

CO-OCCURRENCE OF MICROCYSTIN AND CYLINDROSPERMOPHSIN IN HYPEREUTROPHIC MAHAKANADARAWA AND NACHCHADUWA RESERVOIRS

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ABSTRACT

Microcystins (MCs) and cylindrospermopsin (CYN) are the most abundant toxins produced by cyanobacteria in tropical freshwaters. We studied the spatial distribution of MC and CYN in two multipurpose reservoirs, Mahakanadarawa and Nachchaduwa in Anuradhapura district in Sri Lanka in September 2020. Fourteen water quality parameters, phytoplankton composition, chlorophyll-*a*, MC and CYN concentrations were analyzed in triplicate in 25 sampling sites from each reservoir. Both reservoirs were at hypereutrophic status. *Microcystis* was the dominant cyanobacteria with $0-3.75 \times 10^3$ cell/mL in Mahakanadarawa and $1-7 \times 10^3$ cell/mL in Nachchaduwa. Besides *Microcystis*, no other potential MC-producing cyanobacteria were observed. In Mahakanadarawa, MC was detected in the range of 0.11-1.63 $\mu\text{g/L}$ which was above the WHO permissible level (1.0 $\mu\text{g/L}$) for drinking water. Although comparatively high *Microcystis* cell density was present in Nachchaduwa, its MC concentration was low (0.06-0.17 $\mu\text{g/L}$). The CYN concentration in Nachchaduwa was above the WHO permissible level (0.7 $\mu\text{g/L}$) for drinking water. It was 0.20-1.02 $\mu\text{g/L}$ in Nachchaduwa and 0.03-0.08 $\mu\text{g/L}$ in Mahakanadarawa. We did not observe any potential CYN-producing cyanobacteria in either of the reservoirs. There was no relationship between the spatial distribution pattern of MC and *Microcystis* cell density in both reservoirs. Although the majority of physico-chemical properties of water indicated suitability for drinking, co-occurrence of high concentrations of MC and CYN indicated their unsuitability for drinking. Hence, this study highlights the necessity for routine detection of cyanotoxins in both reservoirs. Further, our findings alarm potential health risks for the local community that relies on Mahakanadarawa and Nachchaduwa reservoirs for drinking, irrigation and fisheries.

Keywords: cyanobacteria, cylindrospermopsin, microcystin, *Microcystis*, spatial distribution

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INTRODUCTION

Cyanobacterial blooms in freshwaters can cause serious ecological and health problems worldwide due to their ability to produce toxic secondary metabolites which are known as cyanotoxins. Cyanotoxins are classified according to their chemical structure mainly into cyclic peptides, alkaloids, lipopeptides, non-protein amino acids and lipoglycans (Du et al. 2019). Some of the cyanotoxins such as microcystin (MC), nodularin (NOD) and cylindrospermopsin (CYN) are hepatotoxins (Benamara et al. 2021). Moreover, CYN is also a genotoxin (Žegura et al. 2011). Anatoxin and saxitoxin are neurotoxins (Testai et al. 2016) while lyngbyatoxin and aplysiatoxin lead to dermatotoxicity (Kaebernick and Neilan et al. 2001). Repeated exposure to cyanotoxins may cause organ failures such as liver, kidney, lung and thyroid and even death (Buratti et al. 2017). Based on the health impacts of cyanotoxins, the World Health Organization (WHO) proposed a provisional permissible concentration limit of 1.0 µg/L for MC (WHO 2020) and 0.7 µg/L for CYN in drinking water (WHO 2019).

MC and its chemical variants are cyclic peptides produced by various species of cyanobacteria such as *Anabaena*, *Anabaenopsis*, *Microcystis*, *Nostoc*, *Oscillatoria* and *Planktothrix* in the major orders of cyanobacteria viz. Chroococcales, Oscillatoriales, Nostocales (Nowruzi and Porzani 2021; Kesari et al. 2022). MC-producing cyanobacteria are found primarily in freshwaters with a wide geographic and ecological distribution worldwide. Hence, MCs are the most frequently reported cyanobacterial toxins. To date, more than 250 different chemical variants of MCs have been identified and of all, the most common and toxicologically most studied MCs is the MC-LR variant (Spoon and Catherine 2017; Bouaïcha et al. 2019). Next to MCs, CYN is the most widely found cyanotoxin worldwide in freshwaters (Antunes et al. 2015), which is chemically an alkaloid with few naturally occurring variants (Ohtani et al. 1992; Kokocinski et al. 2017). CYNs are produced

by various strains of cyanobacterial species, primarily in the order Nostocales (*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis*, *Umezakia*) and Oscillatoriales (*Lyngbya*, *Oscillatoria*) (De la Cruz et al. 2020). In Asia, Australia and New Zealand, *Cylindrospermopsis raciborskii* is a major CYN producer while in Europe and the Americas it is *Aphanizomenon* and *Dolichospermum* (formerly *Anabaena*) (Antunes et al. 2015). In Sri Lanka, MCs and CYN are the most common and frequently reported cyanotoxins (Kulasooriya 2017) as they are the most frequently studied cyanotoxins in Sri Lanka and not much attention was paid on other cyanotoxins.

MCs and CYN occur naturally in surface waters. Therefore, if untreated or insufficiently treated, drinking water is the most likely route of exposure to cyanotoxins (WHO 2020). In addition, bathing, irrigation and consumption of toxin-accumulated food items may also be other exposure pathways. Since recreational activities such as bathing typically take place near-shore where surface blooms usually accumulate, unintentional swallowing of water sometimes can lead to exposure to extremely high concentrations of toxins. In natural water, MCs are mostly confined to intracellular in viable cells (Juttner and Lüthi 2008). Therefore, MC concentrations in water coincide with the abundance of MC-producing cyanobacterial species. MCs can be released from cells by senescence of cells or induced cell lysis for example by cyanophage activity, unfavorable conditions for growth or algicidal treatment (WHO 2020). In contrast to MCs, up to 90% of the CYN in water are released from viable cells (Preußel et al. 2009). Therefore, the highest MC and CYN concentrations occur during the peak of blooms. Previous studies have reported co-occurrence of two or more cyanotoxins in freshwater reservoirs. For instance, co-occurrence of CYN, MC and nodularin in Lake Victoria in Tanzania (Mchau et al. 2021), CYN, MC and saxitoxin in Lake Taihu in China (Li et al. 2022) and anatoxin-a and MC in the Lake Garda in Italy (Cerasino and Salmaso 2020). In Sri Lanka, although there are many reports

on the co-occurrence of toxigenic cyanobacteria in freshwater reservoirs, to our knowledge, the co-occurrence of two or more cyanotoxins has not been reported to date.

In the environment, MCs are moderately resistant to chemical and microbial breakdown whereas CYN is highly resistant to degradation (Chiswell et al. 1999; Massey and Yang 2020). Hence, even if the cell-bound CYN concentration is low, the concentration of dissolved CYN can be high. It can remain long after the CYN-producing cyanobacteria have disappeared (WHO 2019). Therefore, careful monitoring of water is required to identify the factors that can affect the growth of toxin-producing cyanobacteria such as nutrient status and other physico-chemical parameters that favors cyanobacterial growth.

In this study, we selected Mahakanadarawa and Nachchaduwa reservoirs in the North-Central province in Sri Lanka. Both reservoirs provide water for drinking, fisheries and irrigation. The National Water Supplies and Drainage Board (NWSDB) provides long-term and stable drinking water supplies for the local community from these two reservoirs. In addition, Mahakanadarawa reservoir supplies raw water for a recently implemented large-scale drinking water project that aims to resolve long-felt water scarcity in rural areas in the Anuradhapura district (NWSDB, Sri Lanka). The main transmission pipeline extends to 89 km supplying water for the population of around 75,000 in 30 Grama Niladhari divisions in the Medawachchiya, Rambewa and Mihintale Divisional Secretariats. Therefore, a large community in the Anuradhapura district utilize water from both reservoirs for their domestic needs, agriculture and fisheries. Hence, the assessment of water quality and cyanotoxin contamination levels in the above two reservoirs have significant importance. We studied the spatial distribution of MC and CYN cyanotoxins in both Mahanadarawa and Nachchaduwa reservoirs along with their phytoplankton composition and physico-chemical properties to understand potential health risks to the local community.

MATERIALS AND METHODS

Site description: Mahakanadarawa and Nachchaduwa are medium-scale, man-made reservoirs with an area at a full capacity of 6100 and 7000 acres respectively (Irrigation Department, Sri Lanka). These two reservoirs are situated in the Anuradhapura district in the North-Central province ($8^{\circ}23'27.83''$ N & $80^{\circ}32'47.65''$ E and $8^{\circ}14'56.29''$ N & $80^{\circ}28'59.24''$ E respectively) (Figure 1). Mahakanadarawa is mainly fed by rainwater and used for aquaculture and fisheries. Malwathu Oya which makes the second-largest catchment of the river basins in the country provides water to the Nachchaduwa reservoir. Sampling was conducted in September 2020 on two-clear sunny days during the daytime between 9.00 am to 2.00 pm. Twenty-five sites from each reservoir were selected for on-site water quality assessment and laboratory analyses (Figure 1). Sampling was not carried out in some areas of Mahakanadarawa reservoir due to the presence of submerged tree trunks and dense growth of *Potamogeton* sp. in Nachchaduwa reservoir. Therefore, those areas were not included as sampling sites in this study.

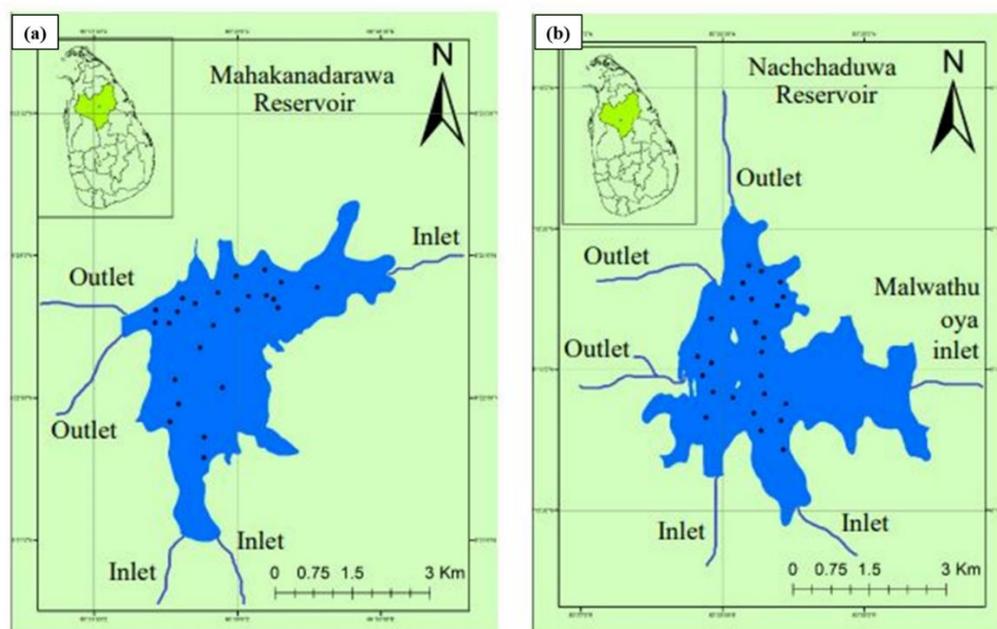


Figure 1: Maps of Mahakanadarawa (a) and Nachchaduwa (b) reservoirs illustrating twenty-five sampling sites.

On-site assessment of water quality: Temperature ($^{\circ}\text{C}$), pH, oxidation-reduction potential (ORP, mV), electrical conductivity (EC, $\mu\text{S}/\text{cm}$), total dissolved solids (TDS, ppm), salinity (psu), pressure (psi), dissolved oxygen (DO, ppm) and turbidity (FNU) were measured *in situ* in triplicate using a multi-parameter water quality meter (HI9829-Hanna Instruments, Romania) and water transparency was estimated using a Secchi disk.

Laboratory analysis of water quality: Water samples for the laboratory analysis were taken with three replicates from the same sites where *in-situ* water quality parameters were measured. For the estimation of chlorophyll-*a*, 50 L of water was filtered through a 30 μm plankton net (Hydro-Bios, Germany). In addition, water samples were collected for the determination of NO_3^- -N, NH_4^+ -N, PO_4^{3-} -P and total suspended solids (TSS). All the samples were collected from the surface of the water into 250 mL glass bottles. Samples were transported to the laboratory following standard protocols of the United States Environmental Protection Agency (US-EPA 2013). All chemical reagents used were from standard manufacturers (Sigma-Aldrich, USA; BDH, UK and Oxoid, UK) unless otherwise mentioned.

The concentrations of NO_3^- -N (ppm) and NH_4^+ -N (ppm) were measured using a nitrate probe (HI7609829-12) and an ammonium probe (HI7609829-10) from Hanna Instruments Inc., Romania respectively. The concentration of PO_4^{3-} -P (ppm) in water was determined using PhosVer3[®] phosphate reagent powder pillows having 0.00 to 2.50 ppm PO_4^{3-} -P detection range (Hatch, USA) following the manufacturer's protocol which is equivalent to the Ascorbic acid method in the US-EPA protocol 365.3 (1978). Absorbance was measured at 881 nm wavelength using a UV/visible spectrophotometer (Genesys 150, Thermo-Scientific, USA). A gravimetric method was used to estimate TSS following the Method 2540D of the American Public Health Association (APHA 1999).

The chlorophyll-*a* concentration of field-collected water samples was immediately measured after returning to the laboratory following the method of Arnon (1949) with slight modifications. Briefly, phytoplankton biomass was collected by low-speed centrifugation at 5000 rpm for 10 minutes. Chlorophyll was extracted into 80% (v/v) acetone. Absorbance was measured at 645 nm and 663 nm wavelengths using a UV/Visible spectrophotometer (Genesys 150, Thermo-Scientific, USA). Chlorophyll-*a* concentration was calculated using the following formula, where OD is the optical density (absorbance), *v* is the volume of acetone, and *V* is the volume of the tested water sample (L). The path length of light (cm) is represented by *d*.

$$\text{Chlorophyll-}a \text{ (ppb)} = \frac{[(12.7 \times \text{OD}_{663}) - (2.69 \times \text{OD}_{645}) \times v]}{(V \times d)} \quad (1)$$

Water samples that were filtered through the plankton net were used for the identification and enumeration of phytoplankton. Cyanobacteria, diatoms and green algae were identified up to the genus or species level with the aid of standard identification keys (Baker et al. 2012; Nienaber and Steinitz-Kannan 2018). The cell density of phytoplankton was estimated following the method described by LeGresley and McDermott (2010). Briefly, water samples were observed within 24 h after collection and enumerated using a Neubauer hemocytometer (Marienfeld, Germany) under the 400x magnification of a bright-field microscope (Nikon *Ci-L*, Japan) and average cell density was determined from three replicates collected from each sampling site.

The concentrations of MC and CYN cyanotoxins were estimated following EPA 546 and 545 protocols (US-EPA 2016 & 2017) respectively within 48 h of sample collection. In order to release intracellular MC, water samples were subjected to three cycles of freezing (-20 °C) and thawing (30 °C) (US-EPA 2016). Thereby, both intracellular and extracellular MC were taken for the measurement. As up to 90% of the total CYN in the surface water is in the dissolved fraction (WHO 2019), water samples were directly measured for CYN

without subjecting to cell lysis. Toxin concentrations were determined using MC and CYN plate kits (96-well), which provide a 0 ppb- 2 ppb min-max detection range (Beacon, USA). All the reactions were performed following the manufacturer's protocols. The absorbance of MC and CYN standard solutions (provided with the kit) and test samples were measured at 450 nm wavelength using a microplate reader (Multiscan FC, Thermo-Scientific, USA). Based on the measured MC and CYN toxin concentrations and cell count of *Microcystis* for each sampling site, their spatial distribution in two reservoirs was mapped using AchGIS 10.6.1 software (Environmental Systems Research Institute Inc., Redlands, CA, USA).

Determination of trophic status: Trophic status of the two reservoirs was determined as Total Trophic State Index (TTSI) with the aid of Secchi depth and chlorophyll-*a* concentration using the following formulae in which TTSI 2.0-2.7, oligotrophic, 3.3-3.7, mesotrophic, 4.5-5.0 eutrophic, and $6.0 \leq$, hypereutrophic (Burns 1999).

$$\text{TTSI} = [\text{TSI (SD)} + \text{TSI (CA)}] / 2 \quad (2)$$

$$\text{TSI for Secchi depth (SD)} = 5.10 + 2.60 \times \log (1/\text{SD} - 1/40) \text{ (meters)} \quad (3)$$

$$\text{TSI for chlorophyll-}a \text{ (CA)} = 2.22 + 2.54 \times \log (\text{CA}) \text{ (}\mu\text{g/L)} \quad (4)$$

Data analysis: Data were statistically analyzed by using a standard statistical software package MINITAB version 18. The measured parameters were presented as arithmetic means \pm SD of three independent measurements. Pearson product correlation coefficient (*r*) was calculated to determine the relationship between water quality parameters, toxin concentration and phytoplankton cell density. The statistical significance was defined at the 0.05 level.

RESULTS

Water quality and trophic status: An algal bloom was not observed at the time of sampling in September 2020 in both Mahakanadarawa and Nachchaduwa reservoirs. The quality of water as indicated by physico-chemical and biological properties is summarized in

Table 1. We observed large site-to-site heterogeneity in ORP, chlorophyll-*a* concentration and the densities of *Melosira* and *Microcystis* in both reservoirs. The Secchi depth and chlorophyll-*a* concentration data indicated that both reservoirs fell into the hypereutrophic (TTSI=6.68 and 6.80) status. When the measured physico-chemical properties of two reservoirs were compared with the Ambient Water Quality Standards of Sri Lanka (CEA, 2019), it revealed that, pH, DO, NO₃⁻-N and PO₄³⁻-P were in the favorable range (pH 6-8.5, DO 3-6 mg/L, min, NO₃⁻-N 10 mg/L, max and PO₄³⁻-P 0.4-07 mg/L, max) for drinking after undergoing simple or general treatment (Category A and D), bathing and contact recreation (Category B), aquatic life (Category C), irrigation and agriculture (Category E) and minimum quality for purposes other than former categories (Category F). However, in both reservoirs, water turbidity and NH₄⁺ concentration exceeded the maximum permissible level (5 NTU, max and 0.22 mg/L, max respectively) for all categories (A to F). When the measured physico-chemical properties of two reservoirs were compared with the WHO (2020) standards for drinking water, pH, TDS, EC and NO₃⁻-N concentration fell within the permissible levels (pH 6.5-8.5, TDS <600 mg/L, EC 750 μS/cm and NO₃⁻-N 10 mg/L) whereas, turbidity and NH₄⁺ concentration exceeded the maximum permissible level (5 NTU, max and 0.20 mg/L, max respectively) for drinking water in both reservoirs. For DO and PO₄³⁻-P, no health-based levels are recommended by the WHO. The concentrations of chlorophyll-*a* in both reservoirs were fairly high and above the Alert Level 2 threshold for drinking water in Alert Level Framework (ALF) which is defined as 12 μg/L when cyanobacteria are present as the dominant phytoplankton (WHO 2020). Further, chlorophyll-*a* in both reservoirs had positive correlation with *Microcystis* cell density (n=25, r = 0.463, p = 0.13 and n=25, r = 0.224, p = 0.441 respectively). Although, *Microcystis* is not the only contributor to chlorophyll-*a*, it was assumed that a higher fraction of chlorophyll-*a* in water is supplied by *Microcystis*, as it is the dominant phytoplankton community observed in both

reservoirs. Further, we did not see a significant correlation between chlorophyll-*a* and either MC or CYN concentrations. Although most measured physico-chemical properties of Mahakanadarawa and Nachchaduwa were in the range of permissible levels for Ambient Water Quality Standards for drinking, irrigation and aquatic life, their turbidity, NH₄⁺-N and chlorophyll-*a* concentrations indicated water is unsuitable for drinking without treatment.

Table 1: Water quality of Mahakanadarawa and Nachchaduwa reservoirs in September 2020 as indicated by physico-chemical and biological properties.

	Mahakanadarawa		Nachchaduwa	
	Mean ± SD (<i>n</i> =25)	Range (min-max)	Mean ± SD (<i>n</i> =25)	Range (min-max)
Secchi depth (cm) transparency	78.66 ± 5.49	50 - 90	77.81 ± 4.46	50 - 85
Temperature (°C)	27.86 ± 0.24	27.4 - 28.3	28.12 ± 0.24	27.7 - 28.9
pH	8.65 ± 0.07	8.5 - 8.7	8.71 ± 0.07	8.56 - 8.97
ORP (mV)	201.05 ± 55.57	160.2 - 230.4	196.01 ± 15.68	164.1-241.1
EC (µS/cm)	560.91 ± 5.38	545 - 679	493.64 ± 21.03	334 - 529
TDS (ppm)	280.37 ± 2.61	273 - 340	248.08 ± 9.54	167 - 262
Turbidity (FNU)	16.40 ± 1.57	14.1 - 24.6	11.22 ± 0.94	9 - 58.7
TSS (mg/L)	0.0065 ± 0.002	0.003- 0.022	0.005 ± 0.001	0.001 - 0.017
DO (mg/L)	9.18 ± 0.44	4.9 - 11	9.16 ± 0.47	3.96 - 10.62
Salinity (psu)	0.260 ± 0.002	0.26 - 0.33	0.23 ± 0.01	0.16 - 0.40
Pressure (psi)	14.57 ± 0.01	14.5 - 14.6	14.53 ± 0.03	14.50 - 15.51
NH ₄ ⁺ -N (mg/L)	1.66 ± 0.04	1.5 - 1.9	1.50 ± 0.05	1.4 - 1.6
NO ₃ ⁻ -N (mg/L)	7.25 ± 1.09	3.7 - 13.0	5.11 ± 1.18	1.2 - 7.2

PO ₄ ³⁻ - P (mg/L)	0.0035 ± 0.002	0.001 - 0.17	0.003 ± 0.002	0.002 - 0.16
Chlorophyll- <i>a</i> (µg/L)	168.28 ± 54.26	61.89	– 216.02 ± 100.19	–
		284.76	73.42	467.47
<i>Melosira</i> filaments/mL	106.858 ± 53.07	28 - 263	172.53 ± 29.032	± 120 - 245
<i>Microcystis</i> (Cells/mL)	1408.65 ± 1182.45	0 - 3750	3490 ± 1492.13	1000 - 7000
MC (µg/L)		0.1-1.6		0.06-0.17
CYN (µg/L)		0.03-0.08		0.20-1.02

Phytoplankton composition: *Microcystis* was the dominant cyanobacteria in both reservoirs (Figure 2 and Figure 3a) and the density ranged from 0-3.75 x10³ cells/mL and 1-7 x 10³ cells/mL in Mahakanadarawa and Nachchaduwa respectively. *Microcystis* was observed in 18 sampling sites in Mahakanadarawa and all 25 sites in Nachchaduwa. The spatial distribution of *Microcystis* in Mahakanadarawa showed an accumulation of high cell densities toward the North-shore whereas in Nachchaduwa no such distinct distribution pattern could be identified (Figure 4 e and f). Besides *Microcystis*, no other cyanobacteria were observed in both reservoirs. Next to *Microcystis*, filamentous diatom *Melosira* (synon. *Aulacoseira*) was found dominant in all sampling sites in both reservoirs (Table 1, Figure 2 and Figure 3b). Several species of the green-algal genus *Pediastrum* were also observed in all sampling sites. Those were identified as *Pediastrum biwae* Negoro, *Pediastrum duplex*

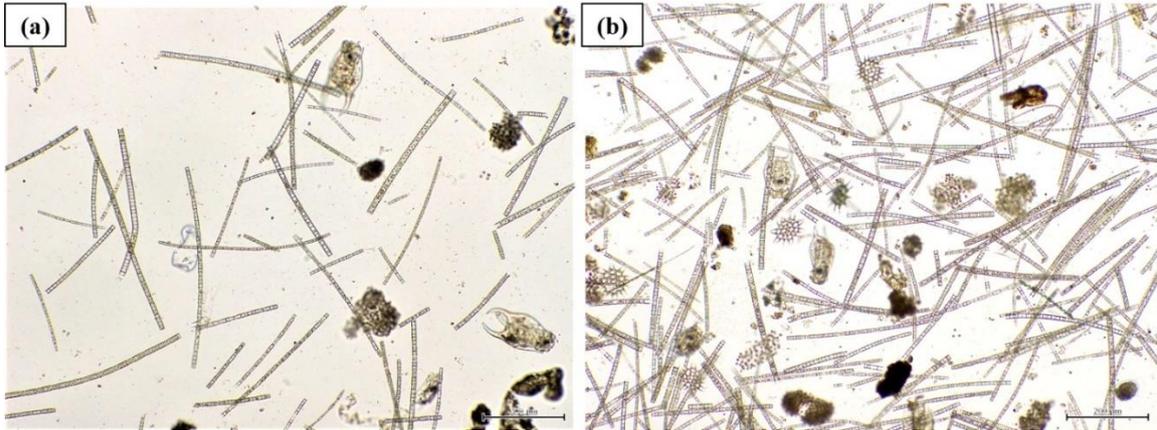


Figure 2. Phytoplankton observed in Mahakanadarawa (a) and Nachchaduwa (b) reservoirs showing the colonies of *Microcystis* and the filamentous diatom, *Melosira* at 40x magnification. Images were captured using a bright-field microscope with an embedded digital camera (Nikon Ci-L, Japan). The scale bar indicates 200 μ m.

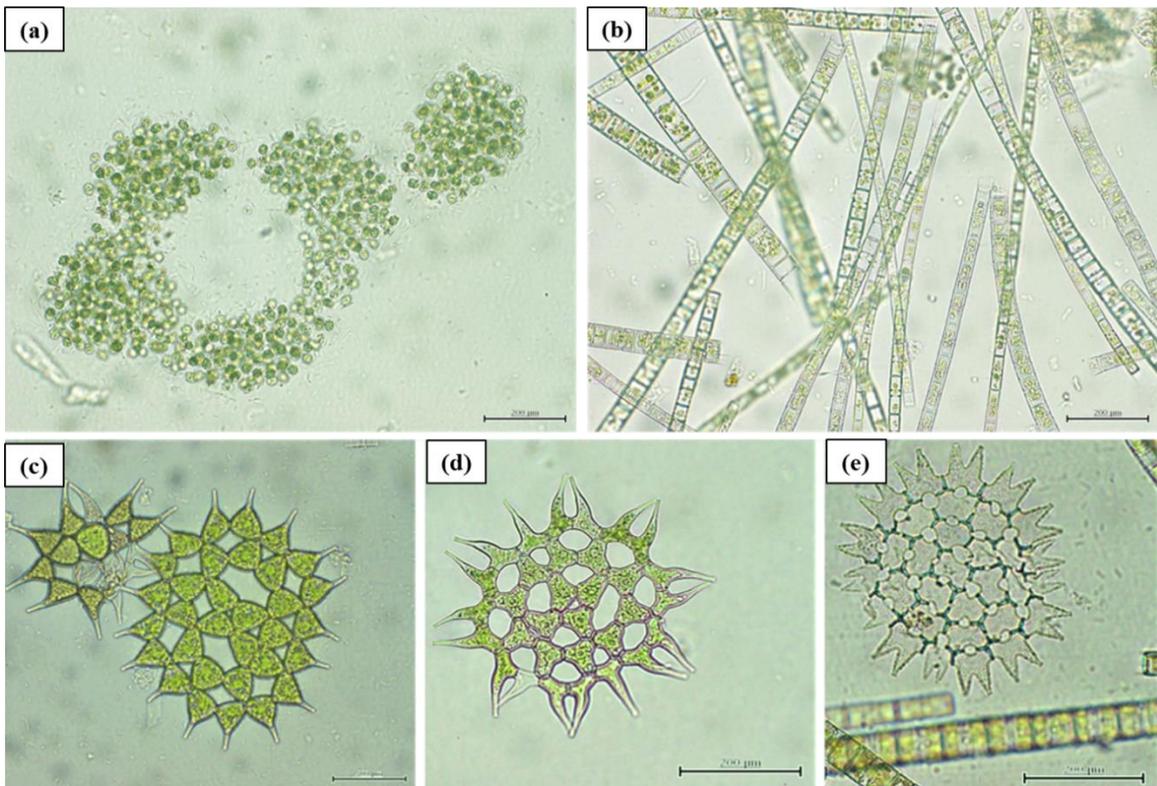


Figure 3. High-resolution images of *Microcystis* (a) and filamentous diatom, *Melosira* (b) and green algae, *Pediastrum biwae* Negoro (c), *P. duplex* Meyen (d) and *P. boryanum* Turpin (e) observed in Mahakanadarawa and Nachchaduwa reservoirs. Images were captured at 400x magnification using a bright-field microscope with an embedded digital camera (Nikon Ci-L, Japan). The scale bar indicates 200 μ m.

Meyen and *Pediastrum boryanum* Turpin based on their cell number and arrangement in colonies (Figure 3c-e).

MC and CYN cyanotoxins and their spatial distribution: The total MC in field-collected water samples in Mahakanadarawa ranged from 0.11-1.63 $\mu\text{g/L}$. Its spatial distribution showed that concentrations $> 0.5 \mu\text{g/L}$ were accumulated toward the direction of the South which was the opposite direction to the accumulation of high densities of *Microcystis* (Figures 4a and 4e). Although comparatively high density ($> 1 \times 10^3 \text{ cell/mL}$) of *Microcystis* was present in all sampling sites in Nachchaduwa reservoir, the MC concentrations in those sampling sites were low (ranging from 0.06-0.17 $\mu\text{g/L}$). In contrast, CYN concentration in Nachchaduwa was higher than that of Mahakanadarawa which was 0.20-1.02 $\mu\text{g/L}$ and it was 0.03-0.08 $\mu\text{g/L}$ in Mahakanadarawa. In Nachchaduwa, CYN concentrations were $> 0.05 \mu\text{g/L}$ in all sites while high concentrations were found accumulated in a single area of the West-shore of the reservoir (Figure 4d). None of the potential CYN-producing cyanobacteria were observed in field-collected water samples from both reservoirs. Further, MC positively correlated with temperature ($r = 0.41, p = 0.17$), DO ($r = 0.61, p = 0.85$) and turbidity ($r = 0.40, p = 0.18$) and negatively correlated with Secchi depth transparency ($r = 0.76, p = 0.81$). Similar to MC, CYN also positively correlated with temperature ($r = 0.25, p = 0.37$) and DO ($r = 0.38, p = 0.18$). However, MC did not correlate with *Microcystis* cell density and chlorophyll-*a* concentration. According to the WHO (2020) guidelines for drinking water, MC and CYN detected in some of the sampling sites in Mahakanadarawa and Nachchaduwa were slightly above the maximum permissible levels for MC (1 $\mu\text{g/L}$) and CYN (0.7 $\mu\text{g/L}$).

MC and CYN in Mahakanadarawa and Nachchaduwa reservoirs

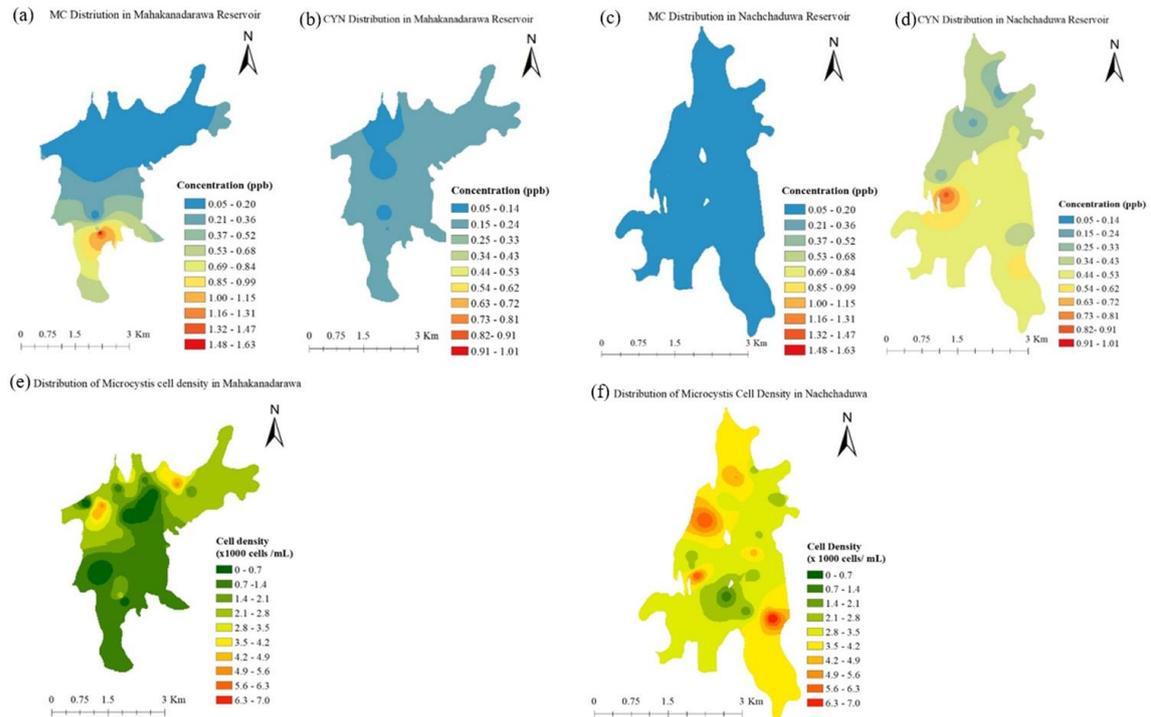


Figure 4. Spatial distribution of MC (a) and (c), CYN (b) and (d) and *Microcystis* (e) and (f) in Mahakanadarawa and Nachchaduwa reservoirs in September 2020.

DISCUSSION

In this study, we report the co-occurrence of two hepatotoxic cyanotoxins, MC and CYN in Mahakanadarawa and Nachchaduwa reservoirs. Unlike in temperate countries where cyanobacterial blooms are likely to be seasonally present, a greater risk is possible for communities in tropical regions with persistent cyanobacterial blooms throughout the year. Therefore, our findings alarm health risks for the local community that rely on Mahakanadarawa and Nachchaduwa for drinking, irrigation and aquaculture due to the potential long-term exposure to the above toxins. Although most measured water quality parameters fell within the favorable range for ambient water quality standards according to the Sri Lanka standard for drinking water (CEA 2019), MC and CYN concentrations exceeded the maximum permissible levels defined by WHO (2020). None of the cyanobacterial toxins including MCs and CYN have been known to affect the taste or odour of water, except some metabolites of certain cyanobacterial species that alter the taste or

odour of water. Hence, the absence of alteration to the taste or odour of raw water is not a reliable indicator of the absence of cyanotoxins. Therefore, unless detected analytically prior to the treatment, the dissolved fractions of MCs and CYN can easily pass-through flocculation and filtration steps in the drinking-water treatment process and finally reach the consumer. Therefore, we highlight the necessity of routine detection of cyanotoxins in source water prior to the treatment for drinking.

According to the findings, total MC concentration did not correlate with either *Microcystis* cell density or chlorophyll-*a* in both reservoirs. This could be due to several reasons. *Microcystis* is not the only contributor for chlorophyll-*a* in these reservoirs as we observed *Melosira* with considerably high density and several other filamentous types which can also contribute for the extracted chlorophyll-*a*. On the other hand, dislocation of *Microcystis* colonies following wind direction (Figure 4) and/or senescence of colonies at the time of sampling leading to the degradation of chlorophyll could also influence chlorophyll-*a* in a particular site. Another possible reason is the contribution of MCs that are accumulated in the benthic environment (Preece et al. 2021) to the detected MC concentrations in the collected water samples in addition to the planktonic *Microcystis*. MCs in the benthic environment can originate from the biomass of MC-producing cyanobacteria that sink to the sediment following a bloom and MC-producing benthic cyanobacteria such as *Phormidium* and *Planothrix* (Magonono et al. 2018). We found that the patterns of the spatial distribution of two toxins in the two reservoirs were highly variable (Figure 4). In Mahakanadarawa, MC accumulated toward the South-shore (Figure 4a) which was opposite to the highly concentrated areas of chlorophyll-*a* were present (Figure 4e). This might be due to the buoyancy of surface blooms such as *Microcystis* and their dislocation at temporary locations following wind direction (Xingqiang et al. 2019). Therefore, it is highly likely that the observed spatial distribution of *Microcystis* in Mahakanadarawa corresponds to the

direction of wind toward the North-shore although its density was not high enough to form surface scum. However, MC did not show a distribution pattern following the downwind. Therefore, it appears that the dissolved fraction of MC resulting from the cell lysis may have contributed more to the detected high concentrations of MC at sites near the South shore. That dissolved fraction of MC may have originated from a previous *Microcystis* bloom or any other MC-producing cyanobacterial community (Magonono et al. 2018; Preece et al. 2021). In contrast, we detected comparatively low levels of MC in Nachchaduwa throughout the water body although it had comparatively high densities of *Microcystis* (Figure 4f). Therefore, it seems that the dissolved fraction of MC had contributed less to the detected concentrations in Nachchaduwa and most likely toxin was intracellular within intact cells. Senescence of those high densities of intact cells could elevate MC in the water body after some time when cells undergo natural or induced lysis releasing their intracellular toxins. Therefore, high *Microcystis* cell density is an indication of a future MC outbreak.

Of the physico-chemical parameters that we measured, MC and CYN in Mahanadarawa and Nachchaduwa reservoirs positively correlated only with temperature and turbidity whereas negatively correlated with Secchi depth transparency. If nutrient and mixing conditions permit, warmer water bodies can sustain more stable toxin (such as MCs) production because thermal stratification is favored by buoyant phytoplankton species such as cyanobacteria (Rigosi et al. 2014). Further, water column stability is an important determinant of the dominance of cyanobacteria and their toxin production (Persaud et al. 2015). Therefore, in countries like Sri Lanka where the warm temperature is prevalent throughout the year, stable cyanotoxin production could be expected. Moreover, according to our data, water temperature within the reservoir in a single day showed a positive correlation with MC and CYN concentrations probably due to the spatial and diurnal variation in temperature where warmer areas accumulated more toxins. The DO is

considered as a potential indicator for the onset of cyanotoxins derived from lysed cells in a blooming population (Hartnell et al. 2020). However, DO of both Mahakanadarawa and Nachchaduwa reservoirs did not show a significant correlation to either MC or CYN. Previous studies have shown that a decrease in DO have a close association with the presence and concertation of MCs (O'Neil et al. 2012; Paerl and Otten 2013). In parallel to our results, a positive correlation between turbidity and MC has been reported in several previous studies (Te and Gin 2011; Graham et al. 2004). Turbidity of surface water is increased with abundance growth of phytoplankton including cyanobacteria such as *Microcystis* resulting in the accumulation of more intracellular MCs (Te and Gin 2011; Lee et al. 2015). Regular monitoring of turbidity could be used as a proxy for MC concentrations in reservoirs, hence perturbation in turbidity is an indication of water contamination (Cunha et al. 2018). It is obvious that turbidity reduces the Secchi depth transparency and it could be the reason for the negative correlation that we obtained in this study for MC and CYN.

Based on the currently available literature, toxin-producing *Microcystis* and *Cylindrospermopsis* were first reported in Sri Lanka in 1907 in northern dry zone reservoirs as non-dominant genera in association with green algae and diatoms (Fritsch 1907a; Fritsch 1907b). Later, between the 1995-1996 periods, *Cylindrospermopsis* has been identified from Tisawewa as the dominant cyanobacteria and in Basawakkulama wewa as an occasionally present species (Weerakoon et al. 1998). At the same time, *Myrocystis* was identified as a dominant genus in northern dry zone reservoirs (Jayawardana et al. 1998). Several later research reported toxigenic genera *Cylindrospermopsis raciborskii* as the dominant cyanobacterial community in several northern dry zone reservoirs and its co-occurrence with *Microcystis* only in Unnichchai tank (Wanigatunge et al. 2014; Kulasooriya 2017 and Yatigamma and Perera 2017). Further, several other toxin-producing cyanobacteria such as *Planktolyngbya*, *Pseudoanabaena*, *Anabaena aphanizomenoides* (Silva and Wijeyaratne

1999), *Phormidium*, *Oscillatoria* (Liyanage et al. 2014) and *Lyngbya* (Shihana et. al. 2012) have been reported from the reservoirs in the northern dry zone. However, we did not observe any of those species in both reservoirs under study. When a lentic water body is eutrophicated, phytoplankton that are sensitive to nutrient pollution gradually disappear and more resistant species survive and tend to become dominant (Carmichael 1992). *Microcystis* has been previously reported dominant in freshwaters that underwent prolonged eutrophication (Krstić and Aleksovski 2016; Li et al. 2021). Therefore, the dominance of *Microcystis* in Mahakanadarawa and Nachchaduwa reservoirs could be a result of eutrophication. There is a limited number of reports on either MC or CYN in reservoirs of Sri Lanka. Only a few reservoirs in the northern dry zone have been tested for MC including Tisawewa, Abyayawewa, Thuruwila, Kalawewa and Mahakanadarawa (Hettiarachchi and Manage 2014) and CYN in Padaviya (Dissananyake et al. 2012). Our findings are also comparable with the studies of Hettiarachchi and Manage (2014) on Nuwarawewa and Rambewa reservoirs in which they did not detect MC, although *Microcystis* was observed as the dominant phytoplankton community with high cell densities in both reservoirs. Further, they reported *Anabaena* as the dominant cyanobacteria in Nachchaduwa and in contrast, we observed *Microcystis* as the dominant species and *Anabaena* was not found at the time of sample collection. Therefore, this is good evidence for variation in composition and diversity of cyanobacteria over time while indicating the importance of frequent monitoring of cyanobacteria and cyanotoxins in these reservoirs.

According to our observations, the spatial distribution pattern of CYN in Mahakanadarawa reservoir is approximately uniform while in Nachchaduwa it showed slight accumulation toward the direction of the South (Figures 4b and 4d). Although we did not observe any of the CYN-producing cyanobacterial species in either of the reservoirs, Wanigatunge et al. (2014) and Yatigamma and Perera (2017) have reported the presence

of potential CYN-producing cyanobacterial species *Cylindrospermopsis raciborskii* in Nachchaduwa. However, in this study, we did not observe *C. raciborskii* or any other CYN-producing species probably due to two reasons, one is the presence of very low densities of such filaments at the time of sampling due to their disappearance. It is known that CYN is persistent in water for a long time after the toxin-producing cyanobacteria have disappeared (Smith et al. 2008). The second reason could be the presence of other CYN-producing species such as *Lyngbya* and *Oscillatoria* in the reservoir (Seifert et al. 2007; Bormans et al. 2014). Hence, those were not captured when collecting water samples from the surface for the analysis of phytoplankton. As such, the mere presence or absence of toxin-producing cyanobacterial species in the water samples is not a reliable indicator for the presence of CYN.

Further, the co-occurrence of multiple toxins in freshwaters can arise the risk of their bioaccumulation in the tissues of aquatic organisms to levels exceeding those of the surrounding water (Mchau et al. 2021). Therefore, the human being at the highest level of the food chain is highly vulnerable to the bioaccumulation of cyanotoxins. As different toxins have different exposure routes, the co-occurrence of multiple toxins enhances the possibility of multiple exposures to the human population through ingestion of contaminated drinking water, consumption of aquatic organisms, fishing and recreational activities (Rastogi et al. 2015). Moreover, the consumption of agricultural products fed with toxin-contaminated irrigational water and meat from livestock that consumes toxin-contaminated water could increase the risk of exposure (Mchau et al. 2021). Not only that, but even in laboratory experiments, it has been proven that the combined effect of MC and CYN induces higher toxicity rather than pure MC or pure CYN (Gutiérrez-Praena et al. 2019). Even at relatively low doses, repetitive and multiple toxin exposures, could result in cumulative liver damage, which may lead to chronic liver diseases in long-term exposure (Chorus et al. 2000).

Therefore, the co-occurrence of multiple toxins can impose high health risks. Therefore, frequent monitoring and implementation of strategies for controlling potential toxigenic cyanobacterial blooms and their accumulation in water supplies and food are essential.

CONCLUSIONS

We detected co-occurrence of hepatotoxic MC and CYN in two multipurpose reservoirs in the Anuradhapura district in Sri Lanka. MC in Mahakanadarawa and CYN in Nachchaduwa were found above the maximum permissible levels for drinking water defined by WHO. *Microcystis* was dominant in both reservoirs and appears to be the MC producing genera. However, we did not observe any potential CYN-producing species in either of the reservoirs. The spatial distribution of two toxins in water bodies was highly variable and not correlated to *Microcystis* cell density, chlorophyll-*a* concentration or any other physico-chemical parameter of water. Most physico-chemical parameters of both reservoirs fell within the favorable range for drinking according to the Ambient Water Quality Standards of Sri Lanka and WHO standards. Although, MC and CYN levels exceeded WHO guidelines in both reservoirs and which indicated the unsuitability of water for drinking without treatment for toxins. Therefore, our findings highlight the necessity of routine monitoring for cyanotoxins in tropical freshwaters to minimize health risks that can be resulted due to long-term exposure to toxins. Further, we recommend more sampling within a year to better understand the spatial distribution pattern of MC and CYN toxins.

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REFERENCES

- Ambient Water Quality Standards, Central Environmental Authority, Sri Lanka (2019), viewed 20 March 2021, <http://www.cea.lk/web/en>
- American Public Health Association (1999), Standard methods for the examination of water and wastewater, Method 2540D Total Suspended Solids Dried at 103–105°C, https://www.researchgate.net/profile/ArvindSingh56/post/what_methods_are_used_in_determination_of_suspended_solids/attachment/59d631c479197b807798f88a/AS%3A367478443134981%401464625096492/download/4.pdf.
- Antunes, J.T., Leao, P.N. & Vasconcelos, V.M. (2015). *Cylindrospermopsis raciborskii*: Review of the distribution, phylogeography, and ecophysiology of a global invasive species. *Frontiers in Microbiology*, 6(13). DOI: <https://doi.org/10.3389/fmicb.2015.00473>
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), pp.1-15. DOI: <https://doi.org/10.1104/pp.24.1.1>
- Baker, A. L., Gretz, P., Coesel, C., Delwiche, S., Sarnoff, A., Silverside, A., Saint-Armand, T., Ryan and Van Egmond, W. (2012). Phycokey-an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects. University of New Hampshire Center for Freshwater Biology.
- Benamara, M., Gómez, E., Dhahri, R., and Serrà, A. (2021). Enhanced photocatalytic removal of cyanotoxins by Al-doped ZnO nanoparticles with visible-LED irradiation. *Toxins* 13(1), pp.66. DOI: <https://doi.org/10.3390/toxins13010066>
- Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E. & Funari, E. (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology*. 91(3), pp.1049–1130.

- Bormans, M., Lengronne, M., Brient, L. & Duval, C. (2014). Cyndrospermopsin accumulation and release by the benthic cyanobacterium *Oscillatoria* sp. PCC 6506 under different light conditions and growth phases. *Bulletin of Environmental Contamination and Toxicology*. 92(2), pp.243-7. DOI: <https://doi.org/10.1007/s00128-013-1144-y>.
- Bouaïcha, N., Mile, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., et al. (2019). Structural diversity, characterization and toxicology of microcystins. *Toxins*. 11:714. DOI: <https://doi.org/10.3390/toxins11120714>
- Burns, N.M., Rutherford, J.C. & Clayton, J.S. (1999). A monitoring and classification system for New Zealand lakes and reservoirs, *Lake and Reservoir Management* 15(4), pp. 255-271. DOI: <https://doi.org/10.1080/07438149909354122>.
- Carmichael, W. (1992). Cyanobacteria secondary metabolites-the cyanotoxins. *Journal of Applied Bacteriology* 72, pp. 445-459. DOI: <https://doi.org/10.1111/j.1365-2672.1992.tb01858.x>.
- Cerasino, L. and Salmaso, N. (2020) Co-occurrence of anatoxin-a and microcystins in Lake Garda and other deep subalpine lakes. *Advances in Oceanography and Limnology*, 11. DOI: <https://doi.org/10.4081/aiol.2020.8677>.
- Chiswell, R.K., Shaw, G.R., Eaglesham, G., Smith, M.J., Norris, R.L., Seawright, A.A. & Moore, M.R. (1999). Stability of cyndrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: Effect of pH, temperature, and sunlight on decomposition. *Environmental Toxicology*. 14, pp.155–161. DOI: [https://doi.org/10.1002/\(SICI\)1522-7278\(199902\)14:1<155::AID-TOX20>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1522-7278(199902)14:1<155::AID-TOX20>3.0.CO;2-Z).
- Chorus, I., I. R. Falconer, H. J. Salas and J. Bartram (2000). Health risks caused by freshwater cyanobacteria in recreational waters. *Journal of Toxicology and*

Environmental Health Part B: Critical Reviews 3(4), pp.323-347. DOI:
<https://doi.org/10.1080/109374000436364>

Cunha, D. G. F., Dodds, W. K. and Loiselle, S. A. (2018). Factors related to water quality and thresholds for microcystin concentrations in subtropical Brazilian reservoirs. *Inland Waters* 8(3), pp.368-380. DOI: <https://doi.org/10.1080/20442041.2018.1492526>

De la Cruz, A. A., N. Chernoff, J. L. Sinclair, D. Hill, D. L. Diggs and A. T. Lynch (2020). Introduction to cyanobacteria and cyanotoxins. *Water treatment for purification from cyanobacteria and cyanotoxins*: 1-35. DOI: <https://doi.org/10.1002/9781118928677.ch1>

Dissananyake, J., W. Ratnayake and P. Radella (2012). Effect of concentrated water from reservoirs of high prevalence area for chronic kidney disease (CKDu) of unknown origin in Sri Lanka on mice. Peradeniya University Research Session PURSE -2011, Proceeding and Abstracts, Vol.16,24th November, 2011, University of Peradeniya, PP. 94.

Du, X., Liu, H., Yuan, L., Wang, Y., Ma, Y., Wang, R., Chen, X., Losiewicz, M.D., Guo, H. and Zhang, H. (2019). The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A Systematic Review. *Toxins* (11) 530. <https://doi.org/10.3390/toxins11090530>.

Francy, D. S., Graham, J. L., Stelzer, E. A., Ecker, C. D., Brady, A. M., Struffolino, P. and Loftin, K. A. (2015). Water quality, cyanobacteria, and environmental factors and their relations to microcystin concentrations for use in predictive models at Ohio Lake Erie and inland lake recreational sites, 2013-14, US Geological Survey. DOI: <https://doi.org/10.3133/sir20155120>

- Fritsch, F. E. (1907a). A general consideration of the subaerial and freshwater algal flora of Ceylon. A contribution to the study of tropical algal ecology. Part 1. Subaerial algae and algae of the inland fresh waters. *Proceedings of the Royal Society London* 79, pp.197-254.
- Fritsch, F. E. (1907b). The subaerial and freshwater algal flora of the tropics. A phytogeographical and ecological study. *Annals of Botany* 21(82), pp.235-275. DOI: <https://www.jstor.org/stable/43235351>
- Graham J.L., Jones J.R., Jones S.B., Downing J.A. & Clevenger T.E. (2004). Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. *Water Research*. 38(20), pp.4395–4404. DOI: 10.1016/j.watres.2004.08.004
- Gutiérrez-Praena, D., R. Guzmán-Guillén, S. Pichardo, F. J. Moreno, V. Vasconcelos, Á. Jos and A. M. Cameán (2019). Cytotoxic and morphological effects of microcystin-LR, cylindrospermopsin, and their combinations on the human hepatic cell line HepG2. *Environmental toxicology* 34(3), pp.240-251. DOI: <https://doi.org/10.1002/tox.22679>
- Hartnell, D.M., Chapman, I.J., Taylor, N.G.H., Esteban, G.F., Turner, A.D. and Franklin, D.J (2020). Cyanobacterial abundance and microcystin profiles in two southern British lakes: The importance of abiotic and biotic Interactions. *Toxins* 12, 503; DOI:10.3390/toxins12080503
- Hettiarachchi, I. U. and Manage, P.M. (2014). Cyanobacterial cell density & intracellular MC-LR levels in drinking/irrigation reservoirs in Anuradhapura, Sri Lanka. The 1st Environment and Natural Resources International Conference, Bangkok, Thailand.
- Department of Irrigation Sri Lanka. Reservoir Status, viewed 21 June 2022. <https://www.irrigation.gov.lk>.

- Jayawardana, B., J. Jayalath, J. Padmasiri and S. Kulasooriya (1998). Occurrence of potential toxin producing cyanobacteria in reservoirs of Sri Lanka. Symposium on Cyanobacterial Toxins in Water. Postgraduate Institute of Science, University of Peradeniya.
- Juttner, F. and Lüthi, H. (2008). Topology and enhanced toxicity of bound microcystins in *Microcystis* PCC 7806. *Toxicon*. 51(3), pp.388-397. DOI: <https://doi.org/10.1016/j.toxicon.2007.10.013>
- Kaebnick, M., & Neilan, B. A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiology Ecology*, 35(1), pp.1-9. DOI: <https://doi.org/10.1111/j.1574-6941.2001.tb00782.x>
- Kesari, V., S. Kumar, I. Yadav, A. Chatterjee, S. Rai and Pandey, S. (2022). Ganga river water quality assessment using combined approaches: physico-chemical parameters and cyanobacterial toxicity detection with special reference to microcystins and molecular characterization of *microcystin synthetase (mcy)* genes carrying cyanobacteria. *Environmental Science and Pollution Research*. 29(9), pp.13122-13140. DOI: <https://doi.org/10.1007/s11356-021-16589-1>
- Kokocinski, M., Maria Cameán, A., Carmeli, S., Guzmán-Guillén, R., Jos, A., Mankiewicz-Boczek, J., Metcalf, J., Moreno, I, Isabel Prieto, A. & Sukenik, A. (2017). Cylindrospermopsin and congeners. *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*. 12, pp.127–137.
- Krstić, S. S., and Aleksovski, B. (2016). Dominance of *Microcystis* spp. in Lake Dojran—a consequence of 30 years of accelerated eutrophication. *Botanica Serbica*, 40(2).
- Kulasooriya, S. A. (2017). Toxin producing freshwater cyanobacteria of Sri Lanka. *Ceylon Journal of Science*. 46(1), pp.3-16. DOI: <http://doi.org/10.4038/cjs.v46i1.7413>

- LeGresley, M., & McDermott, G. (2010). Counting chamber methods for quantitative phytoplankton analysis-haemocytometer, Palmer-Maloney cell and Sedgewick-Rafter cell In: Microscopic and molecular methods for quantitative phytoplankton analysis of IOC Manuals and Guides No. 55 (eds. B. Karlson, C. Cusack and E. Bresnan), pp. 25-30. Paris, UNESCO.
- Li, H., Gu, X., Chen, H., Mao, Z., Shen, R., Zeng, Q. and Ge, Y. 2022. Co-occurrence of multiple cyanotoxins and taste-and-odor compounds in the large eutrophic Lake Taihu, China: Dynamics, driving factors, and challenges for risk assessment. *Environmental Pollution* 294, 118594. DOI: <https://doi.org/10.1016/j.envpol.2021.118594>
- Li, M., Yu, R., Cao, Z., Jin, Y., Sun, H. and Qi, Z. (2021). Analysis of optimal environmental conditions for *Microcystis* blooms in large, shallow, eutrophic lakes, *All Life* 14(1), pp. 340-354, DOI: <http://doi.10.1080/26895293.2021.1919569>
- Liyanage, H. M., Magana-Arachchi, D. N., Chandrasekharan, N. V. and Kulasoorya, S. A. (2014). Detection and quantification of cylindrospermopsin in water reservoirs of Sri Lanka using high performance liquid chromatography (HPLC) In: Illeperuma, O. A. et al. (eds) *Symposium Proceedings, International Symposium on Water Quality and Human Health: Challenges Ahead*, 27 – 28, June, PGIS, Peradeniya, Sri Lanka pp. 25.
- Massey, I. Y. and Yang, F. (2020). A mini review on microcystins and bacterial degradation. *Toxins* 12(4), pp. 268. DOI: <https://doi.org/10.3390/toxins12040268>.
- Mchau, G. J., R. Machunda, M. Kimanya, E. Makule, Y. Y. Gong, E. Mpolya, J. P. Meneely, C. T. Elliott and B. Greer (2021). First report of the co-occurrence of cylindrospermopsin, nodularin and microcystins in the freshwaters of Lake Victoria, Tanzania. *Exposure and Health* 13(2), pp.185-194. DOI: <https://doi.org/10.1007/s12403-020-00372-7>.

- Magonono, M., Oberholster, P., Addmore, S., Stanley, M. and Gumbo, J. (2018). The presence of toxic and non-toxic cyanobacteria in the sediments of the Limpopo River Basin: Implications for human health. *Toxins* 10(269). DOI: <https://10.3390/toxins10070269>.
- National Water Supplies and Drainage Board, viewed 21 June 2022. <http://www.waterboard.lk/>
- Nienaber, M. A. and M. Steinitz-Kannan (2018). A guide to cyanobacteria: identification and impact, University Press of Kentucky.
- Nowruzi, B. and S. J. Porzani (2021). Toxic compounds produced by cyanobacteria belonging to several species of the order Nostocales: A review. *Journal of Applied Toxicology* 41(4), pp.510-548. DOI: <https://doi.org/10.1002/jat.4088>
- Ohtani, I., Moore, R.E. & Runnegar, M. (1992). Cyindrospermopsin: A potent hepatotoxin from the blue-green alga *Cyindrospermopsis raciborskii*. *Chemical Information* 114, pp.7941–7942. DOI: <https://doi.org/10.1021/ja00046a067>
- O’Neil, J.M., Davis, T.W, Burford, M.A., Gobler, C.J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14, pp. 313–334.
- Paerl, H.W. and Otten, T.G. (2013). Blooms bite the hand that feeds them. *Science* 342, pp 433–434.
- Persaud, A.D., Paterson, A.M., Dillon, P.J., Winter, J.G., Palmer, M. & Somers, K.M. (2015). Forecasting cyanobacteria dominance in Canadian temperate lakes. *Journal of Environmental Management* 151, pp.343–352. DOI: <https://doi.org/10.1016/j.jenvman.2015.01.009>.
- Preece, E.P., Hobbs, W., Hardy, F.J., O’Garro, L., Frame, E. and Sweeney, F. (2021). Prevalence and persistence of microcystin in shoreline lake sediments and porewater,

and associated potential for human health risk. *Chemosphere* 272:129581. DOI: <http://10.1016/j.chemosphere.2021.129581>.

Preußel, K., Wessel, G., Fastner, J. & Chorus, I. (2009). Response of cylindrospermopsin production and release in *Aphanizomenon flos-aquae* (Cyanobacteria) to varying light and temperature conditions. *Harmful Algae*. 8, pp.645–650. DOI: <https://doi.org/10.1016/j.hal.2008.10.009>.

Rastogi, R., D. Madamwar and A. Incharoensakdi (2015). Bloom dynamics of cyanobacteria and their toxins: environmental health impacts and mitigation strategies, *Frontiers in Microbiology*. 6 (2015), pp.1–22. DOI: <https://doi.org/10.3389/fmicb.2015.01254>

Rigosi, A., Carey, C.C., Ibelings, B.W. & Brookes, J.D. (2014). The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. *Limnology and Oceanography* 59, pp.99–114.

DOI: <https://doi.org/10.4319/lo.2014.59.1.0099>

Seifert, M., G., McGregor, G., Eaglesham, W., Wickramasinghe, G. & Shaw (2007). First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck. *Harmful Algae*. 6, pp.73-80.

DOI: <https://doi.org/10.1016/j.hal.2006.07.001>

Shihana, F., Jayasekera, J. M. K. B., Dissanayake, D. M., Ratnayake, P., Wickremasinghe, W. and Radella, Y. A. (2012). The short-term effect of cyanobacterial toxin extracts on mice kidney. In: Illeperuma, O. A. et al (eds) *Symposium Proceedings, International Symposium on Water Quality and Human Health: Challenges Ahead*, 22- 23, March PGIS, Peradeniya, Sri Lanka pp.13.

- Silva, E. and Wijeyaratne, M. (1999). The occurrence of cyanobacteria in the reservoirs of the Mahaweli River basin in Sri Lanka. *Sri Lanka Journal of Aquatic Science* 4, pp.51-60.
- Smith, M.J., Shaw, G.R., Eaglesham, G.K., Ho, L. & Brookes, J.D. (2008). Elucidating the factors influencing the biodegradation of cylindrospermopsin in drinking water sources. *Environmental Toxicology*. 23(3), pp.413-21. DOI: <https://doi.org/10.1002/tox.20356>. PMID: 18214886.
- Spoof, L. & Catherine, A. (2017). Table of microcystins and nodularins. In: Meriluoto, J., Spoof, L., Codd, G.A., editors. Handbook of cyanobacterial monitoring and cyanotoxin analysis. Chichester, UK: John Wiley & Sons. pp. 526-37.
- Tammy, A. L., Gretchen, R. W., Stephen, M. B. and Joshua J. F. H. (2015). Environmental influence on cyanobacteria abundance and microcystin toxin production in a shallow temperate lake. *Ecotoxicology and Environmental Safety*. 114, pp. 318-325. DOI: <https://doi.org/10.1016/j.ecoenv.2014.05.004>.
- Te S.H. and Gin K.Y.H. (2011). The dynamics of cyanobacteria and microcystin production in a tropical reservoir of Singapore. *Harmful Algae*. 10, pp. 319–329. DOI: 10.1016/j.hal.2010.11.006
- Testai, E., Scardala, S., Vichi, S., Buratti, F. M., & Funari, E. (2016). Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. *Critical Reviews in Toxicology*. 46(5), pp.385-419. DOI: <https://doi.org/10.3109/10408444.2015.1137865>
- US-Environmental Protection Agency (1978). Method 365.3: Phosphorous, all forms (Colorimetric, Ascorbic Acid, Two Reagent), viewed 2 March 2021. https://www.epa.gov/sites/default/files/2015-08/documents/method_365-3_1978.pdf.

US-Environmental Protection Agency (2013). Surface water sampling, viewed 2 March 2021. <https://www.epa.gov/sites/production/files/2015-06/documents/Surfacewater-Sampling.pdf>

US-Environmental Protection Agency (2016). Method 546: Determination of total microcystins and nodularins in drinking water and ambient water by Adda Enzyme-Linked Immunosorbent Assay, viewed 2 March 2021. <https://www.epa.gov/sites/production/files/2016-09/documents/method-546-determination-total-microcystins-nodularins-drinking-water-ambient-water-adda-enzyme-linked-immunosorbent-assay.pdf>.

US-Environmental Protection Agency (2017). Method 545: Determination of cylindrospermopsin and anatoxin-a in drinking water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS), viewed 2 March 2021. https://www.epa.gov/sites/production/files/2017-10/documents/epa_815-r-15-009_method_545.pdf.

Wanigatunge, R., Magana-arachchi, D., Chandrasekharan, N. & Kulasooriya, S. (2014). Genetic diversity and molecular phylogeny of cyanobacteria from Sri Lanka based on 16S rRNA gene. *Environmental Engineering Research*. 19(4), pp-317-329.

DOI: <https://doi.org/10.4491/eer.2014.035>

Weerakoon, A., Jayawardana, B., Padmasiri, J. and Kulasooriya, S. (1998). Comparison of algal populations of Tisawewa and Basawakulama tanks in Anuradhapura. Symposium on Cyanobacterial Toxins in Water. Postgraduate Institute of Science, University of Peradeniya.

World Health Organization (2017). Water quality and health-review of turbidity: information for regulators and water suppliers, viewed 9 March 2021.

<https://apps.who.int/iris/bitstream/handle/10665/254631/WHO-FWC-WSH-17.01-eng.pdf>.

World Health Organization (2019). Cyanobacterial toxins: cylindrospermopsin. Background document for development of WHO Guidelines for drinking-water quality and Guidelines for safe recreational water environments, viewed 20 April 2021. https://www.who.int/water_sanitation_health/water-quality/guidelines/chemicals/cylindrospermopsin-gdwq-bd-for-review-20191120.pdf.

World Health Organization (2020). Cyanobacterial toxins: microcystins. Background document for development of 2017. Guidelines for drinking-water quality and Guidelines for safe recreational water environments, viewed 20 March 2021. <https://apps.who.int/iris/bitstream/handle/10665/338066/WHO-HEP-ECH-WSH-2020.6-eng.pdf>.

Xingqiang, W., Christian, N., Liu, L. & Andreas, L. (2019). Effects of small-scale turbulence at the air-water interface on microcystis surface scum formation. *Water Research*. 167. DOI: <https://doi.org/10.1016/j.watres.2019.115091>.

Yatigamma, S. & Perera, M. (2017). Distribution of *Cylindrospermopsis raciborskii* (cyanobacteria) in Sri Lanka. *Ceylon Journal of Science* 46(3), pp.65-80. DOI: <http://doi.org/10.4038/cjs.v46i3.7444>.

Žegura, B. Štraser, A. & Filipič, M. (2011). Genotoxicity and potential carcinogenicity of cyanobacterial toxins: a review. *Mutation Research/Reviews in Mutation Research*. 727, pp. 16–41. DOI: <https://doi.org/10.1016/j.mrrev.2011.01.002>