2020). Its main uses are for irrigation and to protect against flooding from the Sinthay River and Yezin Stream (Than et al., 2019). The communities living near the dam use some of the water for domestic purposes and drinking without treatment. The consumption of water from Yezin Dam has been increasing, with increasing development activities and expanding urban areas in the watershed area. Forest degradation and annual forest fires in the Yezin watershed area have gradually increased the rate of sedimentation in the dam since 1975 (Win et al., 2003). Landsat data showed that the water surface area of Yezin Dam decreased by 74 % between 2011 and 2016 due to reduced rainfall in the catchment area and increased domestic water usage (Than et al., 2019). Anthropogenic activities like fishing, bathing, washing clothes with laundry detergents in the dam, poor sanitation and wastewater treatment systems in the watershed area further negatively affect the water quality of the dam.

This study was initiated after we noticed increased algal and cyanobacterial growth in the dam in 2014. We suspected that the presence of cyanobacteria could counteract the societal need for good water quality and could pose serious health problems to the people who directly use the water from the dam. The knowledge about cyanobacteria and cyanotoxins in reservoirs in many tropical countries is still poor. This study aimed therefore to add new information by

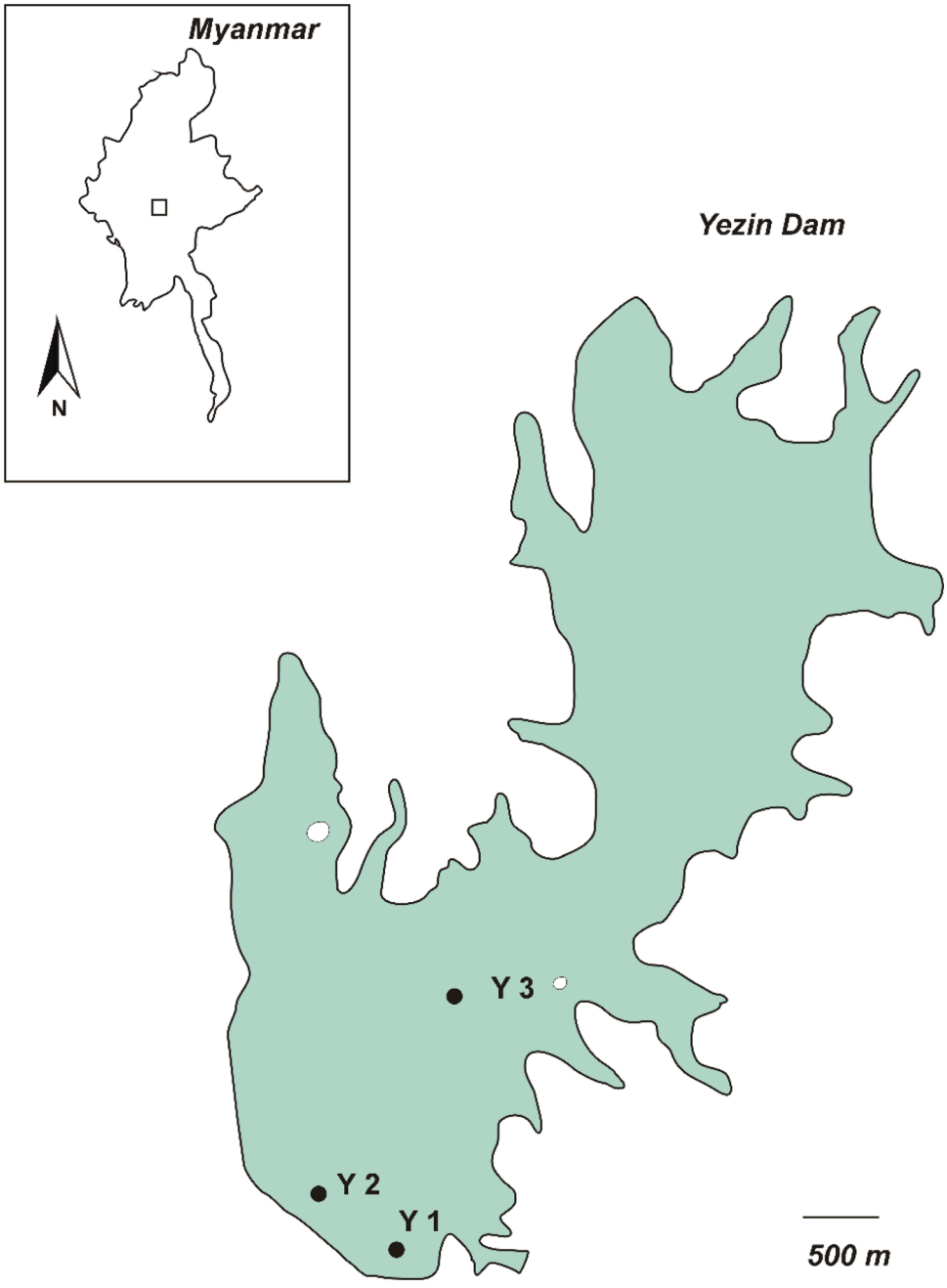


**Fig. 1.** Map of Yezin Dam showing the locations of water sampling (Stations Y1–Y3). The location of Yezin Dam in Myanmar is shown in the inset.

investigating the presence of cyanobacteria and cyanobacterial toxins in Yezin Dam and in cyanobacterial strains isolated from the dam as well as to elucidate in detail the cyanobacterial composition, phylogeny, toxin production and toxin profiles which were investigated in water and cyanobacterial strains isolated from the dam.

## 2. Material and methods

### 2.1. Study area, sampling methods and analyses

Yezin Dam is a man-made reservoir located in the Zeyar Thiri Township, Nay Pyi Taw and situated between Latitudes 19 50′ 36″ N and 19 53′ 08″ N and Longitudes 96 16′ 00″ E and 96 17′ 52″ E, at 133 m above sea level. The dam has been operational since 1976, with a total water storage capacity of 0.074 $km^3$ (FAO, 2020). When filled, it has a surface area of approximately 9.3 $km^2$ (Than et al., 2019).

Three sampling points were selected in the southern part of the dam: near the shore, near the dam, and in the center (Fig. 1). Water sampling could not be performed in the northern part as it is the security area of a military zone. Water samples were collected once in January 2014 for the isolation of cyanobacterial strains, 8 times between March 2017 and June 2018 for phytoplankton analyses and isolation of cyanobacterial strains and once in February 2020 to test for anatoxins, cylindrospermopsins, microcystins and saxitoxins using enzyme-linked immunosorbent assay (ELISA).

Water transparency was measured with a Secchi disk. The water temperature, pH, conductivity and oxygen content were measured in situ with a Hach, HQd Portable Meter (Hach, Loveland, CO, USA). For water chemistry analyses, integrated water samples (1 m steps) were collected from the trophogenic zone (two times the Secchi depth) using a Limnos water sampler (Limnos, Komorów, Poland). Water samples were preserved with 4 M $H_2SO_4$ for nutrient analysis (to 1 % final concentration) and not preserved for analysis of turbidity, total alkalinity, color, calcium and other cations. These samples were analysed at the Water Quality Laboratory in the Forest Research Institute, Yezin, Myanmar.

Subsamples (50 mL) were taken from the integrated samples for quantitative phytoplankton analysis and preserved with acidic Lugol's solution. For qualitative phytoplankton analysis, a concentrated net sample (mesh size 20 μm) was collected and preserved by addition of formaldehyde (to 4 % final concentration). All samples for quantitative and qualitative analysis were stored in the dark until they were analysed. Water samples (50 mL) were taken at each sampling point for isolation of cyanobacteria and kept in a cool shady place and gently shaken twice per day until processing at the Norwegian Institute for Water Research (NIVA) in Norway.

The Lugol-fixed samples were analyzed for phytoplankton composition and biomass using Utermöhl sedimentation chambers (Utermöhl, 1958) and an inverted microscope (Olympus Optical Co-Ltd Japan Model CK2, Olympus, Tokyo, Japan). For cyanobacterial taxa, length and width of 100 vegetative cells, heterocytes and akinetes were measured for morphological characterization. Phytoplankton biomass was calculated by geometrical approximations using the computerized counting program Opticount (SequentiX - Digital DNA Processing, Klein Raden, Germany). The specific density of phytoplankton cells was calculated as 1 g $cm^{-3}$. The taxa were identified to species or genus level using selected identification keys (e.g. Komárek, 2013; Komárek and Anagnostidis, 1999).

### 2.2. Isolation of strains and morphological characterization

Single colonies of *Microcystis* and single filaments of *Dolichospermum* sp. and *Raphidiopsis* sp. were isolated using a microcapillary. They were washed five times and then placed in wells of microtiter plates containing 300 μL Z8 medium (Kotai, 1972). After successful growth, they were placed in 50 mL Erlenmeyer flasks which contained 20 mL Z8 medium and maintained at 22 °C and a photon flux density of 80 mol of photons $m^{-2}$ $s^{-1}$. Classification of the strains was based on morphological traits (Komárek, 2013; Komárek and Anagnostidis, 1999).

The morphology of the isolated strains was identified with a Leica DM2500 light microscope, Leica DFC 450 camera and Leica Application Suite software (LAS) (Leica, Oslo, Norway) based on the: (i) size of vegetative cells and heterocytes, and; (ii) nature and shape of filaments or colonies. The Length and width of 50–250 vegetative cells and of 20–50 heterocytes were measured. All isolated strains used for this study are kept at NIVA.

### 2.3. Genomic DNA extraction, PCR amplification and sequencing

Genomic DNA of all isolated strains was extracted according to Ballot et al. (2016). All PCRs were performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). The 16S rRNA gene of the isolated strains from Yezin Dam was amplified using the primers as described by Ballot et al. (2016) (Table S1). PCR products were visualized by 1 % agarose gel electrophoresis with GelRed staining (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) and UV illumination. The amplified PCR products were purified with Qiagen PCR purifications columns (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Sequencing was performed on both strands of each PCR product, with the same primers used for PCR and intermediate primers as used in Ballot et al. (2016), on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, Oslo, Norway) following the manufacturer's instructions.

### 2.4. Phylogenetic analysis

16S rRNA gene sequences of *Dolichospermum, Raphidiopsis* and *Microcystis* strains were analyzed using the Seqassem software package (version 07/2008) and the Align MS Windows-based manual sequence alignment editor (version 03/2007) (SequentiX - Digital DNA Processing, Klein Raden, Germany).

A 16S rRNA gene set containing 1136 positions was used for the calculation of the *Anabaena/Dolichospermum* phylogenetic tree. Eight *Dolichospermum* sequences from Yezin Dam and 23 additional *Dolichospermum* sequences derived from GenBank were included in the analysis and *Cuspidothrix issatschenkoi* (FN689797) was employed as the outgroup. A 16S rRNA gene set of 1136 positions was used for the calculation of the *Raphidiopsis/Cylindrospermopsis* phylogenetic tree. Five sequences from *Raphidiopsis* strains isolated from Yezin Dam, and 37 additional *Cylindropsermopsis/Raphidiopsis* sequences derived from GenBank were included in the analyses and *Sphaerospermopsis aphanizomenoides* (LN846954) was employed as the outgroup. A set containing 1427 positions was used for the *Microcystis* 16S rRNA gene analysis. *Chroococcus subviolaceus* (MF072353) was employed as the outgroup. Two sequences from *Microcystis* strains isolated from Yezin Dam, and 42 additional *Microcystis* sequences derived from GenBank were included in the analysis.

Phylogenetic trees for 16S rRNA gene sequences were constructed using the maximum likelihood (ML) algorithm in Mega versionX with 1000 bootstrap replicates (Kumar et al., 2018). Evolutionary substitution models were evaluated using Mega version X (Kumar et al., 2018). The HKY + G + I evolutionary model was found to be the best-fitting evolutionary model for the *Anabaena/Dolichospermum* 16S rRNA gene tree, K2+G + I for the *Cylindrospermopsis/Raphidiopsis* 16S rRNA gene tree and T92+G + I for the *Microcystis* 16S rRNA gene tree. The data were submitted to the European Nucleotide Archive (ENA) under the accession numbers listed in Table 1. 16S rRNA gene sequence similarities were calculated separately for the *Dolichospermum*, *Microcystis* and *Raphidiopsis* sequences and are depicted in Table S2.

**Table 1**
Cyanobacterial strains isolated from Yezin Dam, strain codes and European Nucleotide Archive (ENA) accession numbers.

| Taxon | Strain | ENA accession nr. 16S rRNA gene |
|---|---|---|
| *Dolichospermum* | | |
| *D. smithii* | AB2014/01 | LR794159 |
| *D. smithii* | AB2014/02 | LR794160 |
| *D. smithii* | AB2014/03 | LR794161 |
| *D. smithii* | AB2014/04 | LR794162 |
| *D. smithii* | AB2014/05 | LR794163 |
| *D. smithii* | AB2014/06 | LR794164 |
| *D. smithii* | AB2017/17 | LR794165 |
| *D. smithii* | AB2017/18 | LR794166 |
| *Raphidiopsis* | | |
| *R. raciborskii* | AB2017/03 | LR794167 |
| *R. raciborskii* | AB2017/04 | LR794168 |
| R. *raciborskii* | AB2017/06 | LR794169 |
| *R. raciborskii* | AB2017/19 | LR794170 |
| *R. raciborskii* | AB2017/20 | LR794171 |
| *Microcystis* | | |
| *Microcystis* | AB2017/08 | LR794172 |
| *Microcystis* | AB2017/10 | LR794173 |

### 2.5. Toxin analysis

#### 2.5.1. Materials

The standards used for LC–MS analyses were: anatoxin-a (ATX) (Tocris Bioscience, Bristol, UK), homoATX (Novakits, Nantes, France), cylindrospermopsin (CYN) (Vinci Biochem, Vinci, Italy); certified reference materials (CRMs) of saxitoxin (STX), decarbamoylSTX (dcSTX), NeoSTX, gonyautoxins (GTX1, GTX4, GTX5), *N*-sulfocarbamoylgonyautoxins (C1 and C2 toxins), MC-RR, MC-LR (**10**) and [Dha$^7$]MC-LR (**11**) (National Research Council of Canada, Halifax, Canada); reference materials (RMs) of [Leu$^1$]MC-LY (LeBlanc et al., 2020), and of [D-Asp$^3$]MC-RR, [D-Asp$^3$]MC-LR (**8**), MC-YR, MC-HilR (**12**) having traces of MC-FR, MC-WR, and MC-LA prepared from commercial samples (Enzo Life Sciences, Farmingdale, NY, USA) produced at NRC Canada, and; extracts containing an array of other identified MCs were available from previous work (Ballot et al., 2020; Mallia et al., 2019; Miles et al., 2014; Yilmaz et al., 2019). Standards for the Adda-ELISA and for the CYN, ATX and STX ELISAs were provided in the kits (Abraxis LLC, Warminister, PA, USA).

#### 2.5.2. ELISA for MCs, CYNs, ATXs and STXs

Fresh cultures of *Dolichospermum*, *Microcystis*, and *Raphidiopsis* strains isolated from Yezin Dam were frozen and thawed three times. The *Raphidiopsis* strains were tested for CYNs using the Abraxis Cylindrospermopsin ELISA kit following the manufacturer's instructions. The test is a direct competitive ELISA that detects CYN but also recognizes deoxyCYN and 7-*epi*-CYN to varying degrees. The *Microcystis* strains were tested for MCs using the Abraxis Microcystins/Nodularins (ADDA) ELISA kit. The test is an indirect competitive ELISA designed to detect Adda, (3*S*-amino-9*S*-methoxy-2*S*,6,8*S*-trimethyl-10-phenyldeca-4*E*,6*E*-dienoic acid), based on specific recognition of the Adda moiety (Fischer et al., 2001). ADDA is a non-protein amino acid and forms a side chain in the microcystin molecule that is present in about 80 % of reported microcystin variants (Bouaïcha et al., 2019). The Adda-ELISA is reported to have very low sensitivity for MCs containing modified Adda variants (Foss et al., 2020).

All strains were also tested for saxitoxins and anatoxin-a analogues using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits. The saxitoxin ELISA is a direct competitive ELISA that detects STX based on specific antibody recognition but also recognizes other saxitoxins (e.g., dcSTX, GTXs, lyngbyatoxin, and NeoSTX) to varying degrees. The test for ATX-a is a direct competitive ELISA that detects anatoxin-a based on specific antibody recognition and but also recognizes homoATX. In addition, a water sample taken from Yezin Dam in February 2020 was tested for the presence of STXs and ATXs, CYNs, and MCs with the above-mentioned ELISA kits. The colour reaction of all ELISA tests was evaluated at 450 nm on a Perkin Elmer 1420 Multilabel counter Victor3 (Perkin Elmer, Waltham, MA, USA) (strain samples) or a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA) (field samples).

#### 2.5.3. Microcystin analysis by LC–HRMS

Fresh culture material of both *Microcystis* strains was prepared for LC–HRMS by freeze-thawing (3 times), diluting with an equal volume of MeOH, and filtering (0.22 μm) (Miles et al., 2012). LC–HRMS/MS analysis was performed on a Q Exactive-HF Orbitrap mass spectrometer equipped with a HESI-II heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA), using an Agilent 1200 LC system including a binary pump, autosampler and column oven (Agilent, Santa Clara, CA, USA), and a SymmetryShield 3.5 μm C18 column (100 × 2.1 mm; Waters, Milford, MA, USA) as described by Ballot et al. (2020). Extracts were derivatized with a 1:1 mixture of mercaptoethanol and $d_4$-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA), or oxidized with DMSO/Oxone, as described by Ballot et al. (2020) and analyzed by LC-HRMS.

#### 2.5.4. CYNs, ATXs and STXs analysis by LC–MS/MS

Cyanobacterial toxins were extracted from freeze-dried cultures (40 mL), with 6 mL of 50 % methanol and sonicated (Omniruptor4000 probe sonicator, Omni-Inc., Kennesaw, MA, USA) for 10 min in pulsed mode (50 %) using 160 W power (Cerasino et al., 2017b). An aliquot of the solution was then filtered on Phenex RC syringe filters (0.2 m; Phenomenex, Castel Maggiore, Italy) and analyzed by LC–MS/MS using a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore). Chromatographic separation of analytes was performed using a HILIC column (Ascentis Express OH5, 2.7 μm, 50 × 2.1 mm; Merck Life Science S.r.l., Milan, Italy), while MS detection was performed using positive electrospray ionization using scheduled Multiple Reaction Monitoring (Cerasino et al., 2017a). The method was suitable for the detection and quantification of the following toxins: ATX, CYN, STX, dcSTX, NeoSTX, GTX1, GTX4, GTX5 and C1 and C2 (Cerasino et al., 2017a). Quantification limits were 0.2–200 μg $L^{-1}$. Other toxic alkaloids not available as pure standards were screened only for tentative analysis (hydroxy-, epoxy-, and homo-ATXs, deoxyCYN, dcNeoSTX, GTX2/3, dcGTX2/3 and C3 and C4 toxins) using equivalent detection settings to their most similar analogs.

## 3. Results

### 3.1. Physical and chemical parameters

The pH varied between 7.2–8.7, the water temperature between 27.4 °C and 32.5 °C and the conductivity between 77 μS $cm^{-1}$ and 87 μS $cm^{-1}$. The Secchi depth varied between 1 and 1.5 m. The total phosphorous (TP) concentrations ranged from 20 to 57 μg $L^{-1}$ (see Table S3 for further physical and chemical data).

### 3.2. Phytoplankton community

During the study period, 99 phytoplankton taxa belonging to 50 genera and 10 classes were identified in water samples from Yezin Dam. Of those genera, 12 belonged to Chlorophyceae, 11 to Cyanobacteria, 8 to Trebouxiophyceae, 6 to Zygnematophyceae, 4 to Bacillariophyceae, 3 each to Dinophyceae and Euglenophyceae, and 1 genus to each of Synurophyceae, Cryptophyceae and Chrysophyceae.

The phytoplankton community in Yezin Dam showed a patchy distribution at all three sampling sites, with different groups dominating at different sites and time (Fig. 2). In general, cyanobacteria were the

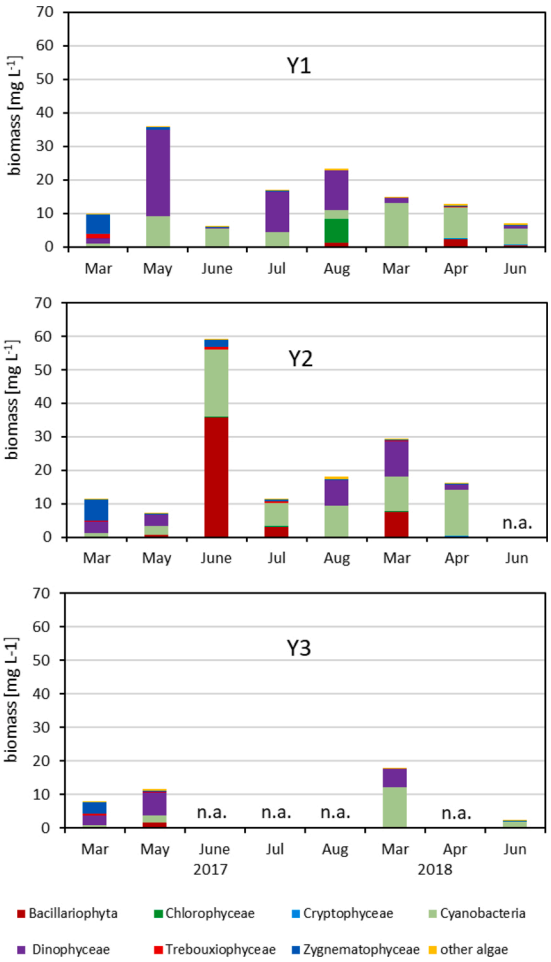


**Fig. 2.** Biomass (mg $L^{-1}$ FW) of phytoplankton groups at all three sampling points in Yezin Dam (March 2017–June 2018). n.a. = not analysed.

dominant phytoplankton organisms, with highest biomasses, recorded at site Y2 in June 2017 and April 2018 and at site Y1 in March 2018, comprising between 34 % and 86 % of the total phytoplankton biomass (Fig. 2, Table S4). Dinophyceae was the most dominant class of algae in May, July and August 2017 at sampling point Y1, while diatoms (Bacillariophyceae) were dominant at sampling point Y2 in June 2017. (Fig. 2, Table S4)

Among the cyanobacteria, *Dolichospermum* was the dominating taxon at all sampling stations throughout the sampling period, followed by *Oscillatoria* spp., *Limnothrix* spp. and *Microcystis*. The *R. raciborskii* biomass was lower than the *Dolichospermum* or *Microcystis* biomasses at all sampling points and sampling dates (Fig. 3, Table S5).

### 3.3. Morphological and phylogenetic characterization

Fifteen potentially toxin-producing cyanobacterial strains were isolated from Yezin Dam (Table 1). According to their morphological features (e.g., presence and form of vegetative cells, heterocytes and akinetes), eight of the isolated strains were morphologically classified as *D. smithii*, five as *R. raciborskii*, and two as *M. aeruginosa*. As genetic methods do not support the morphologically based assignment of *Microcystis* spp., Harke et al. (2016) suggested all

*Microcystis* spp. warrant placement into the same species complex. In the following parts of the manuscript, we use therefore "Microcystis" instead of species names.

The morphological determinations of the isolated strains were confirmed by genetic methods. Phylogenetic relationships of the investigated strains are presented in the maximum likelihood (ML) trees of

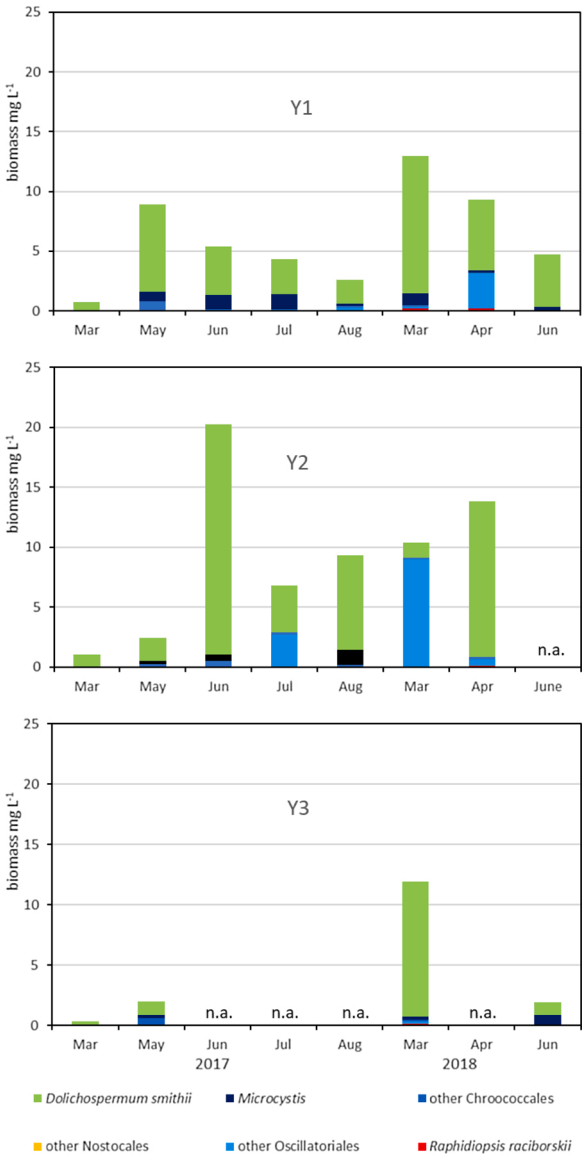


**Fig. 3.** Biomass (mg $L^{-1}$ FW) of the cyanobacterial taxa at all three sampling points in Yezin Dam (from March 2017–June 2018). n.a. = not analysed.

the 16S rRNA gene for *Dolichospermum* (Fig. S1), *Raphidiopsis* (Fig. S2), and *Microcystis* (Fig. S3).

In the ML tree in Fig. S1, all isolated *Dolichospermum* strains from Yezin Dam were grouped in the same subcluster and were assigned to *D. smithii*. The phylogenetic analyses of the 16S rRNA gene revealed that they were most similar to other *D./A. smithii*, but also to *D. spiroides, D. mucosum, D. ucrainicum, D. viguieri, D. circinale* and *D. pseudocompactum* sequences, described from the Czech Republic and Japan. In the *Raphdiopsis* ML tree, the strains from Yezin Dam grouped together with 16S rRNA gene sequences of *Cylindrospermopsis* and *Raphidiopsis* strains from Asia, Europe, Australia and North America. *Raphidiopsis* strains isolated from Meiktila Lake in Myanmar were also found in the same cluster (Fig. S2).

Both *Microcystis* strains from Yezin Dam clustered together with 16S rRNA gene sequences of *Microcystis* from Europe, Asia and Africa (Fig. S3). They differed slightly in their 16S rRNA sequences (99.72 % similarity).

### 3.4. Identification of cyanobacterial toxins

All investigated *D. smithii* strains from Yezin Dam were negative for MCs, CYNs, STXs, and ATXs by ELISA. All five *R. raciborskii* strains from Yezin Dam were also negative for STXs, ATXs and MCs by ELISA, but four of the strains were shown to produce CYNs either by ELISA or LC–MS/MS (Table 2).

Both *Microcystis* strains AB2017/08 and AB2017/10 were negative for CYNs, STXs and ATXs by ELISA, but strain AB2017/08 was identified as MC-producer by ELISA. In culture, 22 microcystin congeners were detected in this strain by LC–HRMS/MS, with a total concentration of 1160 μg $g^{-1}$ FW. Six of the congeners were unidentified or previously unreported variants. The microcystin variants and their concentrations are shown in Tables 3 and S6. In the environmental sample taken from Yezin Dam in February 2020, 0.12 μg $L^{-1}$ CYNs and 0.34 μg $L^{-1}$ MCs were measured by ELISA, but no ATXs and STXs were detected.

## 4. Discussion

The water of Yezin Dam is used for domestic purposes and drinking water by the local people. TP concentrations between 20 and 57 μg $L^{-1}$ demonstrate that Yezin Dam is in a mesotrophic to eutrophic status (Salas and Martino, 1991) and explain the high phytoplankton and cyanobacterial biomasses. Rigosi et al. (2015) have investigated the probability for hazardous cyanobacterial blooms based on water temperature and TP by using Bayesian networks in multiple lake systems. They showed that at temperatures above 24 °C and TP values between 20 and 100 μg $L^{-1}$, which are typical conditions for Yezin Dam, the probability for hazardous cyanobacterial blooms is almost 50 %. Different cyanobacterial taxa have diverse responses to varying nitrogen vs. phosphorus enrichment and cannot be treated as a single group when considering the effects of nutrient loading on the composition of the phytoplankton community (Dolman et al., 2012). Beside the presence or absence of toxin producing species, the amount of toxins is also dependent on the relation of toxic versus non-toxic strains and can therefore not be predicted by biomass of a certain taxon only. The phytoplankton data show clearly that there is a spatial and temporal variation in the distribution of the dominant phytoplankton groups in Yezin Dam. Patchiness on small and bigger scales has been described for phytoplankton distribution in lake and ocean environments, especially for species that have good buoyancy-regulating mechanisms like cyanobacteria or dinoflagellates (Borics et al., 2011; Breier et al., 2018). These spatial and temporal variations make it difficult to relate nutrient values to the biomass of phytoplankton groups.

The low occurrence of aquatic macrophytes and high nutrient loading in Yezin Dam may be the reason for the high algal and cyanobacterial biomasses observed. Only a few aquatic macrophytes, *Hydrilla verticillata* and *Chara fibrosa* are found in the dam due to the excessive removal of aquatic macrophytes since the 1990s (Mjelde et al., 2021; personal communication with local population) and water level regulations. Aquatic macrophytes can through allelopathic effects and competition for nutrients prevent the growth of planktonic algae and potentially toxic cyanobacteria and help to maintain a clear water state (Wojciechowski et al., 2018; Cheng et al., 2017; Hilt and Gross, 2008). Biological management can therefore be a suitable measure to control cyanobacterial blooms, and planting of macrophytes should be considered as a measure to help control the growth of cyanobacteria and other phytoplankton in Yezin Dam.

During the study period *D. smithii* was the dominant cyanobacterium in the phytoplankton of Yezin Dam but no cyanobacterial toxins of the MC, STX, ATX or CYN groups were produced by the isolated *D. smithii* strains. Similarly, no strains of *D. smithii* from other locations worldwide have yet been reported to produce cyanobacterial toxins. *D. smithii* is neither part of the phytoplankton in Meiktila Lake, nor has it been described from other water bodies in Myanmar (Ballot et al., 2020). *D. smithii* is reported of mesotrophic to slightly eutrophic ponds, reservoirs and lakes in temperate areas (Komárek, 2013). Although *D. smithii* is not seen as a tropical species, we assigned the strains isolated from Yezin Dam to this taxon because the morphological characteristics fitted best to *D. smithii,* which is also supported by the phylogenetic analyses. Although the *Dolichospermum* strains isolated from Yezin Dam are grouped in a separate subcluster in the phylogenetic tree in Fig. S1, they are part of a cluster including species of *D. smithii* but also *D. viguieri*, *D. mucosa*, *D. spiroides*, *D. circinale* and *D. ucrainicum*. This demonstrates the difficulty to assign *Dolichospermum* sp. from Yezin Dam to a particular species based on publicly available genetic data only, because reliable use of phylogenetic data from public databases (e.g., NCBI, ENA) depends strongly on their unambiguity. Reports of *D. smithii* from tropical Brazil and Senegal support our assignment (Berger et al., 2005;

**Table 2**
Concentrations (μg $mg^{-1}$ FW) of CYNs (ELISA), and of CYN and deoxyCYN (LC–MS/MS) in *R. raciborskii* strains isolated from Yezin Dam, Myanmar.

| Strain | ELISA | LC-MS/MS | | | |
|---|---|---|---|---|---|
| | CYNs | CYN | deoxyCYN | CYN % | deoxyCYN % |
| AB2017/03 | 0.89 | 0.17 | 0.11 | 61 | 39 |
| AB2017/04 | 3.50 | 0.2 | 0.11 | 65 | 35 |
| AB2017/06 | n.d. | n.d. | n.d | n.d. | n.d. |
| AB2017/19 | 3.18 | 0.75 | 1.84 | 29 | 71 |
| AB2017/20 | 1.86 | 1.03 | 2.19 | 32 | 68 |

Percentages are % of total CYNs measured by LC–MS/MS. n.d. = not detected.

**Table 3**
Microcystins detected by LC-HRMS/MS analysis in *Microcystis* strain AB2017/08 isolated from Yezin Dam, their retention times ($t_R$), concentrations (μg $g^{-1}$ FW), relative abundances (%), and observed *m/z* values in negative ionisation mode.[a]

| | *m/z* | Compound name | Confidence | $t_R$ (min) | Concentration[b] AB2017/08 μg $g^{-1}$ | % |
|---|---|---|---|---|---|---|
| 1 | 1011.5521[c] | Unidentified MC | Unidentified | 5.10 | 1.3 | 0.11 |
| 2 | 1011.5521 | Unidentified MC | Unidentified | 5.85 | 22.4 | 1.93 |
| 3 | 1130.5562 | MC-LR-Cys(O) | Tentative | 6.14 | 0.1 | 0.01 |
| 4 | 1114.5612 | MC-LR-Cys | Probable | 6.16 | 10.3 | 0.89 |
| 5 | 997.5364 | [D-Asp$^3$,Mser$^7$] MC-LR | Tentative | 6.70 | 1.3 | 0.11 |
| 6 | 1011.5521 | [Mser$^7$]MC-LR | Probable | 6.81 | 9.5 | 0.82 |
| 7 | 997.5364 | [D-Asp$^3$,Mser$^7$] MC-LR | Tentative | 6.88 | 1.4 | 0.12 |
| 8 | 979.5258[c] | [D-Asp$^3$]MC-LR | Confirmed | 6.90 | 181.9 | 15.68 |
| 9 | 1011.5521[c] | Unidentified | Unidentified | 7.06 | 7.8 | 0.67 |
| 10 | 993.5415[c] | MC-LR | Confirmed | 7.12 | 891.7 | 76.89 |
| 11 | 979.5258[c] | [Dha$^7$]MC-LR | Confirmed | 7.13 | 9.6 | 0.82 |
| 12 | 1007.5571[c] | MC-HilR | Confirmed | 7.39 | 9.5 | 0.82 |
| 13 | 980.5099[c] | Unidentified | Unidentified | 11.26 | 0.5 | 0.04 |
| 14 | 994.5255[c] | Unidentified | Unidentified | 12.20 | 0.8 | 0.06 |
| 15 | 952.5037[c] | Unidentified | Unidentified | 13.39 | 0.2 | 0.01 |
| 16 | 986.4881[c] | [D-Asp$^3$]MC-LY | Tentative | 14.62 | 0.3 | 0.02 |
| 17 | 1000.5037[c] | MC-LY | Tentative | 15.14 | 1.4 | 0.12 |
| 18 | 1009.5082[c] | [D-Asp$^3$]MC-LW | Tentative | 16.41 | 1.7 | 0.14 |
| 19 | 970.4931[c] | [D-Asp$^3$]MC-LF | Tentative | 16.95 | 0.5 | 0.05 |
| 20 | 1023.5197[c] | MC-LW | Tentative | 17.00 | 3.7 | 0.32 |
| 21 | 984.5088[c] | MC-LF | Tentative | 17.61 | 3.8 | 0.33 |
| 22 | 950.5244[c] | MC-LL | Probable | 18.03 | 0.2 | 0.01 |

[a] A comprehensive version of this Table, including positive and negative ionisation MS data, reactivity towards thiols, proposed formulae, mass error, number of rings plus double-bond equivalents (RDBE), and presence of characteristic ions observed in positive and negative ionisation MS/MS spectra, is in the Supporting Information (Table S1) together with LC–HRMS/MS spectra (Figs. S4–S7). [b] Concentration is expressed per weight of biomass (FW) and as a percentage of the total microcystins detected in each culture; [c] Reacted with mercaptoethanol (1 equivalent).

Sant'Anna, 1991).

ELISA analyses of a water sample from February 2020 confirm the presence of both CYNs and MCs in Yezin Dam. Our study demonstrated clearly that CYN- and deoxyCYN-production can be related to the presence of *R. raciborskii* and MCs production to the presence of *Microcystis*. The biomass of both taxa has been relatively low during the study period. This fact and the co-existence of non-toxin-producing and toxin-producing *Microcystis* and *R. raciborskii* strains can explain the relatively low toxin concentrations in February 2020.

In a recent study, CYNs-producing *R. raciborskii* was also found in Meiktila Lake with up to ten times higher biomasses than in Yezin Dam (Ballot et al., 2020). The *Raphidiopsis* strains from Yezin Dam produce CYN and deoxyCYN is in the same or slightly lower range as found for *R. raciborskii* strains from Meiktila Lake (2.9–9.8 μg $mg^{-1}$ FW) (Ballot et al., 2020). Australian *Raphidiopsis* strains are described to produce up to 3.5 μg $mg^{-1}$ FW of CYNs, but these values comprise intracellular CYNs only (Saker and Griffiths, 2000).

Production of CYNs by *R. raciborskii* has also been reported from lakes in China, Japan, Thailand and Vietnam (Chonudomkul et al., 2004; Hawkins et al., 1997; Nguyen et al., 2017). Brazilian, *Raphidiopsis* strains are known to produce saxitoxins but not CYNs (Hoff-Risseti et al., 2013). Although *R. raciborskii* is also widely distributed in Europe, only one study by Đorđević et al. (2015) has related findings of CYN in Serbian Lake Aleksandrovac to the presence of *R. raciborski.* This was not confirmed by genetic and chemical investigations of isolated strains.

*Microcystis* is widespread in many Asian countries (Harke et al., 2016), but has been reported from only a few water bodies in Myanmar (Ballot et al., 2020; Green, 2011; Naw et al., 2020). Both *Microcystis* strains from Yezin Dam differ slightly in their 16S sequences, which also differ slightly from those of the two *Microcystis* strains AB2017/14 and AB2017/15 isolated from Meiktila Lake (Ballot et al., 2020). The four *Microcystis* strains from Yezin Dam and Meiktila Lake cluster together with strains from Asia, Europe and South America.

Only one of the two *Microcystis* strains isolated from Yezin Dam was found to produce MCs. With 1160 μg $g^{-1}$ FW the MC concentration is in the same range as described for a *Microcystis* strain from South African Hartbeespoort Dam (Ballot et al., 2014). Higher MC concentrations in *Microcystis* strains up to 5.8 μg $mg^{-1}$ dry weight are reported by Vézie et al. (2002). The coexistence of MC-producing and non-producing *Microcystis* strains has also been reported from Hartbeespoort Dam, where only one of 16 isolated strains produced MCs (Ballot et al., 2014). The MC profile of strain AB2017/08 from Yezin Dam differs considerably from those of the strains from Hartbeespoort Dam and Meiktila Lake (Ballot et al., 2014, 2020). Twenty-two MC congeners were produced by AB2017/08, MC-LR and [D-$Asp^3$]MC-LR were the dominant MC congeners, comprising 76.9 % and 15.7 %, respectively, of the total MCs detected. Of the six unidentified MCs (Table 3), the accurate masses of **1**, **2** and **9** correspond to MC-LR + $H_2O$ but had different retention times to [Mser7]MC-LR (**6**). However, due to the lack of MS/MS spectra with adequate signal-to-noise for these minor compounds, they must all be regarded as "unidentified". One interesting feature is that although the majority of the MCs (entries 5–21 have similar relative intensities in both positive and negative ionization modes), the four earliest-eluting compounds were significantly relatively more intense in negative mode (Fig. 4). This may be due to the presence of extra phenolic or carboxylic acid groups, with the latter appearing to be the case for **4** and **3** (the Cys-adduct of MC-LR, and its sulfoxide). With 52 MC congeners, *Microcystis* strain AB2017/14 from Meiktila Lake produces a considerably higher number of MCs, but MC-LR and [D-$Asp^3$]MC-LR together make up only 20 % of the total MC concentration in that strain (Ballot et al., 2020).

The concentrations of CYNs (0.12 μg $L^{-1}$) and MCs (0.34 μg $L^{-1}$) measured in Yezin Dam were well below the provisional short-term drinking-water guideline value (GV) of 3 μg $L^{-1}$ for CYNs and 12 μg

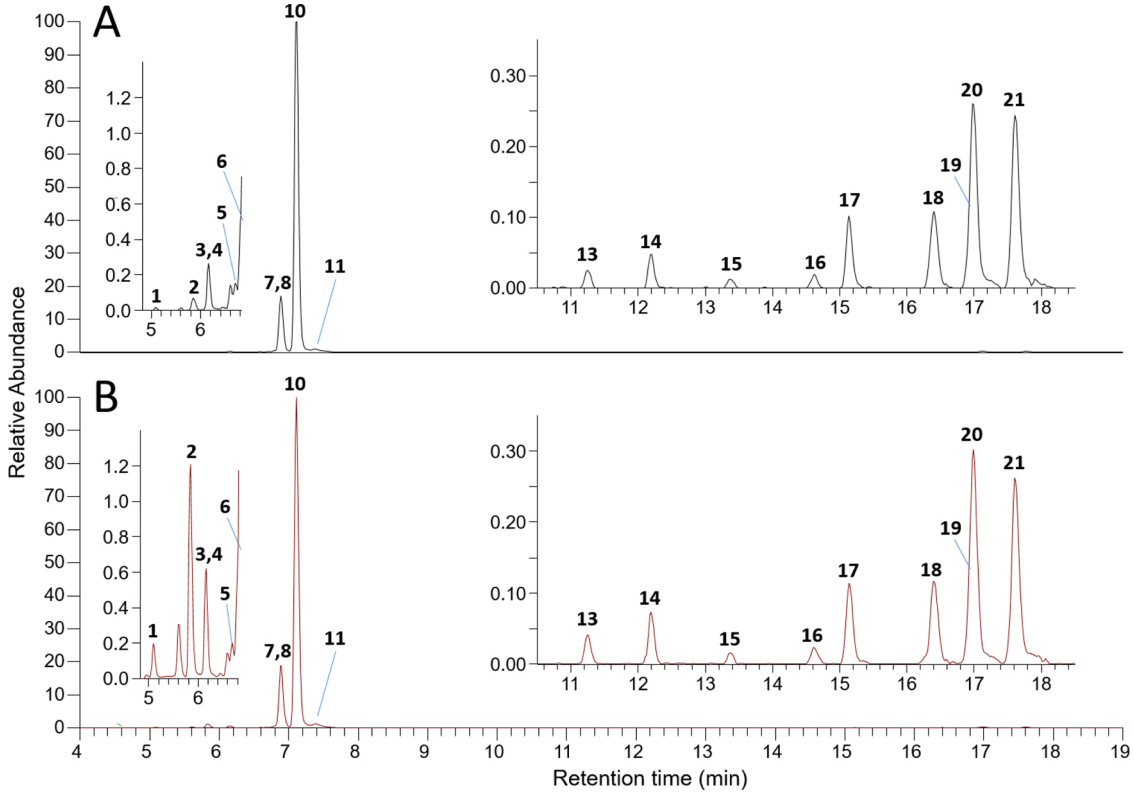


**Fig. 4.** LC–HRMS FS chromatograms, extracted (±5 ppm) for the *m/z* values in Table 3, from analysis of microcystins in an extract from a culture of *Microcystis* strain AB2017/08 isolated from Yezin Dam: A, in positive ionization mode, and; B, in negative ionization mode. The two insets in each chromatogram show vertical expansions that display the minor components. Peaks are labelled with the compound numbers in Table 3, and some of the very minor peaks are not labelled. The intensity scales of both chromatograms are scaled relative to the most intense peak (MC-LR (**10**)).

$L^{-1}$ for MCs and well below the provisional lifetime drinking-water GV of 0.7 μg $L^{-1}$ for CYN and 1 μg $L^{-1}$ for MC-LR (WHO, 2020a, 2020b). The provisional lifetime drinking water GVs would be exceeded after the daily intake of more than 6 L of water (CYNs) or 3 L of Water (MCs). In the event of a bloom of toxic *Raphidiopsis* or *Microcystis* strains, a much lower intake of water would be enough to exceed the above mentioned GVs. However, this assumes also that CYN and deoxyCYN or the MC variants found have similar toxicities. Norris et al. (1999) and Li et al. (2001) did not find any significant contribution of deoxyCYN to the total toxicity of *R. raciborskii*. In contrast, cell viability assays showed the toxicity of deoxyCYN to be only slightly lower than that of CYN and that deoxyCYN and CYN act by same mechanism of toxicity (Neumann et al., 2007) which is also supported by WHO (2020a). The GV values for MC are based on the toxicity of MC-LR. MCs in water samples are often measured as MC-LR equivalents. However, the toxicity has only been investigated for some of the 257 MC variants known today and can differ considerably from that of MC-LR (Bouaïcha et al., 2019). It is to be expected that the relative abundance of the different cyanobacterial taxa in Yezin Dam will vary over time, and a future shift from the dominance of non-toxic *Dolichospermum* to dominance of CYN-producing *Raphidiopsis* or MC-producing *Microcystis* cannot be excluded. An increase in their biomasses would lead to increased concentrations of CYNs and MCs in the water.

## 5. Conclusions

In conclusion, the present study investigates for the first time the occurrence of cyanobacteria and cyanotoxins in Yezin Dam, Myanmar. Although all *Dolichospermum* strains isolated from Yezin Dam were negative for production of cyanotoxins, one of the *Microcystis* strains produced MCs, and four of the five isolated *Raphidiopsis* strains produced CYN and deoxyCYN.

Aside from their toxins being relevant to human health, cyanobacteria have the potential to cause substantial ecological impacts on aquatic food webs. As the dam is an important source for drinking water and irrigation, the monitoring of cyanobacterial blooms and their toxins should be considered as an important basis for the integrated water resource management plan of the country (environmental and health risk assessment plan of the water bodies).

## Authors statement

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.limno.2021.125901.

## References

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J., Miles, C.O., 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Mar. Freshwat. Res. 65, 175–189.

Ballot, A., Cerasino, L., Hostyeva, V., Cirés, S., 2016. Variability in the sxt gene clusters of PSP toxin producing *Aphanizomenon gracile* strains from Norway, Spain, Germany and North America. PLoS One 11, e0167552.

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V., Miles, C.O., 2020. Cylindrospermopsin- and deoxycylindrospermopsin-producing *Raphidiopsis raciborskii* and microcystin-producing *Microcystis* spp. in Meiktila Lake, Myanmar. Toxins 12, 232.

Berger, C., Coute, A., Gansoumana, N., Gugger, M., 2005. Cyanobacterial taxa of the Senegal River system (northern Senegal, West Africa). Algol. Stud. 117, 147–176.

Borics, G., Abonyi, A., Krasznai, E., Várbíró, G., Grigorszky, I., Szabó, S., Déak, C., Tóthmérész, B., 2011. Small-scale patchiness of the phytoplankton in a lentic oxbow. J. Plankt. Res. 33, 973–981.

Bouaïcha, N., Miles, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins 11, 714.

Breier, R.E., Lalescu, C.C., Waas, D., Wilczek, M., Mazza, M.G., 2018. Emergence of phytoplankton patchiness at small scales in mild turbulence. Proc. Natl. Acad. Sci. U. S. A. 115, 12112–12117.

Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Hum. Ecol. Risk Assess. 7, 1393–1407.

Cerasino, L., Capelli, C., Salmaso, N., 2017a. A comparative study of the metabolic profiles of common nuisance cyanobacteria in southern perialpine lakes. Adv. Oceanogr. Limnol. 8, 22–32.

Cerasino, L., Meriluoto, J., Bláha, L., Carmeli, S., Kaloudis, T., Mazur-Marzec, A., 2017b. SOP 6. Extraction of cyanotoxins from cyanobacterial biomass. In: Meriluoto, J., Spoof, L., Codd, G.A. (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Wiley, pp. 350–353.

Cheng, L., Cao, X., Yu, X., 2017. Mechanism of growth inhibition effect of aquatic plants on water cyanobacteria. Insights Biol. Med. 1, 1–9.

Chonudomkul, D., Yongmanitchai, W., Theeragool, G., Kawachi, M., Kasai, F., Kaya, K., Watanabe, M.M., 2004. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. FEMS Microbiol. Ecol. 48, 345–355.

Đorđević, N.B., Simić, S.B., Ćirić, A.R., 2015. First identification of the cylindrospermopsin (CYN)-producing cyanobacterium *Cylindrospermopsis raciborkii* (Woloszyńska) Seenayya & Subba Raju in Serbia. Fresenius Environ. Bull. 24, 3736–3742.

Dolman, A.M., Rücker, J., Pick, F.R., Fastner, J., Rohrlack, T., Mischke, U., Wiedner, C., 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS One 11, e38757.

FAO, 2020. AQUASTAT - FAO’s Global Information System on Water and Agriculture. Water Resources, Myanmar accessed on 11 May 2020. http://www.fao.org/nr/water/aquastat/countries_regions/Profile_segments/MMR-WR_esp.stm.

Fischer, W.J., Garthwaite, I., Miles, C.O., Ross, K.M., Aggen, J.B., Chamberlin, A.R., Towers, N.R., Dietrich, D.R., 2001. Congener-independent immunoassay for microcystins and nodularins. Environ. Sci. Technol. 35, 4849–4856.

Foss, A.J., Miles, C.O., Wilkins, A.L., Rise, F., Trovik, K.W., Cieslik, K., Aubel, M.T., 2020. Analysis of total microcystins and nodularins by oxidative cleavage of their ADMAdda, DMAdda, and Adda moieties. Anal. Chim. Acta: X 6, 100060.

Green, J., 2011. Geographical variation in rotifers associated with *Microcystis* blooms. Hydrobiologia 662, 197–204.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H. W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. Harmful Algae 54, 4–20.

Hawkins, P.R., Chandrasena, N.R., Jones, G.J., Humpage, A.R., Falconer, I.R., 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. Toxicon 35, 341–346.

Hilborn, E.D., Beasley, V.R., 2015. One health and cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. Toxins 7, 1374–1395.

Hilt, S., Gross, E.M., 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic Appl. Ecol. 9, 422–432.

Hoff-Risseti, C., Dorr, F.A., Schaker, P.D.C., Pinto, E., Werner, V.R., Fiore, M.F., 2013. Cylindrospermopsin and saxitoxin synthetase genes in *Cylindrospermopsis raciborskii* strains from Brazilian freshwater. PLoS One 8, 14.

Komárek, J., 2013. Cyanoprokaryota. 3. Heterocytous genera. In: Büdel, B., Krienitz, L., Gärtner, G., Schagerl, M. (Eds.), Süsswasserflora von Mitteleuropa/Freshwater flora of Central Europe. Springer Spektrum Berlin, Heidelberg, p. 1130.

Komárek, J., Anagnostidis, K., 1999. Cyanoprokaryota 1. Chroococcales. – 19/1. In: Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa Gustav Fischer. Jena-Stuttgart-Lübeck-Ulm, p. 548.

Kotai, J., 1972. Instructions for Preparation of Modified Nutrient Solution Z8 for Algae. - Publication B-11/69. Norwegian Institute for Water Research, Blindern, Oslo, Norway.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

LeBlanc, P., Merkley, N., Thomas, K., Lewis, N.I., Békri, K., Renaud, S.L., Pick, F.R., McCarron, P., Miles, C.O., Quilliam, M.A., 2020. Isolation and characterization of [D-Leu$^{1}$]microcystin-LY from *Microcystis aeruginosa* CPCC-464. Toxins 12, 77.

Li, R.H., Carmichael, W.W., Brittain, S., Eaglesham, G.K., Shaw, G.R., Liu, Y.D., Watanabe, M.M., 2001. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria). J. Phycol. 37, 1121–1126.

Mallia, V., Uhlig, S., Rafuse, C., Meija, J., Miles, C.O., 2019. Novel microcystins from *Planktothrix prolifica* NIVA-CYA 544 identified by LC-MS/MS, functional group derivatization and $^{15}$N-labeling. Mar. Drugs 17, 22.

Miles, C.O., Sandvik, M., Nonga, H.E., Rundberget, T., Wilkins, A.L., Rise, F., Ballot, A., 2012. Thiol derivatization for LC-MS identification of microcystins in complex matrices. Environ. Sci. Technol. 46, 8937–8944.

Miles, C.O., Melanson, J.E., Ballot, A., 2014. Sulfide oxidations for LC-MS analysis of methionine-containing microcystins in *Dolichospermum flos-aquae* NIVA-CYA 656. Environ. Sci. Technol. 48, 13307–13315.

Mjelde, M., Swe, T., Langangen, A., Ballot, A., 2021. A contribution to the knowledge of Charophytes in Myanmar; morphological and genetic identification and ecology notes. Bot. Lett. 168, 102–109.

Naw, M.W.D., San, D.P., Aung, Y., 2020. Occurrence of Microcystis Toxic Blooms in Indawgyi Lake, Kachin State. University of Mandalay Res. J. 11, 1–9.

Neumann, C., Bain, P., Shaw, G., 2007. Studies of the comparative in vitro toxicology of the cyanobacterial metabolite deoxycylindrospermopsin. J. Toxicol. Environ. Health, Part A 70, 1679–1686.

Nguyen, T.T.L., Hoang, T.H., Nguyen, T.K., Duong, T.T., 2017. The occurrence of toxic cyanobacterium *Cylindrospermopsis raciborskii* and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam. Environ. Monit. Assess. 189, 11.

Norris, R.L., Eaglesham, G.K., Pierens, G., Shaw, G.R., Smith, M.J., Chiswell, R.K., Seawright, A.A., Moore, M.R., 1999. Deoxycylindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. Environ. Toxicol. 14, 163–165.

Oo, A.T., Van Huylenbroeck, G., Speelman, S., 2017. Differential impacts of an irrigation project: case study of the Swar Dam Project in Yedashe, Bago region of Myanmar. J. Dev. Agric. Econ. 9, 178–189.

Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1, 27–37.

Rigosi, A., Hanson, P., Hamilton, D.P., Hipsey, M., Rusak, J.A., Bois, J., Sparber, K., Chorus, I., Watkinson, A.J., Qin, B., Kim, B., Brookes, J.D., 2015. Determining the probability of cyanobacterial blooms: the application of Bayesian networks in multiple lake systems. Ecol. Appl. 25, 186–199.

Saker, M.L., Griffiths, D.J., 2000. The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. Phycologia 39, 349–354.

Salas, H.J., Martino, P., 1991. A simplified phosphorus trophic state model for warm-water tropical lakes. Water Res. 25, 341–350.

Sant'Anna, C.L., 1991. Two new taxa of *Anabaena* and other Nostocaceae (Cyanophyceae) from the State of São Paulo, southeastern Brazil. Algol. Stud./Arch. Hydrobiol., Suppl. 64, 527–545.

Than, M.M., Yee, K.M., Lint, K., Thu, M.M., Han, M., Zin, M.M., Khaing, E.E., 2019. Water level changes in Yezin Dam, Zayarthiri township, Nay Pyi Taw, using geospatial techniques. Sci. Res. J. 7, 78–83.

Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9, 1–38.

Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., Sivonen, K., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. Microb. Ecol. 43, 443–454.

WHO, 2020a. Cyanobacterial Toxins: Cylindrospermopsins. Background Document for Development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/338063.

WHO, 2020b. Cyanobacterial Toxins: Microcystins. Background Document for Development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/338066.

Win, B.N., Keh, K., Than, M.M., 2003. Application of RS/GIS for the assessment of Yezin Dam Watershed area. In: Annual Research Conference. Forest Research Institute, Ministry of Forestry, Myanmar accessed on 16 September 2020. https://forestdepartment.gov.mm/books/research/application-rsgis-assessment-yezin-dam-watershed-area.

Wojciechowski, J., Burda, T.M., Scheer, M.B., Costa, E.A.D.d., Fernandes, L.F., 2018. Potential effects of mechanically removing macrophytes on the phytoplankton community of a subtropical reservoir. Acta Bot. Bras. 32, 588–594.

Yilmaz, M., Foss, A.J., Miles, C.O., Özen, M., Demir, N., Balci, M., Beach, D.G., 2019. Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of *Microcystis aeruginosa* from a Turkish lake. Toxicon 167, 87–100.