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Diversity of Cyanobacteria and Cyanotoxins in Meikhtila Lake and Yezin Dam

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Diversity of Cyanobacteria and Cyanotoxins in Meikhtila and Yezin Reservoirs

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Abstract

The water from Meiktila and Yezin reservoirs are used for irrigation, domestic purposes and drinking water. Increased pollution due to the development activities with population increases and other factors around the reservoirs has led to the potential for occurrence of more frequent severe cyanobacterial blooms, which would have a negative impact on the use of the reservoirs' water by the residents. In order to provide insight into the extent of cyanobacteria (harmful blue green algae) and cyanotoxins in both reservoirs, water samples were collected between March 2017 and March 2020.Seven potentially toxin-producing cyanobacteria strains from Meiktila Lake (**I**) and 15 strains from Yezin Dam (**II**) were isolated and identified based on morphology and molecular phylogeny using 16S rRNA gene sequences. Phylogenetic analyses using maximum likelihood (ML) trees of 16S rRNA gene confirmed the morphological determination of the isolated *Raphidiopsis* (Woloszynska) Aguilera, Berrendero Go´mez, Kastovsky, Echenique & Salerno 2018, *Microcystis* and *Dolichospermum smithii* (Komárek) Wacklin, L. Hoffmann & Komárek 2009 strains. In the cyanobacterial community of Meiktila Lake, *Raciborskii raciborskii* was the most dominant species while *Dolichospermum* sp. was the most dominant cyanobacterium and found only in Yezin Dam. This study confirmed the presence of toxin producing cyanobacteria (*Microcystis* sp. and *Raphidiopsis* sp.) in both reservoirs by ELISA and LC-MS/MS analysis. The potential, therefore, to cause harmful effects on humans, domestic and wild animals exists for the use of undreated water from Meiktila and Yezin Reservoir. Due to the presence of CYN- and MC-producing cyanobacteria, harmful effects on humans, domestic and wild animals cannot be excluded in these water bodies. As Meiktila and Yezin reservoirs are 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.

Keywords: *Raphidiopsis, Microcystis, Dolichospermum, Raciborskii raciborskii, Dolichospermum* sp, cyanobacterial blooms

မိတ္ထီလာကန်နှင့် ရေဆင်းဆည်တို့အတွင်း စိမ်းပြာရေညှိမျိုးကွဲများနှင့် အဆိပ်အတောက် ပါဝင်မှုအား လေ့လာခြင်း

ဒေါ်သီတာဆွေ၊ လက်ထောက်သုတေသနအရာရှိ
သစ်တောသုတေသနဌာန

စာတမ်းအကျဉ်း

မိတ္ထီလာကန်နှင့် ရေဆင်းဆည်တို့မှ ရေကို စိုက်ပျိုးရေ၊ နေစဉ်သုံးရေနှင့် သောက်သုံးရေတို့အဖြစ်သုံးစွဲလျက်ရှိပါသည်။ အဆိုပါကန်တို့၏ ရေဝေရေလဲဧရိယာအတွင်း လူဦးရေတိုးပွားလာသည်နှင့်အမျှ ဖွံ့ဖြိုးတိုးတက်ရေးလုပ်ငန်းစဉ်များနှင့် အခြားအကြောင်းအမျိုးမျိုးတို့ကြောင့် ကန်ရေများ ညစ်ညမ်းလာခြင်းသည် စိမ်းပြာရေညှိမျိုးများတိုးပွားလာခြင်းကိုဖြစ်စေနိုင်သဖြင့် ကန်ရေကိုမှီခိုသုံးစွဲနေသည့် ဒေသခံများအပေါ်ဆိုးကျိုး သက်ရောက်မှုများဖြစ်ပေါ်စေနိုင်ပါသည်။ သို့ဖြစ်ပါ၍ ကန်တို့အတွင်းအဆိပ်အတောက်ထုတ်လွှတ်နိုင်သည့် စိမ်းပြာရေညှိမျိုးများနှင့်အဆိပ်အတောက်ပါဝင်နေမှုကို သိရှိစေနိုင်ရန် **မိတ္ထီလာ**ကန်နှင့် ရေဆင်းဆည်တို့အတွင်းရေအရည်အသွေးဆန်းစစ်လေ့လာခြင်းကို ၂၀၁၇ ခုနှစ် မတ်လ မှ ၂၀၂၀ မတ်လအထိဆောင်ရွက်ခဲ့ပါသည်။ **မိတ္ထီလာ**ကန်နှင့် ရေဆင်းဆည်တို့မှ အဆိပ်အတောက်ထုတ်လွှတ်ရန် အလားအလာ ရှိသည့် စိမ်းပြာရေညှိ မျိုးစိတ် ၇ မျိုးနှင့် ၁၅ မျိုးတို့အား မွေးမြူ၍ အဆိုပါမျိုးစိတ်များကို မိရိုးဖလာရုပ်သွင်ပြင်ကို အခြေခံသည့်နည်းစနစ်နှင့် မျိုးရိုးဗီဇ နည်းစနစ် (phylogenetic) ကို အသုံးပြု၍မျိုးခွဲခြင်း (identification)များဆောင်ရွက်ခဲ့ပါသည်။ မိရိုးဖလာနည်းစဉ်ဖြင့် မျိုးစိတ်များကို မျိုးခွဲရာတွင် ရေဆင်းဆည်မှ မျိုးစိတ် ၈ ခုကို *Dolichospermum smithii* (Komárek) Wacklin, L. Hoffmann & Komárek 2009 ဟုလည်းကောင်း၊ **မိတ္ထီလာ**ကန်မှ ၅ ခုနှင့် ရေဆင်းဆည်မှ ၅ ခုကို *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Go´mez, Kastovsky, Echenique & Salerno 2018 ဟုလည်းကောင်း၊ **မိတ္ထီလာ**ကန်မှ မျိုးစိတ် ၂ ခုကို *Microcystis aeruginosa* (Kützing) Kützing 1846 နှင့် *Microcystis novacekii* (Komárek–Compere, 1974) ဟုလည်းကောင်း၊ ရေဆင်းဆည်မှ မျိုးစိတ် ၂ ခုကို *Microcystis aeruginosa* (Kützing) Kützing 1846 ဟု လည်းကောင်း ခွဲခြားတွေ့ရှိရပါသည်။ မိရိုးဖလာ နည်းစဉ်ဖြင့် မျိုးစိတ်များ၏အမည်ခွဲခြင်းနှင့် အဆိုပါမျိုးစိတ် များ၏ 16S rRNA ဖြင့် မျိုးရိုးဗီဇနည်းစဉ်ဖြင့် အမည်ခွဲခြင်းတို့တူညီကြောင်းတွေ့ရှိရပါသည်။ မျိုးခွဲတွေ့ရှိချက်အရ **မိတ္ထီလာ**ကန်တွင် *Raphidiopsis raciborskii* နှင့် ရေဆင်းဆည်တွင် *Dolichospermum* စိမ်းပြာရေညှိမျိုးများအပေါများဆုံး ပေါက်ဖွားလျက်ရှိကြောင်းတွေ့ရှိရပါသည်။ ELISA နှင့် LC–MS–MS

တို့ဖြင့် တိုင်းတာချက်အရ **မိတ္ထီလာ**ကန်နှင့် ရေဆင်းဆည်တို့ တွင် *Microcystis* နှင့် *Raphidiopsis* မျိုးစိတ်တို့တွင် အသဲ၊ ကျောက်ကပ်တို့ကို ပျက်စီး စေနိုင်သည့် Cylindrospermopsin (CYN) နှင့် Microcystin (MC) အဆိပ်များ ထုတ်လွှတ် ပေးသော စိမ်းပြာရေညှိမျိုးများဖြစ်ကြောင်း တွေ့ရှိရပါသည်။ အဆိုပါ စိမ်းပြာရေညှိမျိုးများ ပေါက်ဖွား နေသည့် **မိတ္ထီလာ**ကန်နှင့်ရေဆင်းဆည်တို့၏ ရေကိုသန့်စင်ခြင်းမရှိပဲအသုံးပြုခြင်း သည် လူနှင့်တိရစ္ဆာန်တို့ကို ဆိုးကျိုးသက်ရောက်စေနိုင်ပါသည်။ သို့ဖြစ်ပါ၍ **မိတ္ထီလာ**ကန်နှင့် ရေဆင်းဆည်တို့သည် စိုက်ပျိုးရေနှင့်သောက်သုံးရေရရှိနိုင်သောအဓိကအရေးပါသည့် အရင်း အမြစ်များဖြစ်ခြင်းကြောင် နိုင်ငံ၏ရေသယံဇာတဘက်စုံစီမံအုပ်ချုပ်ခြင်း လုပ်ငန်းစဉ်တွင် စိမ်းပြာရေညှိပေါက်ဖွားမှုနှင့် ၎င်းတို့မှထွက်ရှိသည့် အဆိပ်အတောက်များ ပါဝင်မှုကိုစောင့် ကြည့်လေ့လာခြင်းလုပ်ငန်းသည် အရေးကြီးသည့် လုပ်ငန်းစဉ်တစ်ရပ်အဖြစ်ထည့်သွင်းစဉ်း စားသင့်ပါကြောင်း အကြံပြုတင်ပြအပ်ပါသည်။

Diversity of Cyanobacteria and Cyanotoxins in Meikhtila and Yezin Reservoirs

1. Introduction

Many lakes and reservoirs worldwide are affected by periodic cyanobacteria dominance or even cyanobacterial blooms. Such mass development of cyanobacteria are typical for eutrophic condition and are often induced by nutrient enrichment caused by increased agricultural, urban and industrial activities and are also expected to increase due to regional and global climate change (Paerl & Huisman, 2009). Various cyanobacterial species forming such blooms are potential producers or heptotoxic or neurotoxic compounds and their presence is often associated with animal poisonings and a threat to human health (Sivonen, 2009).

Myanmar is characterized by the presence of several natural lakes and numerous man-made reservoirs. Availability of freshwater, however, varies regionally as well as seasonally. 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.

Meiktila Lake is one of the numerous reservoirs in Myanmar and is exposed to sedimentation due to deforestation in the catchment and especially northern part has been partially filled with sediment over a period of more than 100 years (Klink et al., 2015). The priority use of water from Meiktila Lake is drinking water, water for domestic purposes and for irrigation, although the lake is also polluted with domestic waste water, street runoff and solid waste (Klink et al., 2015, Aung, 2015). The ongoing pollution of the lake suggests the potential for occurrence of more frequent severe cyanobacterial blooms, which would have a negative impact on the use of the lake for drinking water and domestic purpose by the residents.

Yezin Dam is a man-made earth embarkment reservoir. 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, 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.

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). However, the knowledge about cyanobacteria and their toxins in lakes and reservoirs in Myanmar is poor.

2. Literature Review

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, Sivonen and Jones, 1999) and according to their chemical structure such as cyclic peptides, alkaloids and lipopolysaccharides (LPS) (Chorus and Welker, 2021, Chorus and Bartram, 2005, Codd et al., 1999, Chorus and Bartram, 1999, Carmichael, 1992).

Microcystins (MCs)

The microcystins (MCs) are cyclic oligopeptides and these toxins were named "Microcystins (MCs)" as they are first isolated from *Microbystis 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).

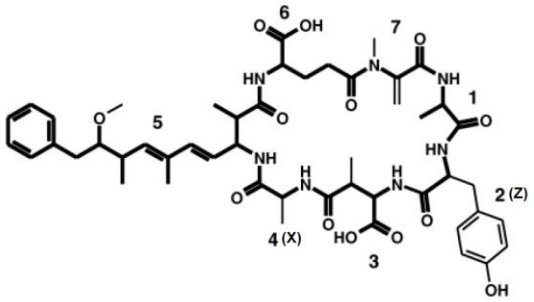

Figure 1. General structure of microcystin (Tillett et al., 2000), the variable L-amino acid residues at position 2 and 4 are indicated by X and Z, respectively

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, Hawkins et al., 1997, Byth, 1980). CYNs can be produced by other cyanobacteria such as *Aphanizomenon, Chrysosporum, Dolichospermum (Anabaena), Lyngbya, Rhaphidiopsis, Oscillatoria,* and *Umezakia* (Akcaalan et al., 2014, Cirés et al., 2014, McGregor et al., 2011, Mazmouz et al., 2010, Brient et al., 2009, Seifert et al., 2007, Preußel et al., 2006, Li et al., 2001, Schembri et al., 2001). 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, Banker et al., 2000, Norris et al., 1999). CYN primarily affects the kidneys, lungs, heart, thymus, spleen, and intestine in mammals (Falconer et al., 1999, Hawkins et al., 1997).

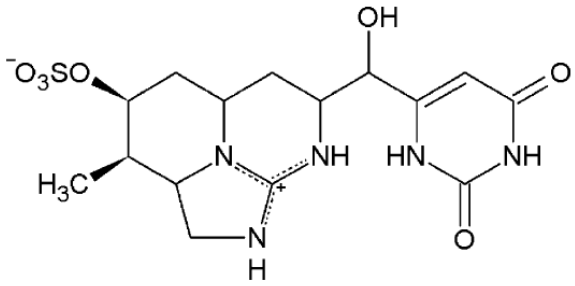

Figure 2. structure of Cylindrospermopsins (CYNs) (Žegura et al., 2011)

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).

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, Harland et al., 2014, Hodoki et al., 2013, Ballot et al., 2010b, Osswald et al., 2009, Cadel-Six et al., 2007a, Selwood et al., 2007, Wood et al., 2007a, Ballot et al., 2005, Gugger et al. 2005, Aráoz et al., 2005, Ballot et al., 2004,)

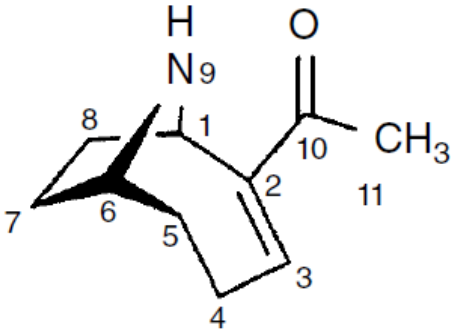

Figure (3) the structure of anatoxin-a (ATX-a) (Bruno et al., 2017)

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, Smith et al., 2012, Soto-Liebe et al., 2012, Smith et al., 2011, Ledreux et al., 2010, Ballot et al., 2010a, Yunes et al., 2009)

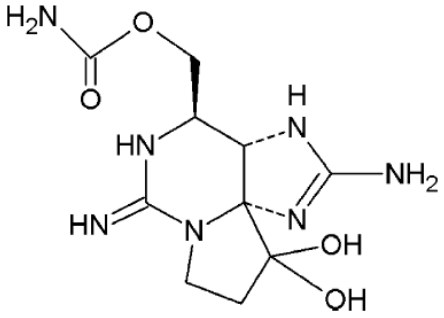

Figure (4) the structure of saxitoxins (Žegura et al., 2011)

3. Objectives

The main objectives of this study are;

- To add new information by investigating the presence of cyanobacteria and their potential toxins in Meiktila Lake and Yezin Dam and in cyanobacterial strains isolated from these waterbodies
- To reveal in detail the cyanobacterial compositions, phylogeny, toxin production and toxin profiles

4. Materials and Methods

4.1 Study areas, sampling methods and analyses

Meiktila Lake is a shallow reservoir, located close to Meiktila city in central Myanmar in the Mandalay region at an altitude of 230 m. It was built in ancient times, dating from an unknown period (Harvey, 1925) but most likely in the reign of King Narapathisithu (1173-1210) (Nyunt, 1995). Today the lake is divided by a dam into a northern and a southern part (Hlaing, 2014). Depending on the season, Meiktila Lake covers an area of around 54 km^2 with a maximum water depth of 10 m. Five sampling points were selected, three in the northern part of Meiktila Lake and two in the southern part (Figure 1-A).

Yezin Dam is a man-made reservoir located in the Zeyar Thiri Township, Nay Pyi Taw. 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-B). 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 once in February 2020 to test for anatoxins, cylindrospermopsisns, microcystins and saxitoxins using exzyme-linket immunosorbent assay (ELISA).

At all sampling points in both reservoirs, water transparency was measured with a Secchi disk. In situ measurements of water temperature, pH, conductivity and dissolved oxygen were conducted, and integrated water samples were taken for the analysis of chemical parameters, phytoplankton composition and biomass and for the isolation of cyanobacterial strains. For quantitative phytoplankton analysis, a 50 ml subsample was removed from a sample taken from integrated samples and preserved with Lugol's solution and a concentrated net sample (mesh size 20 µm) was taken and preserved by addition of formaldehyde (4% final concentration) for qualitative analysis. A 50 ml water sample for isolation of cyanobacteria was taken at each sampling point and kept in a cool shady place and gently shaken twice per day before further treatment in Norway.

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 gcm^{-3}. The taxa were identified to species or genus level using selected identification keys (e.g. Kom´arek, 2013; Kom´arek and Anagnostidis, 1999).

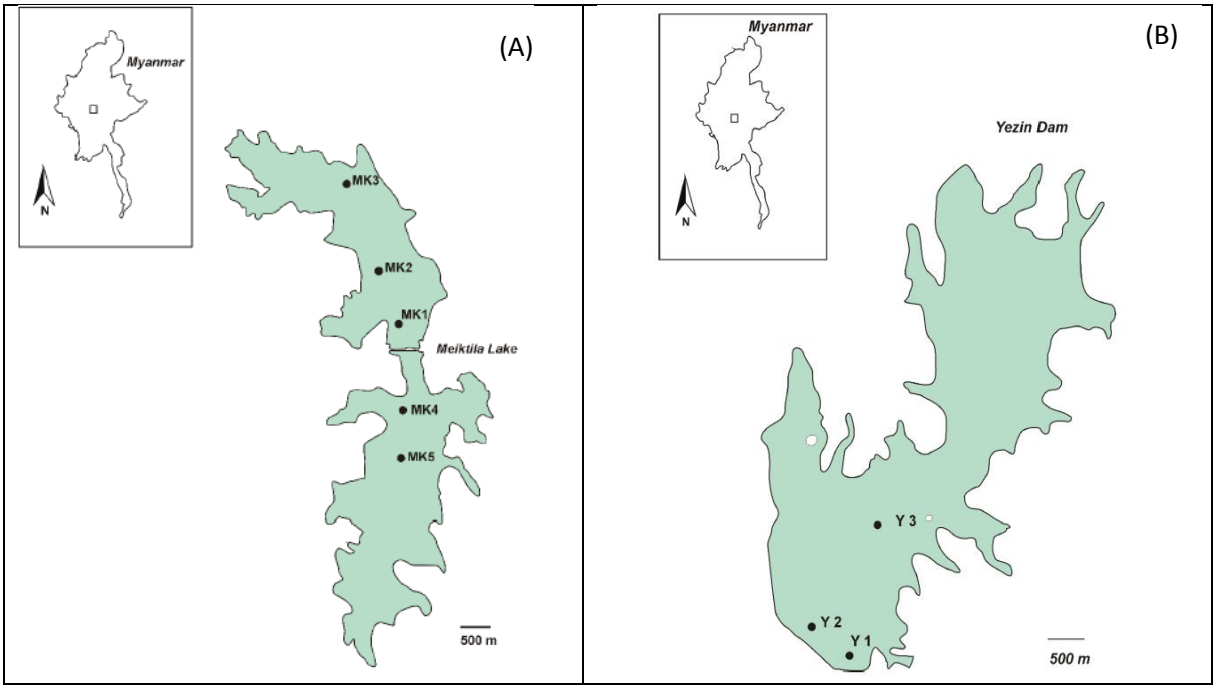

Figure 1. Maps of (A) Meiktila Lake and (B) Yezin Dam. The maps show the locations of water sampling; stations MK1 – MK5 in Meiktila Lake and stations Y1 – Y3 in Yezin Dam. The locations of Meiktila Lake and Yezin Dam are shown in the inset.

4.2 Isolation of strains and Morphological Characterization

Using a microcapillary, filaments of Dolichospermum from Yezin Dam and single colonies of *Microcystis* and *Raphidiopsis* from Meiktila Lake and Yezin Dam were isolated. 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 filament 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.

The isolated potentially toxin producing cyanobacterial strains from Meiktila Lake and Yezin Dam were as described in Table 1.

Table 1. Cyanobacteria species and strains isolated from Meiktila Lakes and Yezin Dam

Species	Strain Code	Lake/Reservoir
Dolichospermum		
D. smithii	AB2017/01	Yezin Dam
D. smithii	AB2017/02	Yezin Dam
D. smithii	AB2017/03	Yezin Dam
D. smithii	AB2017/04	Yezin Dam
D. smithii	AB2017/05	Yezin Dam
D. smithii	AB2017/06	Yezin Dam
D. smithii	AB2017/17	Yezin Dam
D. smithii	AB2017/18	Yezin Dam
Raphidiopsis		
R. raciborskii	AB2017/05	Meiktila Lake

Species	Strain Code	Lake/Reservoir
R. raciborskii	AB2017/09	Meiktila Lake
R. raciborskii	AB2017/12	Meiktila Lake
R. raciborskii	AB2017/13	Meiktila Lake
R. raciborskii	AB2017/16	Meiktila Lake
R. raciborskii	AB2017/14	Yezin Dam
R. raciborskii	AB2017/14	Yezin Dam
R. raciborskii	AB2017/14	Yezin Dam
R. raciborskii	AB2017/14	Yezin Dam
R. raciborskii	AB2017/14	Yezin Dam
Microcystis		
Microcystis	AB2017/14	Meiktila Lake
Microcystis	AB2017/15	Meiktila Lake
Microcystis	AB2017/14	Yezin Dam
Microcystis	AB2017/15	Yezin Dam

4.3 Toxin analysis

4.3.1. 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); certifie reference materials (CRMs) of saxitoxin (STX), decarbamoylSTX (dcSTX), NeoSTX, gonyautoxins (GTX1, GTX4, GTX5), N-sulfocarba [Dha7] moylgonyautoxins (C1 and C2 toxins), MC-RR, MC-LR (10) and MC-LR (11) (National Research Council of Canada, Halifax, Canada); [Leu1 reference materials (RMs) of]MC-LY (LeBlanc et al., 2020), and of [D-Asp3] MC-RR, [D-Asp3]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 (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).

4.3.2. 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 Microcystis strains were tested for MCs using the Abraxis Microcystins/Nodularins (ADDA) ELISA kit. All strains were also tested for saxitoxins and anatoxin-a analogues using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits.

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).

4.3.3. 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 d4-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA), or oxidized with DMSO/Oxone, as described by Ballot et al. (2020) and analyzed by LC-HRMS.

5. Results and Discussions

5.1 Physical and Chemical Parameters

Meiktila lake is moderate alkaline and turbid; the southern basin is more turbid than the northern basin. Yezin Dam is a low to moderate alkaline and turbid reservoir with large water level fluctuation. Meiktila Lake is characterized by the average TP values of less than 20 μgL^{-1} while higher TP values between 20 – 57 µg L^{-1} were also observed in Yezin Dam during our study period.

5.2. Cyanobacteria composition

Thirteen phytoplankton groups were recorded at Meiktila Lake while 10 classes at Yezin Dam. The cyanobacteria were common phytoplankton groups in both reservoirs. The other phytoplankton groups were Bacillariophyceae, Cryptophyceae, Chlorophyceae and Euglenophyceae.

Among the cyanobacterium groups, *Raphidiopsis raciborskii* was the most dominant, which comprised biomasses between 0.2 and 1.9 mgL^{-1} fresh weight (FW), or 27% - 91 % of the total cyanobacterial biomass at the sampling points MK1, MK2, MK4 and MK5 in Meiktila Lake. The *Microcystis* biomass was lower than the *Raphidiopsis* biomass at all sampling points and sampling dates and ranged from 0.003 to 0.16 mg L^{-1} (data not shown).

In Yezin Dam, *Dolichospermum* sp. was the dominating taxon at all sampling stations throughout the sampling period, followed by *Oscillatoria* sp., *Limnothrix* sp. and *Microcystis*. The lowest biomass of *R. raciborskii* was observed at all sampling points and sampling dates.

The dominance of cyanobacteria in the phytoplankton community in both reservoirs indicates the eutrophic condition of these water bodies (Ke et al., 2009, Zhang and Zang, 2015). The presence of R. raciborskii has never been documented from Myanmar water bodies before this study, although it has been shown in other subtropical and tropical Southeast Asian freshwater habitats and other temperate water bodies worldwide (Nguyen et al. 2017, Antunes et al. 2015, Dao et al. 2010, Li et al. 2001).

The highest biomass of *Dolichospermum smithii* was 6.7 mgL^{-1}. This species was recorded only in Yezin Dam and was not observed in Meiktila Lake and other water bodies such as Inlay Lake, Indawgyi Lake, Taungthaman Inn, Kyet Mauk Taung Reservoir, Ngalaik Reservoir. The occurrence of *D. smithii* has been reported from mesotrophic to slightly eutrophic ponds, reservoirs and lakes in temperate areas but also in tropical Brazil and Senegal (Komárek, 2013, Berger et al. 2005, Sant'Anna, 1991). Low amounts of *Microcystis* were found in both studied areas. The presence of *Microcystis* has described from only a few water bodies in Myanmar, however, it is described from many Asian countries (Naw et al., 2020, Harke et al., 2016, Mowe 2015, Green 2011).

The higher TP concentrations demonstrated that Yezin Dam is in a mesotrophic to eutrophic status (Salas and Martino, 1991) and explain the high phytoplankton and cyanobacterial biomasses.

5.3 Morphological Characterization on isolated cyanobacterial strains

Seven and fifteen potentially toxin-producing cyanobacteria strains from Meiktila Lake and Yezin Dam were isolated and identified based on morphology and molecular phylogeny using 16S rRNA gene sequences.

On the basis of morphological features e.g., presence and form of colonies or filaments, vegetative cells and heterocytes, eight of the isolated strains from Yezin Dam were identified as *Dolichospermum smithii* (Komárek) Wacklin, L. Hoffmann & Komárek 2009. Five strains from Meiktila Lake and five strains from Yezin Dam were identified as *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Go´mez, Kastovsky, Echenique & Salerno 2018. Two strains from Meiktila Lake were identified as *Microcystis aeruginosa* (Kützing) Kützing 1846 and *Microcystis novacekii* (Komárek-Compere, 1974) while two strains from Yezin Dam were identified as *M. aeruginosa* (Kützing) Kützing 1846 respectively. As Harke et al. (2016) suggested that all *Microcystis* warrant placement into the same species, "*Microcystis*" is used in the following parts of this paper instead of species names.

5.4 Identification of cyanobacterial toxins and toxin producing strains

Dolichospermum smithii was the dominant taxon in the phytoplankton communities of Yezin Dam. However, all of these strains did not produce any toxins confirmed by ELISA. This agrees with other studies worldwide that no strains of *D. smithii* produce any cyanobacterial toxins such as MCs, STXs, ATXs or CYNs.

According to ELISA analysis, three of four *Raphidiopsis* strains from Meiktila Lake and four of five from Yezin Dam were shown to produce variable amount of CYNs. However, all of these *Raphidiopsis* strains did not produce STXs, ATXs or MCs. The concentrations of CYNs in the *Raphidiopsis* strains from Meiktila Lake ranged from 1.84 to 4.3 $\mu g\ mg^{-1}$ while from Yezin Dam varied between 1.84 $\mu g\ mg^{-1}$ and 3.5 $\mu g\ mg^{-1}$.

According to LC-MS/MS analysis, these CYNs producing strains produced two variants; CYN and deoxy-CYN. The CYNs and deoxy-CYN concentrations of *Raphidiopsis* strains from Meiktila Lake varied between 1.7 and 2.5 $\mu g\ mg^{-1}$ FW and 1.3 – 7.3 $\mu g\ mg^{-1}$ FW, respecitively while that of *Raphidiopsis* strains from Yezin Dam were between 0.17 and 1.03 $\mu g\ mg^{-1}$ FW and 0.11 – 2.19 $\mu g\ mg^{-1}$ FW (Table 2).

Table 2. Cylindrospermopsin (CYN) and deoxycylindrospermopsin (deoxyCYN) concentrations in cultured *Raphidiopsis raciborskii* strains isolated from Meiktila Lake and Yezin Dam

Lake/ Reservoir	**Strains**	**ELISA**	**LC-MS/MS**			
		µg CYN/mg FW	**µg CYN/mg FW**	**µg deoxyCYN /mg FW**	**CYNs %[#]**	**deoxyCYNs %**
Meiktila	AB2017/09	2.18	-*	-*	38	62
	AB2017/05	n.d.	n.d.	n.d.	n.d.	n.d.
	AB2017/16	1.84	1.65	1.25	57	43
	AB2017/12	n.d.	n.d.	n.d.	n.d.	n.d.
	AB2017/13	4.31	2.46	7.29	25	75
Yezin	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.
	AB2017/19	3.18	0.75	1.84	29	71
	AB2017/20	1.86	1.03	2.19	32	68

*=biomass not determined, n.d. = not detected, FW = fresh weight

[#]=Percentages are % of total CYNs measured by LC-MS/MS.

Two *Microcystis* strains from Meiktila Lake and one *Microcystis* strains from Yezin Dam produce MCs as determined by ELISA. However, none of these strains produced CYNs, STXs, or ATXs. The highest MC concentration of 14000 µg g^{-1} FW was detected in one *Microcystis* strain from Meiktila Lake. The MC concentrations of the other one *Microcystis* strains from Meiktila Lake and the strain from Yezin Dam were 1100 µg g^{-1} FW and 1160 µg g^{-1}, which were in the same range as described for a *Microcystis* strains from Hartbeespoort Dam from South Africa (Ballot et al., 2014).

Table 3. Identities of microcystins detected by LC-HRMS/MS analysis in *Microcystis* strains AB2017/14 and /15 isolated from Meiktila Lake, their retention times (t_R), concentrations, relative abundances (%), and observed *m*/*z* values in negative ionisation mode

					Concentration[a]			
					AB2017/14		AB2017/15	
	m/*z*	Compound name	Confidence	ʀ (min)	µg g^{-1}	%	µg g^{-1}	%
1	1022.5443	[D-Asp[3]]MC-RR[b]	Confirmed	4.18	2.4	0.21	1002.4	7.21
2	1022.5454	[Dha[7]]MC-RR[b]	Probable	4.53	0.1	0.01	31.8	0.23
3	1036.5597	MC-RR[b]	Confirmed	4.55	19.4	1.74	1458.0	10.49
4	995.4858	[D-Asp[3]]MC-ER[b]	Probable	6.14	ND	—	119.6	0.86
5	1114.5657	MC-LR–Cys[c]	Probable	6.16	0.1	0.01	ND	—
6	1045.5378	MC-(H2)YR[b]	Probable	6.18	32.4	2.90	ND	—
7	1031.5224	[D-Asp[3]]MC-(H2)YR[b]	Probable	6.20	32.9	2.95	ND	—
8	1033.5381	[D-Asp[3]]MC-(H4)YR[b]	Probable	6.27	0.6	0.05	148.1	1.07
9	1009.5015	MC-ER[b]	Probable	6.55	0.6	0.05	37.8	0.27
10	1029.5072	[D-Asp[3]]MC-YR[b]	Probable	6.59	ND	—	144.1	1.04
11	1047.5540	MC-(H4)YR[b]	Probable	6.64	6.4	0.57	310.8	2.24
12	1011.5530	[Mser[7]]MC-LR	Probable	6.80	0.4	0.04	13.3	0.10
13	979.5273	[D-Asp[3]]MC-LR[b]	Confirmed	6.89	40.8	3.65	1870.3	13.45
14	1043.5224	MC-YR[b]	Confirmed	6.99	9.1	0.81	317.4	2.28
15	1011.4999	MC-MR[b,c,d]	Probable	6.99	3.6	0.32	ND	—
16	1073.5329	MC-Y(OMe)R[b]	Probable	7.07	7.2	0.64	ND	—
17	993.5435	MC-LR[b]	Confirmed	7.12	183.0	16.38	3332.5	23.97
18	979.5289	[Dha[7]]MC-LR[b]	Confirmed	7.13	0.8	0.07	37.4	0.27

19	1080.5170	Unidentified MC[b]	Unidentified	7.15	59.0	5.28	ND	—
20	1070.5332	MC-KynR[b]	Probable	7.35	4.5	0.40	ND	—
21	1007.5582	MC-HilR[b]	Confirmed	7.38	41.8	3.74	17.9	0.13
22	1080.5169	Unidentified[b]	Unidentified	7.38	59.6	5.34	ND	—
23	1082.5311	MC-OiaR[b]	Tentative	7.40	02.4	9.17	ND	—
24	995.4857	[D-Asp[3]]MC-RE[b]	Probable	7.43	ND	—	110.1	0.79
25	1027.5269	MC-FR[b]	Confirmed	7.48	55.2	22.85	ND	—
26	1066.5380	MC-WR[b]	Confirmed	7.64	0.7	0.06	ND	—
27	1009.5015	MC-RE[b]	Probable	7.79	3.8	0.34	1281.0	9.21
28	951.4955	MC-RA[b]	Probable	8.24	13	1.16	ND	—
29	965.5116	MC-RAbu[b]	Probable	8.83	7.1	0.64	ND	—
30	946.4577	MC-(H2)YG[b]	Probable	9.06	5.4	0.48	ND	—
31	1011.4966	MC-RM[b,c,d]	Probable	9.23	0.2	0.02	ND	—
32	960.4738	MC-(H2)YA[b]	Probable	9.34	10.1	0.90	ND	—
33	953.4783	Unidentified MC[b]	Unidentified	9.50	12	1.07	ND	—
34	967.4942	Unidentified MC[b]	Unidentified	9.78	2.3	0.21	ND	—
35	984.4735	Unidentified MC[b]	Unidentified	9.96	3.7	0.33	ND	—
36	968.4272	[D-Asp[3]]MC-EE[b]	Probable	11.43	ND	—	403.3	2.90
37	982.4432	MC-EE[b]	Probable	12.58	0.9	0.08	1533.5	11.03
38	952.4690	[D-Asp[3]]MC-LE[b]	Probable	12.82	1.2	0.11	358.8	2.58
39	894.4631	[D-Asp[3]]MC-LA[b]	Probable	13.32	6.5	0.58	ND	—
40	966.484	MC-LE[b]	Probable	13.69	12.9	1.15	1376.	9.90

	9						1	
41	908.4788	MC-LA[b]	Confirmed	14.87	44.8	4.01	ND	—
42	922.4942	MC-HilA[b]	Probable	15.29	5.9	0.53	ND	—
43	981.4737	MC-WA[b]	Probable	15.88	4.4	0.39	ND	—
44	942.4629	MC-FA[b]	Probable	15.95	42.3	3.79	ND	—
45	922.4943	MC-LAbu[b]	Probable	16.09	16.6	1.49	ND	—
46	936.5110	MC-HilAbu[b]	Probable	16.55	2.2	0.20	ND	—
47	995.4898	MC-WAbu[b]	Probable	17.20	13.4	1.20	ND	—
48	956.4784	MC-FAbu[b]	Probable	17.39	26.4	2.36	ND	—
49	936.5106	MC-LV[b]	Probable	17.62	3.6	0.32	ND	—
50	950.5254	MC-LL[b]	Probable	17.93	3.9	0.35	ND	—
51	970.4944	MC-FV[b]	Probable	18.40	0.7	0.06	ND	—
52	950.5262	*iso*-MC-LL[b]	Tentative	18.49	1.2	0.11	ND	—
53	1009.5041	MC-WV[b]	Probable	18.54	2.9	0.26	ND	—
54	1023.5210	MC-WL[b]	Probable	18.74	2.8	0.25	ND	—
55	970.4942	*iso*-MC-FV[b]	Tentative	18.79	2.1	0.19	ND	—
56	1023.5216	*iso*-MC-WL[b]	Tentative	19.16	1.3	0.12	ND	—

[a] Concentration expressed per weight of biomass (FW) and as a percentage of total microcystins detected in each culture); ND = not detected; [b] Reacted with mercaptoethanol; [c] Oxidised by Oxone/DMSO; [d] HRMS/MS of oxidation product.

Table 4. Microcystins detected by LC-HRMS/MS analysis in *Microcystis* strain AB2017/08 isolated from Yezin Dam, their retention times (t_R), concentrations, relative abundances (%), and observed *m*/*z* values in negative ionisation mode

					Concentration [a] AB2017/08	
	m/*z*	Compound name	Confidence	$_R$ (min)	μg g^{-1}	%
1	1011.5521	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	[D-Asp^3]MC-LR	Confirmed	6.90	181.9	15.68
9	1011.5521	[Mser^7]MC-LR	Tentative	7.06	7.8	0.67
10	993.5415	MC-LR	Confirmed	7.12	891.7	76.89
11	979.5258	[Dha^7]MC-LR	Confirmed	7.13	9.6	0.82
12	1007.5571	MC-HilR	Confirmed	7.39	9.5	0.82
13	980.5099	[D-Asp^3]MC-LCit	Tentative	11.26	0.5	0.04
14	994.5255	Unidentified	Unidentified	12.20	0.8	0.06
15	952.5037	Unidentified	Unidentified	13.39	0.2	0.01
16	986.4881	[D-Asp^3]MC-LY	Tentative	14.62	0.3	0.02
17	1000.5037	MC-LY	Tentative	15.14	1.4	0.12
18	1009.5082	[D-Asp^3]MC-LW	Tentative	16.41	1.7	0.14
19	970.4931	[D-Asp^3]MC-LF	Tentative	16.95	0.5	0.05
20	1023.5197	MC-LW	Tentative	17.00	3.7	0.32
21	984.5088	MC-LF	Tentative	17.61	3.8	0.33
22	950.5244	MC-LL	Probable	18.03	0.2	0.01

[a] Concentration is expressed per weight of biomass (FW) and as a percentage of the total microcystins detected in each culture.

Regarding the LC-MS/MS analysis on the MC producing strains, altogether 52 MC congeners including 22 previously undescribed variants and 22 MC congeners including 6 previously unidentified MC variants were detected from the *Microcystis* strains from Meiktila Lake and Yezin Dam respectively. The toxicity of these MC congeners varied from highly toxic variants such as MC-LR or MC-LA to lesser toxic variants such as MC-RR (Rinehart et al., 1994). As shown in the table 3 and 4, the MC profiles of the strains from Yezin Dam highly differ from that of the strains from Meiktila Lake. Although the strain of Yezin Dam

produced 22 MC congeners, MC-LR and [D-Asp3]MC-LR, the highly toxic variant, were the dominant MC congeners, containing 76.9% and 15.7% of the total MCs, respectively. On the other hand, although the *Microcystis* strain (AB2017/14) isolated from Meiktila Lake produce higher number of MCs (52 MC congeners), the highly toxic variant like MC-LR and [D-Asp3]MC-LR were only 20% of the total MC concentrations. Since the toxicity of several of the MC

The toxicity of several of the MC variants found in this study has not yet been described, which makes a full risk assessment difficult. It is recommended, therefore, that future research should focus on the toxicity of these unidentified or previous unreported variants.

6. Conclusions

This study clearly demonstrates for the first time the presence of CYN- and deoxyCYN-producing *R. raciborskii* and MC-producing *Microcystis* in the phytoplankton community of Meiktila Lake and Yezin Dam in Myanmar. Although all *Dolichospermum* strains isolated from Yezin Dam were negative for production of cyanotoxins, one of the *Microcystis* strains from Yezin Dam and both *Microcystis* strains from Meiktila Lake produced variable amount of MCs. Then four of the five isolated *Raphidiopsis* strains from Yezin Dam and three out of five *Raphidiopsis* strains from Meiktila Lake produced CYN and deoxyCYN similar to other *Raphidiopsis*/*Cylindrospermopsis* strains from other Aisan countries and Australia.

Aside from their toxins being relevant to human health, cyanobacteria have the potential to cause substantial ecological impacts on aquatic food webs. As both investigated water bodies are 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.

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