Leaflet No.

The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation

Evaluation of Harmful Algal Blooms and Cyanotoxin Levels in Yezin Dam

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March, 2026

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Dr. Thida Swe, Research Officer

Abstract

Yezin Dam, an artificial reservoir near Yezin Village, Zay Yar Thiri Township, Nay Pyi Taw, supplies water for irrigation, domestic use, and drinking purposes within the Yezin watershed area. Anthropogenic development pressures in the Yezin Reservoir area have led to a decline in water quality, promoting the proliferation of harmful algal bloom. These Harmful Algal Blooms (HABs) are rapid, excessive growths of blue green algae or cyanobacteria in water bodies that produce toxins, deplete oxygen, or damage ecosystems, causing sickness in people, pets, and marine life. Therefore, the occurrence of cyanobacteria and their toxin production in Yezin Dam were evaluated based on water samples collected on twelve occasions between 2014 and 2025. The water samples were analyzed for physicochemical and biological parameters. The analysis revealed a total of 99 phytoplankton taxa distributed across 50 genera, including several potentially toxin-producing cyanobacterial species. *Dolichospermum* sp. dominated throughout the study period, followed by Microcystis and. Fifteen cyanobacterial strains isolated from Yezin Dam were identified as *Dolichospermum smithii* (Komárek) Wacklin, L.Hoffmann & Komárek, *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera & al., and *Microcystis* spp., and analyzed for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins (STXs), and anatoxins (ATXs) using LC–MS/MS and ELISA. No toxins were detected in *Dolichospermum* strains, whereas four out of five *Raphidiopsis* strains produced CYN and deoxyCYN, and a single *Microcystis* strain synthesized 22 MC congeners. Environmental water samples contained 0.12 μg L^{-1} CYNs and 0.34 μg L^{-1} MCs. These findings indicate potential health risks associated with the use of untreated water from Yezin Dam.

Key words: *Yezin Dam, Harmful Algal Blooms, cyanobacteria, Dolichospermum* smithii, *Raphidiopsis raciborskii*, *Microcystis, MCs, CYNs, STXs, health risk*

ရေဆင်းဆည်အတွင်း အန္တရာယ်ဖြစ်စေသောရေညှိမျိုးများနှင့် စိမ်းပြာရေညှိမှ ထုတ်လွှတ်သော အဆိပ်အတောက် ပါဝင်မှုအား ဆန်းစစ်လေ့လာခြင်း

ဒေါက်တာသီတာဆွေ၊ သုတေသနအရာရှိ

စာတမ်းအကျဉ်း

ရေဆင်းဆည်သည် နေပြည်တော်ကောင်စီနယ်မြေ၊ ဇေယျာသီရိမြို့နယ်၊ ရေဆင်းကျေးရွာအနီး တည်ရှိပြီး စိုက်ပျိုးရေ၊ နေ့စဉ်သုံးရေနဲ့ သောက်သုံးရေတို့ကို ထောက်ပံ့ ပေးလျက် ရှိပါသည်။ ရေဆင်းဆည် ရေဝေရေလဲဇရိယာအတွင်း လူတို့၏ လုပ်ဆောင်မှုများကြောင့် ရေ၏ အရည်အသွေးကျဆင်းလာခြင်းသည် ဘေးအန္တရာယ်ဖြစ်စေသော ရေညှိမျိုးစိတ်များ ပေါက်ဖွားခြင်း ကို ဖြစ်ပေါ်စေလာပါသည်။ ဘေးအန္တရာယ်ဖြစ်စေသော ရေညှိမျိုးစိတ်များသည် ရေထုအတွင်း အလွန်အကျွံ ပေါက်ဖွားနိုင်ခြင်း၊ အဆိပ်အတောက်များ ထုတ်လွှတ်ပေးခြင်း၊ ရေထဲတွင် အောက်စီဂျင် ပျော်ဝင်မှု လျော့နည်းစေခြင်းနှင့် ဂေဟစနစ်ကို ပျက်စီးစေပြီး လူ၊ တိရစ္ဆာန်နှင့် ရေနေ သတ္တဝါများအပေါ် ဆိုးကျိုး သက်ရောက်စေပါသည်။ ထို့ကြောင့် ၂၀၁၄ ခုနှစ်မှ ၂၀၂၅ ခုနှစ် အတွင်း ရေဆင်းဆည်မှ ရေနမူနာ ၁၂ ကြိမ်ကောက်ယူခဲ့ပြီး ရေ၏ ရူပ၊ ဓာတုနှင့် ဇီဝ ဂုဏ်သတ္တိများကို ဓာတ်ခွဲဆန်းစစ်ပြီး ဆည်အတွင်း Cyanobacteria ပေါက်ဖွားမှုနှင့် အဆိပ် အတောက် ထုတ်လွှတ်မှု အခြေအနေဆန်းစစ်ခြင်း လုပ်ငန်းအား ဆောင်ရွက်ခဲ့ပါသည်။ စမ်းသပ်မှုအရ ဆည်အတွင်း ရေညှိ မျိုးစိတ် ၉၉ မျိုး တွေ့ရှိရပြီး၊ အချို့မှာ အဆိပ်ထုတ်နိုင်သည့် cyanobacteria မျိုးစိတ် ဖြစ်ကြောင်း တွေ့ရှိရပါသည်။ Cyanobacteria မျိုးစိတ်များ အတွင်း *Dolichospermum* sp. သည် အများဆုံး ပေါက်ဖွားလျက်ရှိကြောင်း ဆန်းစစ်တွေ့ရှိခဲ့ပါသည်။ Cyanobacteria မျိုးစိတ်၁၅ မျိုးကို မွေးမြူမျိုးခွဲပြီး မျိုးရိုးဗီဇ ဆန်းစစ်ခြင်းနည်းလမ်းဖြင့် *Dolichospermum smithii*၊ *Raphidiopsis raciborskii* နှင့် *Microcystis* မျိုးစိတ်များ ဖြစ်ကြောင်း အတည်ပြုနိုင်ခဲ့ ပါသည်။ LC–MS/MS နှင့် ELISA ကိုအသုံးပြုပြီး အဆိုပါ မျိုးစိတ်များတွင် ပါဝင်သည့် Cyanotoxins ပါဝင်မှုကို ဆန်းစစ်ခဲ့ရာ *Dolichospermum* မျိုးစိတ်များတွင် အဆိပ်အတောက် ထုတ်လွှတ်ခြင်းမရှိသော်လည်း *Raphidiop-sis* မျိုးစိတ် ၅ မျိုးအနက် ၄ မျိုး သည် CYN နှင့် deoxyCYN အဆိပ်အတောက်နှင့် *Microcystis* မျိုးစိတ်မှ မျိုးကွဲ ၂၂ ခု ရှိသည့် MC အဆိပ်အတောက်ကို ထုတ်လွှတ်ကြကြောင်း တွေ့ရှိ ခဲ့ရပါသည်။ အဆိုပါ အဆိပ်အတောက်ပါသော ရေကိုသောက်သုံးခြင်း၊ ရှူရှိုက်မိခြင်း သို့မဟုတ် အရေပြားနှင့် ထိတွေ့ခြင်းတို့မှတဆင့် လူ၏ကျန်းမာရေးကို ထိခိုက်စေနိုင်ပြီး အရေပြားယားယံမှုမှ ပြင်းထန်သည့် အသည်းပျက်စီးမှုနှင့် အာရုံကြောဆိုင်ရာ ချို့ယွင်းချက်များအထိ ဖြစ်ပေါ်စေနိုင်ပါသည်။ သို့ဖြစ်ပါ၍ ဤသုတေသန တွေ့ရှိချက်သည် ရေဆင်းဆည်ရေအပေါ် မှီခိုနေသည့် လူနှင့်ရေနေသတ္တဝါများ၏ ကျန်းမာရေး အန္တရာယ်ကို ခြိန်းခြောက်လျက်ရှိကြောင်း ညွှန်ပြနေပါသည်။

Evaluation of Harmful Algal Blooms and Cyanotoxin Levels in Yezin Dam

1. Introduction

Myanmar's economy is predominantly agriculture-based, and extensive investments have been made in irrigation infrastructure to secure water availability during the dry season and enhance socioeconomic development (Oo et al., 2017). To date, approximately 240 dams have been constructed nationwide (Oo et al., 2017). However, many reservoirs—particularly those located near urban areas—are increasingly affected by eutrophication driven by rapid population growth, untreated sewage discharge, and agricultural runoff. These pressures promote harmful algal and cyanobacterial blooms, leading to declining water quality and ecosystem degradation. 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).

Yezin Dam, a key irrigation and water supply reservoir, exhibited notable algal and cyanobacterial proliferation in 2014, raising concerns about potential cyanotoxin contamination and associated public health risks for communities directly dependent on the dam for water supply. Despite the growing global recognition of cyanobacterial blooms as an emerging environmental and health issue, comprehensive data on cyanobacterial diversity, phylogeny, and toxin production in tropical reservoirs—particularly in Myanmar—remain scarce.

Previous studies, such as in Meiktila Lake near the city of Meiktila, documented cylindrospermopsin (CYN)- and deoxy-cylindrospermopsin (deoxyCYN)-producing *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*), as well as microcystin-producing *Microcystis aeruginosa* and *Microcystis novacekii*, as dominant toxin-producing cyanobacteria (Ballot et al., 2020). These findings highlight the occurrence of harmful cyanobacteria in Myanmar's freshwater systems and underscore the need for similar investigations in other critical reservoirs, including Yezin Dam, where comparable environmental conditions may favor toxin-producing species.

To address this knowledge gap, the present study investigated the occurrence of cyanobacteria and their associated toxins in Yezin Dam using long-term monitoring data collected between 2014 and 2025. The study further aimed to characterize the cyanobacterial community structure, phylogenetic relationships, toxin production, and toxin profiles through integrated analyses of water samples and isolated cyanobacterial strains.

2. Objectives

This study aims to –

- Assess the occurrence of cyanobacteria and their toxins in Yezin Dam using long-term data (2014–2025).
- Characterize the cyanobacterial community in terms of composition, phylogeny, and toxin production.
- Determine cyanotoxin types and profiles through analyses of both water samples and isolated strains.

3. Literature Review

3.1 Cyanobacteria

Cyanobacteria, which is also known as blue-green algae, blue-green bacteria, cyanoprokaryota, or cyanophytes, are primarily caused by the harmful algae blooms in lakes and reservoirs worldwide. Cyanobacteria have a long evolutionary history and documented

fossil records about 3500 million years ago (Schopf, 2000). They can survive in almost every terrestrial and aquatic system while they are most abundant in aquatic habitats as part of the plankton, and some can be found tightly or loosely attached to surfaces of plants, rocks and sediments.

They may be unicellular or have cells arranged in colonies and some form filaments in single trichomes or filaments with false or true branching. Cyanobacteria have high nutrient affinity and it is widely accepted that the availability of nitrogen and phosphorous are important factors in enhancing cyanobacterial growth (Mur et al., 1999).

Cyanotoxins

Cyanotoxins are bioactive secondary metabolites produced by cyanobacteria. Humans may be exposed to cyanotoxins via contact through recreational activities such as bathing in contaminated water and by consuming untreated, or unsuitably treated drinking water, food, or some dietary supplements (Roy-Lachapelle et al., 2017, Testai et al., 2016, Funari and Testai, 2008). These toxins can cause gastroenteritis, skin reactions and liver failure in humans and cause skin lesions, nervous system problems, and liver damage in domestic and wild animals.

Cyanobacterial toxins are grouped according to their function or their toxicological properties – e.g., hepatotoxins, neutotoxins, dermatotoxins (Codd et al., 2005) and according to their chemical structure such as cyclic peptides, alkaloids and lipopolysaccharides (LPS) (Chorus and Welker, 2021).

3.2 Microcystins (MCs)

The microcystins (MCs) are cyclic oligopeptides and these toxins were named "Microcystins (MCs)" as they are first isolated from Microcystis aeruginosa (WHO 1999, Carmichael et al., 1988). It was estimated that 66% of Microcystis blooms produced MCs (Green, 2011). MCs can be produced by other planktic and benthic cyanobacteria taxa such as Dolichospermum/Anabaena, Fischerella, Gloeotrichia, etc. MCs are hepatotoxins as they mainly affect the liver and but they can also affect the kidneys and reproductive system (Liu et al., 2018). There are 279 variants structurally characterized (Bouaïcha et al., 2019) while Microcystin-LR (MC-LR) is the most common microcystin variants and has potent carcinogenic to humans (IARC, 2010).

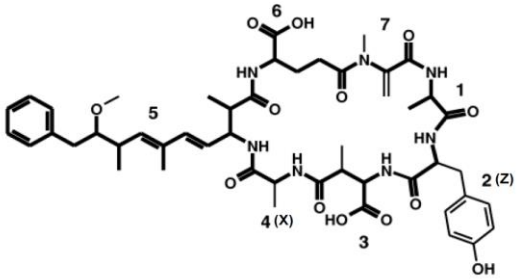

Figure 1. Chemical structure of microcystin (Tillett et al., 2000), the variable L-amino acid residues at position 2 and 4 are indicated by X and Z, respectively

3.3 Cylindrospermopsins (CYNs)

The cylindrospermopsin (CYN) is cyclic alkaloid and was first isolated from a culture of *Cylindrospermopsis raciborskii* (now *Raphidiopsis raciborskii*) (Wołszyńska, 1912) Seenayya et Subba Raju (1972) from a reservoir in tropical northern Australia (Kuiper-Goodman and Fitzgerald, 1999). Their chemical structure was characterized by Ohtani et al. (1992). Toxicity of CYN was first recorded in 1979 on Palm Island, Australia as more than 100 persons were hospitalized due to the consumption of drinking water contaminated by C. raciborskii (Griffiths and Saker, 2003). CYNs can be produced by other cyanobacteria such as Aphanizomenon, Chrysosporum, Dolichospermum (Anabaena), Lyngbya, Rhaphidiopsis,

Oscillatoria, and Umezakia (Akcaalan et al., 2014). CYN has four structurally variants and they are 7-epi-cylindrospermopsin (7-epi-CYN), 7-deoxy-cylindrospermopsin (7-deoxy-CYN), 7-deoxy-desulpho-cylindrospermopsin and 7-deoxy-desulpho-12-acetylcylin-drospermopsin (Wimmer et al., 2014). CYN primarily affects the kidneys, lungs, heart, thymus, spleen, and intestine in mammals (Falconer et al., 1999).

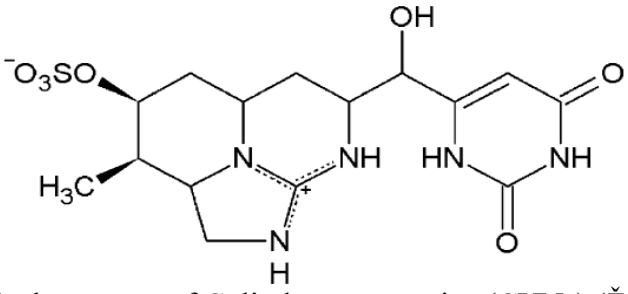

Figure 2. Chemical structure of Cylindrospermopsins (CYNs) (Žegura et al., 2011)

3.4 Anatoxin-a (ATX-a)

The anatoxin-a (ATX-a) is a neurotoxin and also called the very fast death factor (VFDF) as it can cause rapid death to animals (Bruno et al., 2017). ATX-a is a bicyclic secondary amine, 2-acetyl-9-azabicyclo-[4.2.1] non-2-ene (Figure 3) (Dow and Swoboda, 2000, Carmichael, 1988, Devlin et al., 1977, Huber, 1972). Homoanatoxin-a (HANTX) is a toxic analogue of ATX-a and has an additional methyl group (CH) on carbon atom 12 (C12).

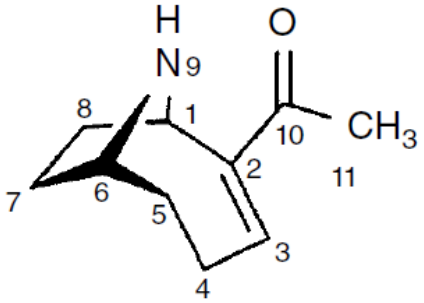

Figure 3. Chemical structure of anatoxin-a (ATX-a) (Bruno et al., 2017)

These toxins can be produced by *Anabaena sp., Aphanizomenon flos-aquae, Aphanizomenon sp, Arthrospira fusiformis, Cuspidothrix issatschenkoi, Dolichospermum circinale, D. flos-aquae, D. macrosporum, D. mendotae, D. planctonicum, D. spiroides, Microcoleus autumnalis, Microcystis aeruginosa, Microcystis sp., Oscillatoria sp., Phomidium favosum, P. formosum, Phomidium sp., Pseudanabaena limnetica, Tychonema bourrellyi* (Shams et al. 2015, Ballot et al., 2005, Ballot et al., 2004,).

3.5 Saxitoxins (STXs)

Saxitoxins (STXs), Paralytic Shellfish Poisoning (PSP) toxins or Paralytic Shellfish toxins (PSTs), are neutotoxic alkaloids and produced by marine eukaryotic dinoflagellates, such as Alexandrium, Gymnodinium and Pyrodinium (Lefebvre et al., 2008, Usup et al., 1994, Oshima et al., 1993) and freshwater prokaryotic cyanobacteria such as Aphanizomenon gracile, Aphanizomenon sp., Cuspidothrix issatschenkoi, Dolichospermum circinale, Lyngbya wollei, Raphidiopsis brookii, Cylindrospermum sp., Phormidium uncinatum., Geitlerinema sp., Limnothrix redekei, Scytonema cf. crispum (Borges et al., 2015, Casero et al., 2014)

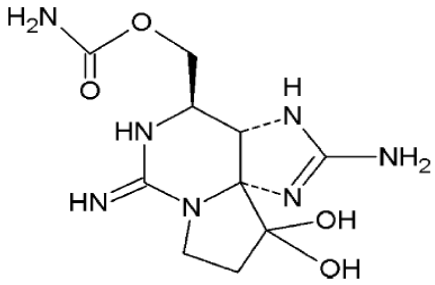

Figure 4. Chemical structure of saxitoxins (Žegura et al., 2011)

4. Materials and Methods

4.1. 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 (Figure 5). 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, close to the dam structure, and at the center (Fig. 5). Sampling in the northern part was not possible as it falls within a restricted military zone. Water samples were collected at different times for various analyses: two times in January 2014 and May 2017 for isolation of cyanobacterial strains, eight times between May 2017 and June 2025 for phytoplankton analysis and further cyanobacterial isolation, and two times in February 2020 and June 2025 to test for anatoxins (ATXs) , cylindrospermopsins (CYNs), microcystins (MCs), and saxitoxins (STXs) using enzyme-linked immunosorbent assay (ELISA).

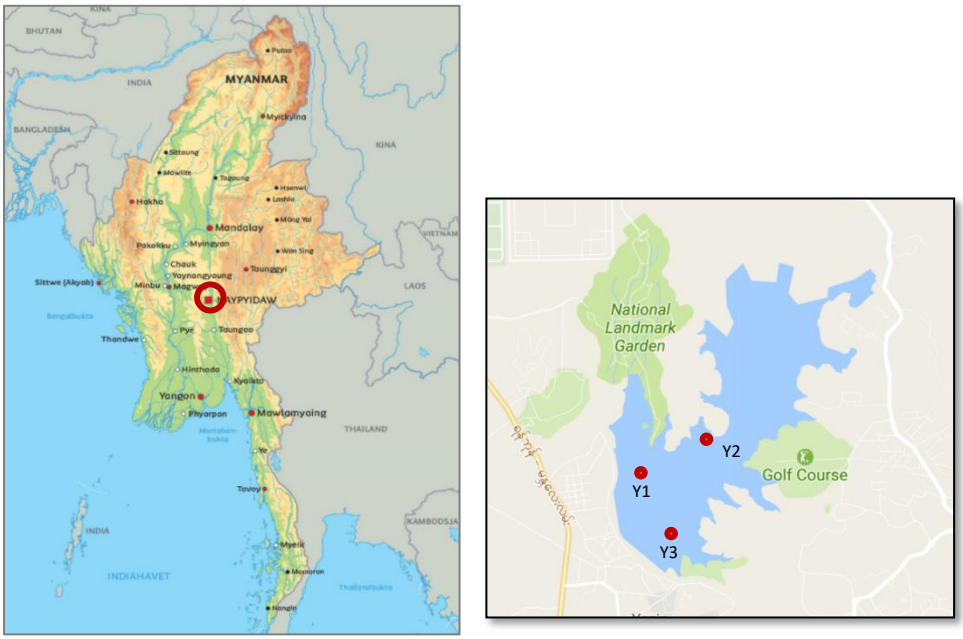

Figure 5. Map of Yezin Dam and Sampling points

Water transparency was measured using a Secchi disk, while in situ measurements of water temperature, pH, conductivity, and dissolved oxygen were taken with a Hach HQd Portable Meter (Hach, Loveland, CO, USA) and Aquaread multiparameter field kit (Model - AP-2000-D/UK). For chemical analyses, integrated water samples were collected from the

trophogenic zone (down to twice the Secchi depth) in 1-meter increments using a Limnos water sampler (Limnos, Komorów, Poland). Samples for nutrient analysis were preserved with 4 M H_2SO_4 to reach a final concentration of 1%, whereas samples intended for turbidity, total alkalinity, color, calcium, and other cation analyses were not preserved. All chemical analyses were conducted at the Water Quality Laboratory of the Forest Research Institute, Yezin, Myanmar.

For phytoplankton analysis, 50 mL subsamples from the integrated water samples were preserved with acidic Lugol's solution for quantitative analysis. For qualitative assessment, concentrated net samples (mesh size 20 µm) were collected and preserved with formaldehyde to a final concentration of 4%. All samples were stored in the dark until analysis. Water samples (50 mL) collected in 2014 for cyanobacterial isolation were kept in a cool, shaded place and gently shaken twice daily until processing at the Norwegian Institute for Water Research (NIVA), Norway.

Lugol-fixed samples were analyzed for phytoplankton composition and biomass using Utermöhl sedimentation chambers (Utermöhl, 1958) and an inverted microscope (Olympus CK2, Olympus, Tokyo, Japan). For cyanobacterial taxa, the length and width of 100 vegetative cells, heterocytes, and akinetes were measured for morphological characterization. Phytoplankton biomass was estimated using geometrical approximations with the computerized counting program Opticount (SequentiX – Digital DNA Processing, Klein Raden, Germany), assuming a specific cell density of 1 g cm^{-3}. Taxa were identified to the genus or species level using standard identification keys (Komárek, 2013, Komárek and Anagnostidis, 1999).

4.2. Isolation of strains and morphological characterization

Single colonies of *Microcystis* and individual filaments of *Dolichospermum* sp. and *Raphidiopsis* sp. were isolated using a microcapillary. Each isolate was washed five times before being transferred into wells of microtiter plates containing 300 µL of Z8 medium (Kotai, 1972). Once growth was established, the cultures were scaled up to 50 mL Erlenmeyer flasks containing 20 mL of Z8 medium and maintained at 22 °C under a photon flux density of 80 µmol photons m^{-2} s^{-1}. Strain classification was based on morphological characteristics following Komárek (2013) and Komárek and Anagnostidis (1999).

The morphology of the isolated strains was examined using a Leica DM2500 light microscope equipped with a Leica DFC 450 camera and Leica Application Suite (LAS) software (Leica, Oslo, Norway). Identification was based on (i) the size of vegetative cells and heterocytes, and (ii) the structure and shape of filaments or colonies. Measurements were taken for 50–250 vegetative cells and 20–50 heterocytes to characterize the isolates.

4.3. Genomic DNA extraction, PCR amplification and sequencing

Genomic DNA from all isolated strains was extracted following the protocol of Ballot et al. (2016). PCR amplification was carried out 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 isolates from Yezin Dam was amplified using primers described by Ballot et al. (2016). PCR products were visualized on a 1% agarose gel stained with GelRed (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) under UV illumination.

Amplified products were purified using Qiagen PCR purification columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing of both strands of each PCR product was performed using the same primers as for PCR, along with intermediate primers following Ballot et al. (2016). Sequencing was conducted on an ABI 3730 Avant

genetic analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, Oslo, Norway), following the manufacturer's protocol.

4.4. Phylogenetic analysis

The detailed procedures for the analysis of the 16S rRNA gene sequences of *Dolichospermum, Raphidiopsis*, and *Microcystis* strains 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) have been previously described in Thida Swe's doctoral thesis (2023), University of South-Eastern Norway (Swe, 2023).

For the *Anabaena/Dolichospermum* phylogenetic analysis, a 16S rRNA gene alignment containing 1,136 positions was used. This dataset included eight sequences from *Dolichospermum* strains isolated from Yezin Dam and 23 additional Dolichospermum sequences obtained from GenBank. *Cuspidothrix issatschenkoi* (FN689797) served as the outgroup. Similarly, a 16S rRNA gene alignment of 1,136 positions was used for the *Raphidiopsis/Cylindrospermopsis* phylogenetic analysis, including five sequences from Yezin Dam isolates and 37 sequences from GenBank, with *Sphaerospermopsis aphanizomenoides* (LN846954) as the outgroup. For Microcystis, a dataset of 1,427 positions was used, comprising two sequences from Yezin Dam isolates and 42 sequences from GenBank, with *Chroococcus subviolaceus* (MF072353) employed as the outgroup.

Phylogenetic trees were constructed using the maximum likelihood (ML) method in MEGA version X with 1,000 bootstrap replicates (Kumar et al., 2018). Evolutionary substitution models were evaluated in MEGA version X, resulting in the best-fitting models: HKY+G+I for the *Anabaena/Dolichospermum* tree, K2+G+I for the *Cylindrospermopsis*/ *Raphidiopsis* tree, and T92+G+I for the Microcystis tree. The sequence data were submitted to the European Nucleotide Archive (ENA), with accession numbers listed in Table 1.

Table1. Cyanobacterial strains isolated from Yezin Dam, strain codes and European Nucleotide Archive (ENA) accession numbers

Species	Strain Code	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	AB2017/14	LR794167
R. raciborskii	AB2017/14	LR794168
R. raciborskii	AB2017/14	LR794169
R. raciborskii	AB2017/14	LR794170
R. raciborskii	AB2017/14	LR794171
Microcystis		
Microcystis	AB2017/14	LR794171
Microcystis	AB2017/15	LR794172

4.5 Toxin analysis

4.5.1 Materials

The standards used for LC–MS analyses included: anatoxin-a (ATX) (Tocris Bioscience, Bristol, UK), homoATX (Novakits, Nantes, France), and cylindrospermopsin (CYN) (Vinci Biochem, Vinci, Italy). Certified reference materials (CRMs) from the National Research Council of Canada (Halifax, Canada) were used for saxitoxin (STX), decarbamoyl STX (dcSTX), NeoSTX, gonyautoxins (GTX1, GTX4, GTX5), N-sulfocarbamoyl gonyautoxins (C1 and C2), MC-RR, MC-LR, and [Dha7] MC-LR. Reference materials (RMs) included [Leu1] MC-LY (LeBlanc et al., 2020), [D-Asp3] MC-RR, [D-Asp3] MC-LR, MC-YR, and MC-HilR, which contained trace amounts of MC-FR, MC-WR, and MC-LA, prepared from commercial samples (Enzo Life Sciences, Farmingdale, NY, USA) and produced at NRC Canada. Additional extracts containing other identified microcystins were obtained from previous studies (Ballot et al., 2020, Mallia et al., 2019, Miles et al., 2014, Yilmaz et al., 2019). Standards for the Adda-ELISA, as well as for the CYN, ATX, and STX ELISAs, were supplied with the respective kits (Abraxis LLC, Warminster, PA, USA).

4.5.2. ELISA for MCs, CYNs, ATXs and STXs

Fresh cultures of *Dolichospermum*, *Microcystis*, and *Raphidiopsis* strains were subjected to three cycles of freezing and thawing. *Raphidiopsis* strains were tested for cylindrospermopsins (CYNs) using the Abraxis Cylindrospermopsin ELISA kit according to the manufacturer's instructions. This direct competitive ELISA detects CYN and also cross-reacts to varying degrees with deoxy-CYN and 7-epi-CYN. *Microcystis* strains were analyzed for microcystins (MCs) using the Abraxis Microcystins/Nodularins (ADDA) ELISA kit, an indirect competitive ELISA that specifically recognizes the Adda moiety (3S-amino-9S-methoxy-2S,6,8S-trimethyl-10-phenyldeca-4E,6E-dienoic acid) of the microcystin molecule (Fischer et al., 2001). Adda is a non-protein amino acid present in approximately 80% of reported microcystin variants (Bouaïcha et al., 2019), and the Adda-ELISA has very low sensitivity for MCs containing modified Adda variants (Foss et al., 2020).

All strains were also screened for saxitoxins (STXs) and anatoxin-a (ATX) analogues using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits. The saxitoxin ELISA is a direct competitive assay that detects STX based on specific antibody recognition but can also cross-react with other saxitoxins (e.g., dcSTX, GTXs, lyngbyatoxin, and NeoSTX). The ATX-a ELISA is a direct competitive assay that detects anatoxin-a, with some cross-reactivity toward homoATX.

In addition, a water sample collected from Yezin Dam in February 2020 and June 2025 was analyzed for STXs, ATXs, CYNs, and MCs using the same ELISA kits. Colorimetric reactions from all ELISA assays were measured at 450 nm using a Perkin Elmer 1420 Multilabel Counter Victor3 (Perkin Elmer, Waltham, MA, USA) for strain samples, and a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA) for field samples.

4.5.3. Microcystin Analysis by LC–HRMS

Fresh cultures of both Microcystis strains were prepared for LC–HRMS analysis by subjecting them to three freeze-thaw cycles, diluting with an equal volume of methanol (MeOH), and filtering through 0.22 μm membranes (Miles et al., 2012). LC–HRMS/MS was carried out on a Q Exactive-HF Orbitrap mass spectrometer equipped with a HESI-II heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA), coupled to an Agilent 1200 LC system with a binary pump, autosampler, and column oven (Agilent, Santa Clara, CA, USA). Chromatographic separation was performed using a SymmetryShield 3.5 μm

C18 column (100 × 2.1 mm; Waters, Milford, MA, USA), following the protocol described by Ballot et al. (2020). Extracts were either derivatized with a 1:1 mixture of mercaptoethanol and d4-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA) or oxidized using DMSO/Oxone, as detailed by Ballot et al. (2020), and subsequently analyzed by LC–HRMS.

4.5.4. CYNs, ATXs, and STXs Analysis by LC–MS/MS

Cyanobacterial toxins were extracted from 40 mL freeze-dried cultures using 6 mL of 50% methanol and sonicated for 10 min in pulsed mode (50%) at 160 W using an Omniruptor4000 probe sonicator (Omni-Inc., Kennesaw, MA, USA) (Cerasino et al., 2017b). The resulting solutions were filtered through 0.2 µm Phenex RC syringe filters (Phenomenex, Castel Maggiore, Italy) and analyzed using a Waters Acquity UPLC system coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore).

Chromatographic separation was achieved on a HILIC column (Ascentis Express OH5, 2.7 µm, 50 × 2.1 mm; Merck Life Science S.r.l., Milan, Italy), while mass spectrometric detection employed positive electrospray ionization with scheduled Multiple Reaction Monitoring (Cerasino et al., 2017a). This method enabled detection and quantification of ATX, CYN, STX, dcSTX, NeoSTX, GTX1, GTX4, GTX5, and C1/C2 toxins, with quantification limits ranging from 0.2 to 200 µg L–1. Other cyanotoxins lacking pure standards were screened tentatively—including hydroxy-, epoxy-, and homo-ATXs, deoxyCYN, dcNeoSTX, GTX2/3, dcGTX2/3, and C3/C4—using the detection settings of their closest analogs.

5. Results and Discussions

5.1. Physical and Chemical Parameters

Water quality measurements in Yezin Dam revealed moderately alkaline conditions, with pH values ranging from 7.2 to 8.7, and temperatures between 27.4 °C and 32.5 °C. Conductivity varied slightly from 77 to 87 µS cm^{-1}, and Secchi depth readings indicated moderate water transparency (1–1.5 m). Total phosphorus (TP) concentrations fluctuated from 20 to 57 µg L^{-1}, reflecting nutrient availability that could support phytoplankton growth.

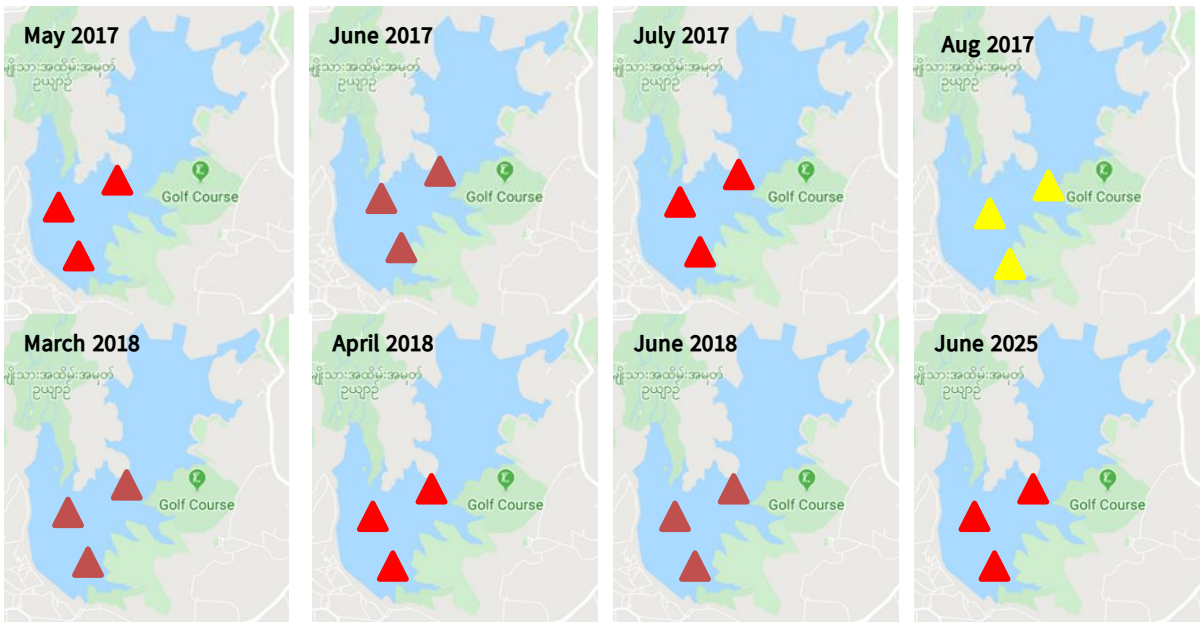

Figure 6. Ecological status of Yezin Dam in 2017-2025 determined using phytoplankton biomass, PTI and cyanbacteria biomass (cyanomax)

In Figure 6, the ecological status of Yezin Dam for 2017 and 2025 is illustrated based on trophic indices and phytoplankton data. Analysis of phytoplankton composition and biomass, along with calculation of the phytoplankton trophic index (PTI), indicates that the dam was generally classified as being in poor to bad ecological status throughout the sampling period, with the exception of August 2017 when it was assessed as moderate. Notably, the 2025 assessment clearly indicates that the measured water quality remained unsatisfactory, reflecting a continued deterioration of ecological conditions in the reservoir.

The evaluation follows the classification system outlined in RAPPORT L.NR. 7301-2018 (Ballot et al., 2018), using the type: lowland, siliceous, clear, deep, where yellow represents moderate status, orange represents poor status, and red represents bad status. This decline in water quality, together with the presence of toxin-producing cyanobacteria, highlights the potential for increased cyanotoxin production during bloom events. The findings underscore the importance of continuous monitoring and the implementation of proactive water management strategies to prevent further ecological deterioration and to safeguard the safety of drinking and irrigation water from Yezin Dam.

5.2. Phytoplankton Community Structure

Analysis of the phytoplankton community across the study period identified 99 taxa spanning 50 genera and 10 classes. Among these, Chlorophyceae (12 genera) and Cyanobacteria (11 genera) were the most diverse, followed by Trebouxiophyceae (8), Zygnematophyceae (6), Bacillariophyceae (4), and Dinophyceae and Euglenophyceae (3 each). Single genera were recorded for Synurophyceae, Cryptophyceae, and Chrysophyceae.

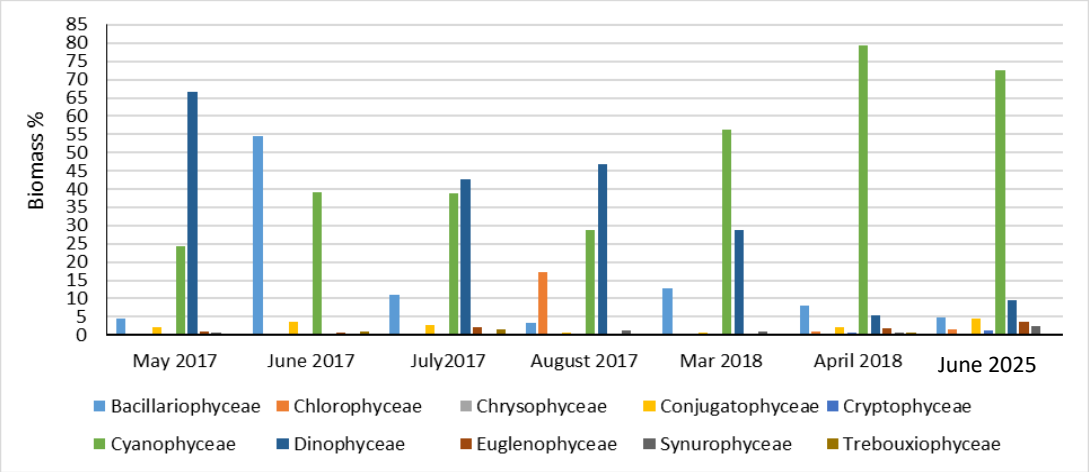

Fig. 7. Temporal variation in phytoplankton biomass in Yezin Dam during the period 2017–2025.

Phytoplankton distribution exhibited notable spatial and temporal variability across the three sampling sites (Fig 7). Cyanobacteria dominated the community throughout the study, comprising 34–86 % of the total biomass, with the highest concentrations recorded at site Y2 in June 2017 and April 2018, and at site Y1 in June 2025. Dinophyceae were temporarily dominant at site Y1 in May, July, and August 2017, whereas diatoms (Bacillariophyceae) peaked at site Y2 in June 2017.

Within the cyanobacterial assemblage, *Dolichospermum* consistently dominated across all sampling sites and periods, followed by *Oscillatoria* spp., *Limnothrix* spp., and *Microcystis*, whereas *Raphidiopsis raciborskii* exhibited comparatively lower biomass throughout the study. This persistent dominance of *Dolichospermum* indicates that environmental conditions in Yezin Dam continue to favor bloom-forming cyanobacteria. The observed community

structure reflects a stable cyanobacterial prevalence within the reservoir and is consistent with the overall trophic status assessment during the study period.

5.3. Environmental Conditions and Phytoplankton Community

Water quality measurements in Yezin Dam indicated moderately alkaline conditions, with pH ranging from 7.2 to 8.7, temperatures between 27.4 °C and 32.5 °C, and conductivity from 77 to 87 $\mu S\ cm^{-1}$. Secchi depth varied between 1 and 1.5 m, reflecting moderate transparency, and total phosphorus (TP) concentrations ranged from 20 to 57 $\mu g\ L^{-1}$, indicating mesotrophic to eutrophic conditions (Salas & Martino, 1991). These nutrient levels likely supported the high phytoplankton and cyanobacterial biomass observed during the study.

A total of 99 phytoplankton taxa, representing 50 genera across 10 classes, were identified. Chlorophyceae (12 genera) and Cyanobacteria (11 genera) were the most diverse, followed by Trebouxiophyceae (8), Zygnematophyceae (6), Bacillariophyceae (4), and Dinophyceae and Euglenophyceae (3 each). Single genera were recorded for Synurophyceae, Cryptophyceae, and Chrysophyceae. Phytoplankton distribution was patchy across the three sampling sites, with cyanobacteria generally dominating (34–86 % of total biomass). Peak cyanobacterial biomass occurred at site Y2 in June 2017 and April 2018, and at site Y1 in March 2018 and June 2025. Dinophyceae dominated temporarily at site Y1 in May, July, and August 2017, whereas Bacillariophyceae peaked at site Y2 in June 2017. These spatial and temporal variations, typical for buoyancy-regulating species such as cyanobacteria and dinoflagellates, complicate direct correlations between nutrient concentrations and biomass (Borics et al., 2011; Breier et al., 2018).

5.4. Cyanobacterial Isolation, Morphology, and Phylogeny

Fifteen potentially toxin-producing cyanobacterial strains were isolated from Yezin Dam (Table 1). Morphological examination identified eight strains as *Dolichospermum smithii* (Komárek) Wacklin, L.Hoffmann & Komárek, five as *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera & al, and two as *Microcystis aeruginosa* (Kützing) Kützing. Due to genetic ambiguity in Microcystis species, all strains were collectively referred to as Microcystis (Harke et al., 2016).

Phylogenetic analyses of the 16S rRNA gene supported these morphological assignments. *Dolichospermum* isolates clustered within a single subcluster of *D. smithii*, showing similarity to sequences from temperate regions including the Czech Republic and Japan (Fig. S1). *Raphidiopsis* strains grouped with sequences of Raphidiopsis and *Cylindrospermopsis* from Asia, Europe, Australia, North America, and previously collected strains from Meiktila Lake, Myanmar. Both *Microcystis* strains clustered with sequences from Europe, Asia, and South America, with 99.72 % sequence similarity. Although *D. smithii* is typically a temperate species, reports from Brazil and Senegal confirm its presence in tropical systems (Berger et al., 2005, Sant'Anna, 1991).

5.5. Cyanotoxin Production

ELISA analysis of a water sample from February 2020 and June 2025 confirmed the presence of both CYNs and MCs in Yezin Dam. Our data indicated that CYN and deoxyCYN production is associated with *R. raciborskii*, whereas MC production is linked to *Microcystis*. Both taxa exhibited relatively low biomass during the study period, and the coexistence of toxin-producing and non-toxin-producing strains explains the low toxin concentrations.

Raphidiopsis strains from Yezin Dam produced CYN and deoxyCYN in amounts similar to strains from Meiktila Lake (2.9–9.8 $\mu g\ mg^{-1}$ FW) (Ballot et al., 2020). Australian strains produce up to 3.5 $\mu g\ mg^{-1}$ FW of intracellular CYNs (Saker & Griffiths, 2000), and production has also been reported in lakes of China, Japan, Thailand, and Vietnam

(Chonudomkul et al., 2004, Hawkins et al., 1997, Nguyen et al., 2017). Brazilian strains produce saxitoxins but not CYNs (Hoff-Risseti et al., 2013), and evidence from Europe is limited (Đorđević et al., 2015).

Among *Microcystis* strains, only AB2017/08 produced MCs, with 22 congeners detected by LC–HRMS/MS totaling 1160 µg g^{-1} FW, dominated by MC-LR and [D-Asp³]MC-LR (76.9 % and 15.7 %, respectively. The MC profile differs from that of strains from Hartbeespoort Dam, South Africa, and Meiktila Lake, illustrating strong strain-specific variation (Ballot et al., 2014, 2020).

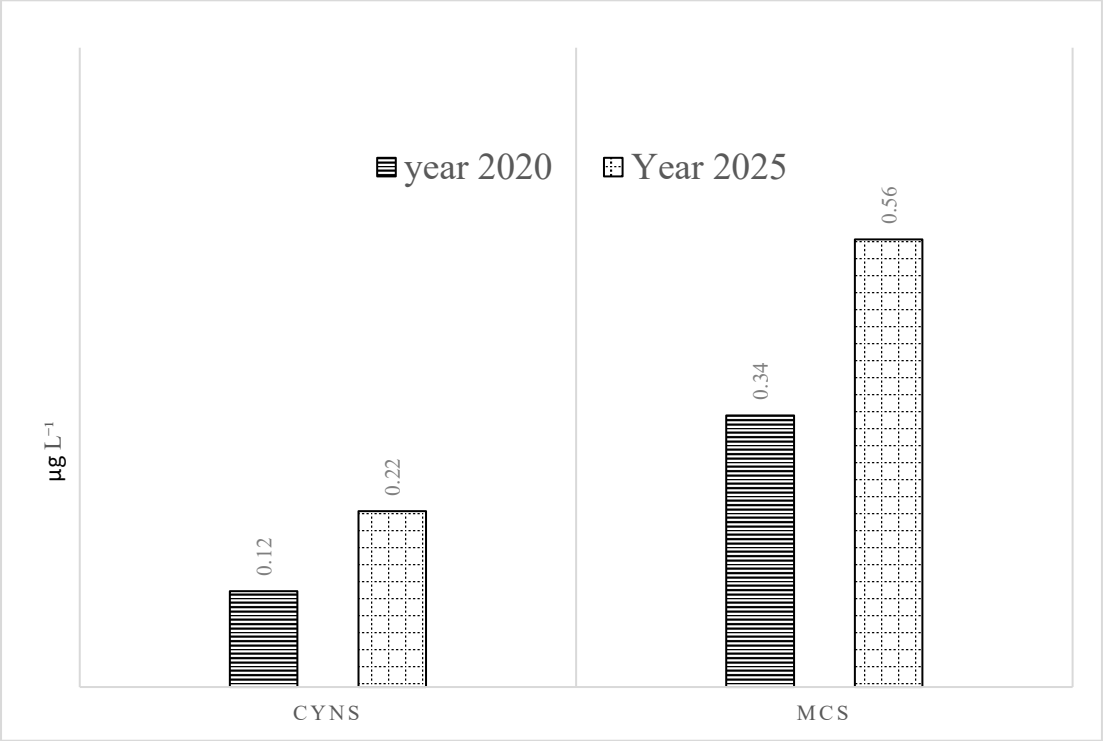

Fig. 8. Cyanotoxins (CYNs, MCs) occurrence in Yezin Dam between 2020 and 2025.

Measured CYN (0.12 µg L^{-1}) and MC (0.34 µg L^{-1}) concentrations in 2020 were well below the WHO provisional short-term guideline values (3 µg L^{-1} for CYNs and 12 µg L^{-1} for MCs) and lifetime guideline values (0.7 µg L^{-1} for CYNs and 1 µg L^{-1} for MC-LR) (WHO, 2020a, 2020b). Although the concentrations measured in 2025 (CYN: 0.22 µg L^{-1}; MC: 0.56 µg L^{-1}) were higher than those recorded in 2020, they remained below both the short-term and lifetime WHO guideline thresholds. Nevertheless, the observed upward trend highlights the importance of continuous monitoring and regular risk assessment to ensure the long-term safety of drinking water and to support proactive water resource management.

Importantly, cyanobacterial blooms dominated by toxic strains could rapidly lead to toxin concentrations exceeding these guideline values. While deoxyCYN is considered slightly less toxic than CYN, both compounds share the same mode of action (Neumann et al., 2007, WHO, 2020a). Similarly, MC toxicity assessments are primarily based on MC-LR, although other congeners may vary substantially in potency (Bouaïcha et al., 2019). Therefore, potential shifts in species composition—particularly from currently non-toxic Dolichospermum to toxin-producing Raphidiopsis or Microcystis—could result in a marked increase in cyanotoxin levels, posing greater risks to public health and water supply safety.

6. Conclusions

This study provides the comprehensive evidence of the occurrence of cyanobacteria and cyanotoxins in Yezin Dam, Myanmar, based on monitoring data collected between 2014 and 2025. Although measured CYN and MC concentrations in both 2020 and 2025 remained below WHO health-based guideline values, the higher concentrations observed in 2025 indicate a gradual upward trend. While current levels do not pose an immediate health concern under existing international standards, the increasing pattern warrants careful attention.

Importantly, the potential for rapid bloom development dominated by toxin-producing strains represents a significant future risk. A possible shift in species composition from currently non-toxic Dolichospermum to toxin-producing Raphidiopsis or Microcystis could substantially elevate cyanotoxin concentrations. Furthermore, considering that different toxin congeners vary in potency and that compounds such as CYN and deoxyCYN share similar toxic mechanisms, precautionary management is justified even when concentrations remain below guideline thresholds.

Given that Yezin Dam serves as an important source of drinking water and irrigation, these findings underscore the need to institutionalize routine cyanotoxin surveillance within Myanmar's national water safety framework. Integrating systematic long-term monitoring, early warning systems for bloom development, and periodic environmental and health risk assessments into the country's integrated water resources management plan will strengthen preventive capacity, safeguard public health, and ensure the sustainable use of water resources under changing environmental conditions.

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