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Using Phycocyanin as Cyanobacterial Biomass Indicator to Determine Potentially- Toxic Bloom: An Example from a Malaysia Reservoir

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Abstract

Cyanobacterial bloom is a serious threat to the safety of public drinking water resources due to potential cyanotoxin microcystin contamination. Water operators inadequately monitor occurrences and dynamics of cyanobacterial blooms and their toxic potential. An effective monitoring and risk assessment can be challenging as the commonly used cyanobacterial biomass indicator might not explain the actual bloom's toxicity. This study aimed to determine a reliable cyanobacterial biomass indicator for monitoring toxigenic blooms in the reservoir used for public drinking water supply. The reservoir was sampled for 12 months at three sampling sites to determine its water physicochemical properties, cyanobacterial biomass abundance, and toxigenic potential estimated by *mcyE* gene copy numbers. Cyanobacterial biomass was quantified as total chlorophyll-*a* and blue-green algae phycocyanin (BGA-PC). The results showed that total chlorophyll-*a* and BGA-PC are highly variable on temporal and spatial scales. *Microcystis* spp. was the dominant toxigenic cyanobacteria, with the mean *mcyE* gene concentrations ranging from 29.5 to 1,180,144.06 copies/mL. Meanwhile, *Anabaena* spp. and *Planktothrix* spp. were also detected in several samples, with the mean *mcyE* gene concentrations ranging from 28.25 to 3,877.05 copies/mL and 18.45 to 3,017.4 copies/mL, respectively. Pearson correlation analysis revealed that *mcyE* gene was only significantly correlated to BGA-PC ($R = 0.553$, $P < 0.05$). Hence, BGA-PC can be utilized as a reliable cyanobacterial biomass indicator for monitoring toxigenic cyanobacterial blooms. This first report on toxigenic cyanobacterial biomass in the reservoir signifies the importance of bloom monitoring and microcystin risk assessment using blue-green algae phycocyanin pigment.

Keywords: Cyanobacterial harmful algal blooms (CHABs); Cyanotoxins; Risk assessment; *mcyE* gene; Photosynthetic pigments

1. Introduction

Cyanobacterial blooms have been widely reported worldwide and primarily affect water quality and disturb water ecosystem stability (Kubickova *et al.,* 2019; Méresse *et al.,* 2022; Ren *et al.,* 2022). Cyanobacterial blooms have become more severe due to climate change that emerges in all the continents. High cyanobacterial biomass in freshwater bodies used for drinking water supply could pose a significant health risk to water consumers due to possible cyanotoxin contamination. Cyanotoxins, particularly microcystins, have raised health risk concerns as it is known to have hepatotoxic effects.

In the freshwater ecosystem, cyanobacteria often grow and dominate as a mixed assemblage of cyanobacterial species (Zhang *et al.,* 2021). The most common cyanobacterial species found in freshwater include toxic and nontoxic

strains of *Microcystis* spp., *Anabaena* spp., *Planktothrix* spp., and *Nostoc* spp. (Massey *et al.,* 2022; Pham & Utsumi, 2018). In response to this issue, many efforts have been made globally to understand the dynamics and environmental triggers of cyanobacterial growth, abundance, and toxicity in various freshwater ecosystems (Zhang *et al.,* 2021; Barçante *et al.,* 2020; Hu *et al.,* 2021; Park *et al.,* 2021; Pham *et al.,* 2021). A wide range of water physicochemical and biological factors, climatic and hydrological factors are known to influence the dynamics of cyanobacteria and its toxic potential in freshwater bodies (Pham *et al.,* 2021; Gophen, 2021; Kimambo *et al.,* 2019; Pound *et al.,* 2021; Sukchinda *et al.,* 2019). Nevertheless, no single factor can fully explain their growth, abundance, and toxicity, as different cyanobacterial species may respond differently to environmental factors. Hence, it is difficult to control the growth and determine the toxicity of cyanobacterial communities in freshwater bodies (Huo *et al.,* 2021).

Uncertain threats posed by cyanobacterial blooms in freshwater ecosystems imply that monitoring and risk assessment should be prioritized in the water bodies with possible human exposure to cyanotoxin microcystins, such as drinking water supply reservoirs. Cyanobacterial blooms and their toxicity should be monitored and controlled by water supply operators to ensure safe drinking water for the end users. According to the monitoring guidelines established by WHO, risks of cyanotoxin exposure can be determined through the quantification of microcystins concentration or cyanobacterial biomass in the form of chlorophyll-*a* concentration (Chorus & Welker, 2021; WHO, 2020). Cyanobacterial biomass quantification, usually measured as species biovolumes or total chlorophyll-*a* concentrations, is a preferred monitoring and risk assessment parameter as it is rapid and inexpensive over direct microcystins quantification (Felip & Catalan, 2000). However, the total biomass may not depict the actual toxic potential of cyanobacterial communities due to the coexistence of toxic and non-toxic species (Ninio *et al.,* 2020). Besides, water operators have not prioritized the monitoring of toxigenic cyanobacterial blooms in drinking water resources over the past years.

In recent years, molecular techniques have been the most promising method to determine and quantify toxigenic cyanobacterial species. Polymerase Chain Reactions (PCR) and quantitative real-time PCR (qPCR) are widely used to identify and quantify microcystin-producing genes in the mixed assemblage of cyanobacterial communities commonly occurring during freshwater blooms. Microcystin synthetase gene cluster containing ten genes, namely *mcyA* to *mcyJ*, is responsible for synthesizing microcystins in cyanobacteria (Dittmann & Börner, 2005; Rantala *et al.,* 2006). The detection of these microcystin biosynthetic genes usually indicates the presence of potentially toxic cyanobacteria in the mixed communities. Among other microcystin biosynthetic genes, *mcyE* is rather unique as this gene encodes an enzyme and polyketide peptide synthetase, which is responsible for the synthesis of the two most toxic amino acids in the microcystins molecule (Chen *et al.,* 2021).

In certain areas in Malaysia, the reservoir serves as important freshwater sources for residential and industrial supplies. Despite the comprehensive treatment of raw water from the reservoir before distribution, it is crucial to examine the potential cyanobacterial growth within the reservoir. Such investigation holds immense importance for ensuring water safety and security. To date, the reports of cyanobacterial abundance in the local reservoir are rather limited. For instance, cyanobacterial dominance has been reported in Semberong Barat Dam, Johor Bahru and Air Itam Dam, Penang, Malaysia. However, no cyanobacterial biomass quantification was reported in these studied reservoirs (Omar et al., 2017; Rohaslinda, 2017).

This study was conducted to understand the dynamics of water physicochemical properties, and the relationship of cyanobacterial biomass and *mcyE* gene copy number in the reservoir used for water supply. This study also aimed to validate the most reliable cyanobacterial biomass parameters for predicting the toxicity of cyanobacterial communities. The authors hypothesized that

blue-green algae phycocyanin (BGA-PC) concentration can be an alternative and reliable cyanobacterial biomass indicator for predicting and monitoring the potentially toxic blooms. This study is the first to report on the occurrence and dynamics of cyanobacterial abundance and potentially toxic blooms in Malaysia reservoir. The results of this study will provide researchers and water operators with a better understanding of the temporal and spatial variations of cyanobacterial biomass, as well as an alternative parameter for cyanobacterial biomass quantification for risk assessment and monitoring purposes.

2. Methodology

2.1 Study location

This study was conducted in one of the reservoir facilities in Peninsular Malaysia. This reservoir is experiencing cyanobacterial blooms throughout the year. The studied reservoir has formed from the confluence of two rivers and is located within the catchment area with mixed land use, including residential, plantation, and industrial areas as shown in Figure 1. The studied reservoir has a 52 hectares' catchment area with a total storage of 10.6 million cubic meters and 37 meters' height. The study area has a tropical climate with an average air temperature of 26.3 °C and the annual rainfall of 2512 mm.

2.2 Sample collection

Sampling was conducted bimonthly from June 2022 to June 2023. Throughout this sampling period, 24 sampling events were carried out. In the reservoir, three sampling sites were selected to represent the whole hydrological environment of the water body. These three sites were located on the east and south sides of the reservoir as the west side is not accessible. The distance between the selected sampling sites is at least 500 m. In detail, Site A is located near the agricultural land, while Site B is in the middle of the reservoir, where the facility's water sampling and reservoir monitoring tower are located. Site C is located within 1km from the raw water intake (Figure 1). During each sampling event, water samples were collected in triplicate at each sampling site by using a water sampler. Samples were taken from approximately 15 cm below the surface to avoid dirt and plant debris. Upon collection, water samples were stored in 1L cleaned high-density polyethylene (HDPE) bottles and were kept in an ice box (~ 4 °C) before being transported to the laboratory for further analyses.

2.3 Water physicochemical properties

Physicochemical properties of the water samples, including water temperature, conductivity, pH, and dissolved oxygen, were

Figure 1. Map of the studied reservoir showing the three sampling sites

analyzed on-site and in the laboratory. Water temperature and conductivity were measured using a portable Aquapro digital water tester (HM Digital, Inc.). The dissolved oxygen was measured using a portable Thermoscientific® Eutech Dissolved Oxygen meter. The water pH was analyzed using a benchtop pH meter (Sartorius®).

2.4 Cyanobacterial biomass quantification

Cyanobacterial biomass was determined based on concentrations of chlorophyll-*a* and blue-green algae phycocyanin (BGA-PC). Chlorophyll-*a* is commonly used for measuring algae biomass in waterbodies and its spatial and temporal variability. It also can be used to classify the trophic condition of a waterbody. Meanwhile, BGA-PC is specifically used for measuring blue-green algae biomass. This water-soluble and photosynthetic pigment gives a blue color to cyanobacteria and functions as light energy capture during photosynthesis (Simkin *et al.,* 2022).

In this study, both chlorophyll-*a* and blue-green algae phycocyanin (BGA-PC) were measured triplicate using Aquaprobe sensors (Aquaread®) in 1000 mL of water samples. The aquaprobe sensors are fixed response fluorometers in which these parameters were measured primarily through fluorescence detection. These sensor probes provide excitation at specific wavelengths which is 470 nm for chlorophyll-*a* and 590 nm for BGA-PC. The aquaprobe sensors also detected any resultant fluorescence above 630 nm and 655 nm, respectively. In brief, the electrodes on the aquaprobe sensors will induce the chlorophyll-*a* and phycocyanin to fluoresce, then the longer wavelength light emitted as a result of the fluorescence will be measured and converted into µg/L and cells/mL, respectively.

2.5 Quantification of mcyE gene copy number

2.5.1 DNA extraction from water samples

The *mcyE* gene copy numbers were quantified from the composite samples combining sites A, B, and C for each sampling date. In total, 24 samples were processed for *mcyE* gene quantification. Cyanobacterial cells were harvested on a glass microfibre filter, 0.45 µm (Whatman®), from 500 to 1000 mL of water samples. The used water volumes depend on the cyanobacterial biomass concentration measured using an Aquaread® AP-Lite probe. The filter papers containing the harvested cyanobacterial cells were frozen at -20 °C until the extraction procedure. The DNA extraction was carried out within 48 hours after the filtration.

Cyanobacterial DNA samples were extracted from the frozen filter papers using the PrimeWay Genomic DNA extraction kit (1st Base). The DNA extraction methods were performed according to the manufacturer's instructions. The concentrations and purities of the extracted DNA were measured using a spectrophotometer with an absorbance ratio of 260 nm/280 nm (A260/A280).

2.5.2 PCR

Microcystin synthetase E gene (*mcyE* gene) PCR amplifications were performed using primer sets developed by the previous studies. The PCR assays target the genus-specific *mcyE* gene (Vaitomaa *et al.,* 2003; Rantala *et al.,* 2006). The assays were optimized to detect the target gene from the collected water samples. All amplifications were performed in 50 µL reaction volume using ThermoScientific™ Dream Taq PCR Master Mix. Each PCR reaction containing 25 µL of PCR master mix, 0.1 – 1.0 µM of forward and reverse primers, 10 pg – 1 µg of DNA template, and nuclease-free water up to 50 µL of reaction volume. The thermal cycling condition was performed with an initial denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 s, annealing at 46.1°C (*Anabaena mcyE*, 47 °C (*Microcystis mcyE*), 44.4 °C (*Planktothrix mcyE*) for 30 s with 40 cycles, extension at 72 °C for 1 min, and final extension at 72 °C for 15 min.

2.5.3 qPCR

The concentrations of toxigenic *Anabaena* spp., *Microcystis* spp., and *Planktothrix* spp. (equivalent to *Anabaena mcyE*, *Microcystis mcyE*, and *Planktothrix mcyE*) were assayed by SYBR Green-based qPCR. The primers

sequences of the genus-specific *mcyE* gene are listed in Table 1. The qPCR mixtures containing SYBR Green qPCR Master Mix, 10 µL, forward and reverse primers, 0.8 µL each, DNA template, 1 µL, and nuclease-free water up to 20 µL reaction volume. The thermal cycling protocol was performed with an initial denaturation at 95 °C for 2 min, denaturation at 95 °C for 5 s, and annealing/ extension at 62 °C for 20 s with 40 cycles.

2.5.4 qPCR standard curve preparation

In this study, the reference strains used for positive control were *Microcystis aeruginosa* NIES 89, *Anabaena* sp. BIR259, and *Planktothrix agardhii* NIVA-CYA127. A standard curve as shown in Figure 2 was plotted from the reference strains to quantify the copy number of the *mcyE* gene of the selected cyanobacterial species in the water samples and the analysis was run in duplicate. The copy number of each gene was calculated according to its molecular weight and concentration and then converted into a copy number based on Avogadro's number (1 mol = 6.02214 x 10^{23}) (Baxa *et al.,* 2010; Rinta-Kanto *et al.,* 2005). For the toxic cyanobacterial species (*Anabaena*, *Microcystis*, and *Planktothrix*), the number of *mcyE* gene copy numbers was directly utilized as the number of cell equivalents, since there was only one copy of the *mcyE* gene per genome (Baxa *et al.,* 2010).

2.6 Data processing and Statistical analyses

Statistical analyses were done using IBM Statistical Package for the Social Sciences (SPSS Inc. Version 23). The data for physicochemical properties and cyanobacterial biomass were expressed as mean ± standard deviation for each sampling date and site. The mean and standard deviations were calculated from the measured triplicate samples. A data normality test was performed before statistical analysis. The normality of data was assessed based on the Shapiro-Wilk test. Data were then log-transformed to meet the assumption of normality. Pearson correlation analysis was conducted to identify correlations between *mcyE* gene copy numbers and cyanobacterial biomass measured as chlorophyll-*a*, and BGA-PC concentrations were used as the average.

Table 1. Primers sequences for genus-specific *mcyE* gene

No.	Primers	Primer sequence	Reference
1	Genus-specific *Microcystis* spp.	Forward primer sequence (Microcystis_mcyE_F1): 5'- GAA ATT TGT GTA GAA GGT GC - 3' Reverse primer sequence (Microcystis_mcyE_R1): 5'- CAA TGG GAG CAT AAC GAG - 3'	(Vaitomaa *et al.,* 2003)
2	Genus-specific *Anabaena* spp.	Forward primer sequence (Anabaena_mcyE_F1): 5' – GAA ATT TGT GTA GAA GGT GC - 3' Reverse primer sequence (Anabaena_mcyE_R1): 5' – CAA TCT CGG TAT AGC GGC - 3'	(Vaitomaa *et al.,* 2003)
3	Genus-specific *Planktothrix* spp.	Forward primer sequence (Planktothrix_mcyE_F1): 5' - GAA ATT TGT GTA GAA GGT GC - 3' Reverse primer sequence (Planktothrix_mcyE_R1): 5'- CTC AAT CTG AGG ATA ACG AT - 3'	(Vaitomaa *et al.,* 2003; Rantala *et al.,* 2006)

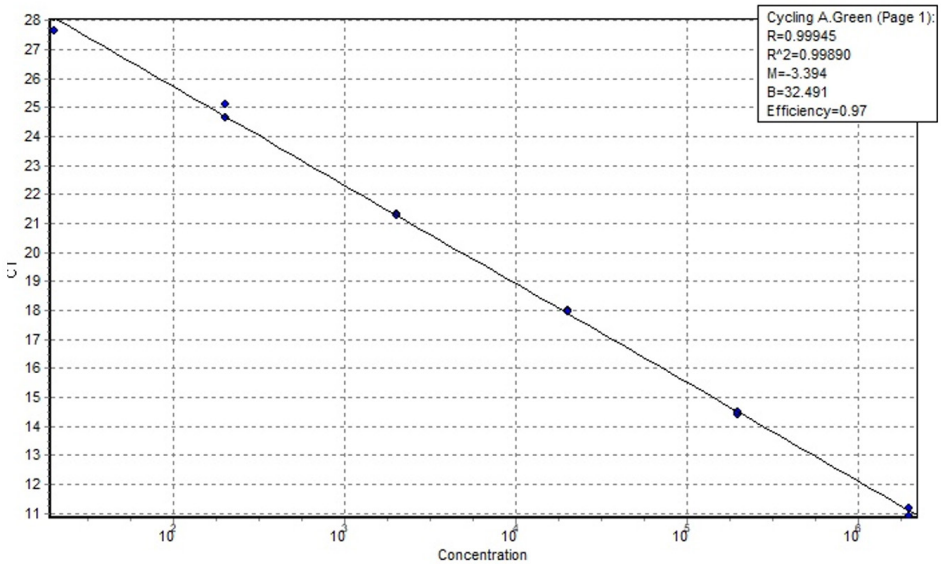


Figure 2. A standard curve of the *Microcystis mcyE* gene was plotted with concentrations ranging from 2000000 to 20 DNA copies (log (DNA copy) on the x-axis), R^2: 0.9989, and qPCR efficiency: 97%

3. Results and Discussion

3.1 Water physicochemical parameters of the reservoir

In general, water physicochemical parameters fluctuated throughout the sampling period. Figure 3 (a-d) shows the variabilities of water temperature, pH, dissolved oxygen, and conductivity in sites A, B, and C of the studied reservoir. Water temperature fluctuate along the sampling period, with mean values ranging from 26.0 to 30.8 °C (Figure 3a). The lowest and highest water temperatures were recorded in September 2022 and June 2022, respectively. The dissolved oxygen levels also displayed temporal variations with mean values ranging from 5.89 to 10.28 mg/L, with the lowest and highest values recorded in December 2022 and February 2023, respectively (Figure 3b). Meanwhile, shifts in pH levels throughout the sampling period were also observed with mean values ranging from 5.66 to 8.5 (Figure 3c). The lowest and highest mean pH values were observed in August 2022 and May 2023, respectively. In contrast to pH, variations in conductivity showed an opposite temporal pattern (Figure 3d). The mean water conductivity ranged from 105.3 to 134.00 mS/m, with the lowest and highest being observed in January 2023 and September 2022, respectively.

These fluctuations indicate the dynamics of physicochemical properties in the studied reservoir, whereby the dynamics are influenced by many factors. The prolonged dry period, becoming more common due to climate change will lead to fluctuations in water temperatures (Palinkas & Wong, 2020). Changes in water temperature may promote cyanobacterial blooms and further enhance aquatic ecosystem distress (Kazmi *et al.,* 2022). It has been reported that cyanobacterial growth rates may be enhanced by warming waters more quickly compared to other phytoplankton, even in low nutrient concentrations (Freeman *et al.,* 2020; Visser *et al.,* 2016). Mullin *et al.* (2020) have reported that surface water temperatures above 25 °C may trigger the formation of cyanobacterial blooms. Different cyanobacterial species may have maximum growth rates at different temperatures. Several studies have reported an association between cyanobacterial abundance and high water temperatures, which showed the optimum growth of certain cyanobacterial species at different temperatures (Richardson *et al.,* 2019; Smucker *et al.,* 2021). For example, temperatures around 30.6 ± 2.3 °C may favor the growth of *Anabaena* spp. (Nalley *et al.,* 2018), while temperatures between 28°C and 37 °C may promote the growth of *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii* (Thomas & Litchman, 2016).

Apart from temperature, dissolved oxygen is another key indicator of health and stability of freshwater ecosystems. Its concentration is vital in aquatic existence and water body components' variation and transition (Yu *et al.,* 2020). In this reservoir, the dissolved oxygen levels were reported above 5 mg/L which indicates a healthy ecosystem for aquatic organisms to survive. Monitoring dissolved oxygen concentration in the water bodies is essential to indirectly assess any pollutants which will impact the level of dissolved oxygen. Besides, the fluctuation of pH levels may be influenced by carbon dioxide concentration in the water (Prakash, 2021). pH levels increase during the photosynthesis process of algae and aquatic plants, reducing the hydrogen ions in the water, while the decomposition and respiration process lowers the pH levels (Dey *et al.,* 2021). The highest pH was recorded at sampling point A during the occurrence of the highest cyanobacterial biomass.

Regarding water conductivity, its temporal variabilities highlighted the shifts in dissolved ions and contaminants throughout the sampling period. In the wet season, pollutants from the basin are flushed into water bodies in high quantities, resulting in water quality changes (Wang *et al.,* 2020). Matej-Łukowicz *et al.* (2023) explained that the significant pollutant deposition in water bodies may have originated from the high inflow of pollutant load for a short period during the wet season. Besides, the underlying geology of the water body also plays an essential

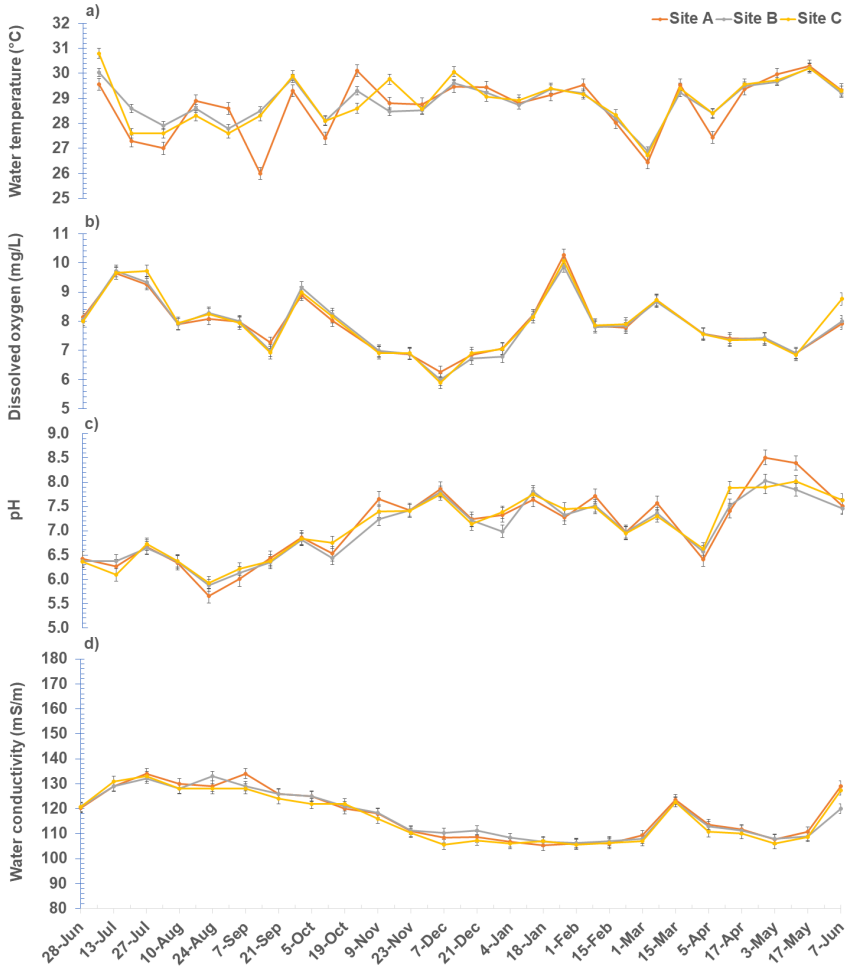


Figure 3. Variability of water physicochemical parameters in sites A, B, and C

role in determining water conductivity, as the soluble ions are different based on the topography characteristics of the area (Lintern *et al.,* 2018; Mainali & Chang, 2021). In this study, fluctuations of these major water physicochemical parameters depict the dynamic nature of the studied reservoir ecosystem and the complex interplay of various factors influencing the water quality (Akhtar *et al.,* 2021).

3.2 Abundance of cyanobacterial biomass

The presence of cyanobacterial biomass in the studied reservoir was determined by cyanobacterial-specific pigments detection, including total chlorophyll-*a* and BGA-PC. Figure 4(a) and Figure 4(b) showed the concentrations of chlorophyll-*a* and BGA-PC at sites A, B, and C throughout the study period. The mean total chlorophyll-*a* concentrations varied from 95.95 to 857.67 µg/L, as shown in Figure 4(a). Meanwhile, the highest and lowest mean chlorophyll-*a* concentrations were recorded in October 2022 and June 2023, respectively.

Besides, the BGA-PC concentrations also showed temporal variability in all sampling sites. In Figure 4(b), the mean total BGA-PC concentrations varied from 2,944.67 to 83,521.33 cells/mL, while the highest and lowest mean BGA-PC concentrations were recorded in August 2022 and February 2023. On a spatial basis, the concentrations of BGA-PC at sites A, B, and C showed trend fluctuations along the sampling period.

In general, site A recorded higher chlorophyll-*a* and BGA-PC concentrations compared to site B and C. Based on Figure 5 (a-b), the highest concentration of chlorophyll-*a* and BGA-PC were recorded at site A, with the maximum value being 857.7 µg/L and 82,375 cells/mL, respectively. Certain environmental factors impacting the cyanobacterial abundance may explain the observed temporal and spatial variabilities.

Many recent studies have highlighted the significance of meteorological and hydrological conditions, such as wind speeds, residence time, outflows, and water level fluctuations, on cyanobacterial abundance (Rao *et al.,* 2021; Yang *et al.,* 2018). Site A showed the highest concentrations of cyanobacterial biomass compared to other sites, potentially due to a slight difference in the hydrological characteristic of the sampling site. Through observation, Site A has lower circulation or stagnant water than Site B and Site C. Furthermore, the tendency of cyanobacterial biomass to accumulate in site A is high due to wind direction. Besides, higher cyanobacterial biomass in site A may

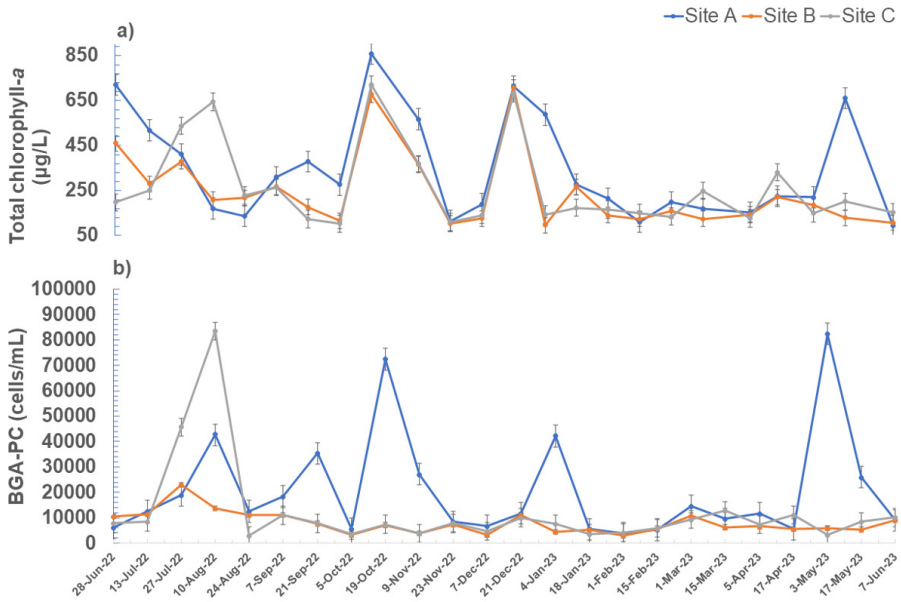
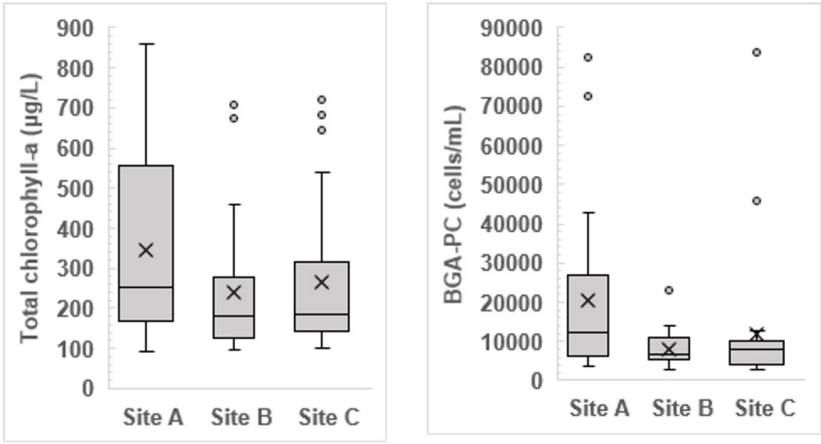


Figure 4. (a,b) The temporal variability of chlorophyll-*a* and BGA-PC in sites A, B, and C

be explained by potentially nutrient-rich water as this site is located near agricultural land. An excessive nutrient runoff from the agricultural land as well as the internal total phosphorus and total nitrogen loading in the reservoir lead to cyanobacterial proliferation since this species is capable to thrive in nutrient-rich environments and sustain nitrogen fixation (Aeriyanie *et al.,* 2021; Richardson *et al.,* 2019; Zou *et al.,* 2020). Hence, different environmental factors may explain the dynamics of cyanobacterial abundance at these sampling sites.

3.3 mcyE gene quantification

The results of PCR assays used for *mcyE* gene quantification are shown in Table 2. In general, cyanobacterial cells were detected consistently throughout the sampling period using primers targeting the cyanobacteria 16S rRNA gene. Meanwhile, *Microcystis mcyE* gene was detected in all (100%) water samples throughout the sampling period, suggesting the dominance of *Microcystis* spp. in this studied reservoir. However, *Anabaena mcyE* and *Planktothrix mcyE* were detected only at certain sampling periods. *Anabaena mcyE* and *Planktothrix mcyE* were detected in 50% and 33.3% of water samples, respectively. *Anabaena mcyE* was detected between November 2022 and June 2023, while *Planktothrix mcyE* was detected between late October 2022 and May 2023. This data shows the variability of the toxic potential of cyanobacterial species in the studied reservoir at different sampling periods.



(a) (b)

Figure 5. (a,b) Boxplot for estimated chlorophyll-*a* concentrations (µg/L) and BGA-PC concentrations (cells/mL) at sampling sites A, B, and C. The box length shows the interquartile range and 50% of cases of the variable. The line in the box indicates the median value, while extended lines from the box are the smallest and largest values. The mean values are represented by x. Cases extended more than the box lengths are represented by a circle ○

Table 2. The number of PCR-positive samples for cyanobacterial 16S rRNA, *Microcystis mcyE, Anabaena mcyE*, and *Planktothrix mcyE* detection

	Cyanobacterial 16S rRNA	*Microcystis mcyE*	*Anabaena mcyE*	*Planktothrix mcyE*
Total sample	24	24	24	24
PCR-positive	24	24	12	8
% of PCR-positive	100	100	50	33.3

All qPCR assays showed linear amplifications for sample concentration between 20 and 2 x 10^6 gene copies per mL. *Microcystis mcyE* was reported from 29.5 to 1,180,144.06 copy numbers per mL (Figure 6). The highest *Microcystis mcyE* copy number was detected in August 2022. Meanwhile, *Anabaena mcyE* and *Planktothrix mcyE* concentrations ranged from 28.25 to 3,877.05 copies/mL and 18.45 to 3,017.4 copies/mL, respectively. The highest *Anabaena mcyE* and *Planktothrix mcyE* copy numbers were detected in December 2022. The linear regression equations for *Microcystis mcyE*, *Anabaena mcyE*, and *Planktothrix mcyE* qPCR assays were: $y = -3.394x + 32.49$ ($R^2 = 0.999$, efficiency = 97%, $P < 0.0005$), $y = -3.406x + 34.01$ ($R^2 = 0.999$, efficiency = 97%, $P < 0.0005$), and $y = -3.246x + 33.23$ ($R^2 = 0.99$, efficiency = 103%, $P < 0.0005$) respectively, where y is the $\log_{10}$ of the gene copy number and x is the corresponding C_T value. All the DNA template concentrations were measured between 1.8 and 2.0 based on A260/A280 ratios.

This study successfully optimized harmful cyanobacterial species for monitoring via qPCR assays. A highly sensitive qPCR assay is much more reliable for cyanobacterial detection and quantification than the conventional PCR assay since it can quantify the *mcyE* genes even at the lowest detection limit of 18.45 gene copies/mL. This result was supported by the previous studies on detecting *mcyE* genes in the freshwater ecosystems where *mcyE* gene was used to rapidly quantify species-specific harmful cyanobacterial cells as well as an early detection on the cyanobacterial cells proliferation (Padovan *et al.,* 2023; Stoyneva-Gärtner *et al.,* 2020). In this studied reservoir, *Microcystis mcyE* copy numbers were most abundant compared to *Anabaena mcyE* and *Planktothrix mcyE* copy numbers, hence, *Microcystis* spp. is potentially the primary microcystin producer in the reservoir.

The distribution of cyanobacterial species has been reported to be influenced by precipitation, which impacts the water column (Ho & Michalak, 2020). The mixing of the water column due to precipitation will cause the benthic cyanobacteria, *Planktothrix* spp., to integrate with surface cyanobacterial species, including *Microcystis* spp. and *Anabaena* spp. (Visser *et al.,* 2016; Ho & Michalak, 2020). Several studies have investigated the impacts of water mixing on water quality, including phytoplankton distribution and nutrients dispersions (Wang *et al.,* 2022), contaminants (Noori *et al.,* 2021), and dissolved oxygen (Perello *et al.,* 2017).

Extreme precipitation also increases nutrient runoff, which is favorable for cyanobacterial growth (Michalak *et al.,* 2013; Reichwaldt & Ghadouani, 2012). In Malaysia, the Southwest Monsoon and the Northeast Monsoon occur from late May to September and from November to March, respectively. These monsoons eventually affect the water volume of the studied reservoir and contribute to the mixing of cyanobacterial communities in the water column. Based on Figure 6, the highest *mcyE* gene copies for *Microcystis* spp., *Planktothrix* spp., and *Anabaena* spp. were recorded within the monsoon seasons. The results imply that during monsoon season, the mixing of cyanobacterial communities occurred within the water column and potentially increased nutrient load due to precipitation, which could explain the increased abundance of cyanobacterial biomass and *mcyE* gene copy number.

3.4 Association between cyanobacterial abundance and mcyE gene copies

Pearson correlation analysis was conducted to determine the strength of the association between *mcyE* gene copies and cyanobacterial abundance. This analysis was done using log-transformed data. *Microcystis* spp. was dominant with the highest *mcyE* gene copies and detected throughout the sampling period. Therefore, the correlation analysis was performed only on *Microcystis mcyE* gene. The *mcyE* gene copy number for *Planktothrix* spp. and *Anabaena* spp. is either detected in very low concentration or undetected compared to *Microcystis* spp. As such, the presence of both *Planktothrix* and *Anabaena* are not frequent during the sampling period. Hence, the correlation of *mcyE* gene copy number for *Planktothrix* spp. and *Anabaena* spp. against cyanobacterial abundance is not conducted. The analysis results are presented in Table 3.

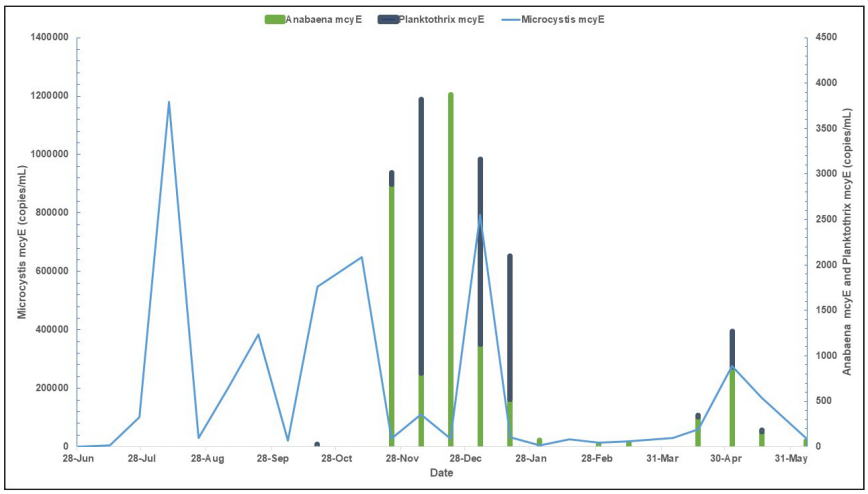


Figure 6. *Microcystis, Anabaena*, and *Planktothrix* microcystin synthetase gene E (*mcyE*) copy numbers from samples collected throughout the sampling period

Table 3. Correlations between *Microcystis mcyE* copies, chlorophyll-*a*, and blue-green algae (phycocyanin) data from the SPSS analysis

	Microcystis mcyE copies	Total chlorophyll-*a*	BGA-PC (phycocyanin)
Microcystis mcyE copies	-	0.113	0.553*

BGA-PC and *mcyE* copies are expressed per mL, while chlorophyll-*a* is expressed as µg/L.
*$P < 0.05$.

Microcystis mcyE copies only correlated significantly with BGA-PC (R = 0.553, $P < 0.05$) but not with chlorophyll-*a*. Hence, it can be suggested that cyanobacterial biomass measured as BGA-PC is a better biomass indicator to predict the toxicity of cyanobacterial communities in the studied reservoir. Though many studies have reported that chlorophyll-*a* as the general biomass indicator, phycocyanin should be included in the regular monitoring of cyanobacterial blooms since this photosynthetic pigment is specifically found in cyanobacteria, rhodophytes, cryptophytes, and glaucophytes (Binding *et al.,* 2021; Eriksen, 2008; Glazer, 1994; Zou *et al.,* 2020). Phycocyanin is a water-soluble pigment that can absorb light, which makes this pigment easily measurable in freshwater using any devices or sensors that emit light, such as algae sensors. In contrast, chlorophyll-*a* is a water-insoluble pigment that requires organic solvents such as acetone or alcohol during extraction. This pigment embedded in a membrane does not absorb and emit light in the water as phycocyanin does. Hence, this factor might influence the uncorrelation of the chlorophyll-*a* and *mcyE* gene copies. All in all, the positive correlation of *mcyE* gene copies and BGA-PC in this study showed the reliability of this biomass indicator for measuring the potential toxicity of mixed cyanobacterial communities in freshwater bodies. This present study provides a new insight of harmful cyanobacterial abundance specifically in Malaysia's reservoir and it is important for further examination on the notable cyanotoxins production by the harmful cyanobacteria.

4. Conclusion

Water physicochemical parameters in the studied reservoir fluctuated throughout the sampling period. In these conditions, the reservoir was consistently dominated by toxigenic *Microcystis* spp. The biomass of *Microcystis* spp. was highly variable on temporal and spatial scales, and significantly correlated with the *mcyE* gene copy numbers. Therefore, our results suggest and justify

the possible threats of toxic cyanobacterial blooms and the need for regular monitoring in any reservoir systems being used for public drinking water supply. Additionally, our data also suggests that the toxigenic potential of the cyanobacterial community can be significantly explained by BGA-PC concentrations instead of the most commonly used chlorophyll-*a*. Hence, BGA-PC can be utilized as reliable indicators for water operators to diagnose the potential risk associated with toxigenic cyanobacteria and cyanotoxins in drinking water sources. A more comprehensive study is needed to further validate the relationship between BGA-PC and microcystins during cyanobacterial blooms in drinking water sources.

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References

Aeriyanie AR, Sinang SC, Nayan N, Poh KB. Role of Internal and External Nutrients Loading in Regulating In-Lake Nutrient Concentrations in a Eutrophic Shallow Lake. EnvironmentAsia 2021; 14(1).

Akhtar N, Syakir Ishak MI, Bhawani SA, Umar K. Various natural and anthropogenic factors responsible for water quality degradation: A review. Water 2021;13(19):2660.

Barçante B, Nascimento NO, Silva TF, Reis LA, Giani A. Cyanobacteria dynamics and phytoplankton species richness as a measure of waterbody recovery: Response to phosphorus removal treatment in a tropical eutrophic reservoir. Ecological Indicators 2020; 117: 106702.

Baxa DV, Kurobe T, Ger KA, Lehman PW, Teh SJ. Estimating the abundance of toxic Microcystis in the San Francisco Estuary using quantitative real-time PCR. Harmful Algae 2010; 9(3): 342-349.

Binding CE, Pizzolato L, Zeng C. EOLakeWatch; Delivering a comprehensive suite of remote sensing algal bloom indices for enhanced monitoring of Canadian eutrophic lakes. Ecological Indicators 2021; 121: 106999.

Chen M, Xu C, Wang X, Wu Y, Li L. Nonribosomal peptide synthetases and nonribosomal cyanopeptides synthesis in Microcystis: A comparative genomics study. Algal Research 2021; 59: 102432.

Chorus I, Welker M. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. Boca Raton, Crc Press/ Taylor & Francis Group. 2021.

Dey S, Botta S, Kallam R, Angadala R, Andugala J. Seasonal variation in water quality parameters of Gudlavalleru Engineering College Pond. Current Research in Green Sustainable Chemistry 2021; 4: 100058.

Dittmann E, Börner T. Genetic contributions to the risk assessment of microcystin in the environment. Toxicology and Applied Pharmacology 2005; 203(3): 192-200.

Eriksen NT. Production of phycocyanin a pigment with applications in biology, biotechnology, foods and medicine. Applied Microbiology and Biotechnology 2008; 80: 1-4.

Felip M, Catalan J. The relationship between phytoplankton biovolume and chlorophyll in a deep oligotrophic lake: decoupling in their spatial and temporal maxima. Journal of Plankton Research 2000; 22(1): 91-106.

Freeman EC, Creed IF, Jones B, Bergström AK. Global changes may be promoting a rise in select cyanobacteria in nutrient-poor northern lakes. Global Change Biology 2020; 26(9): 4966-4987.

Glazer AN. Phycobiliproteins—a family of valuable, widely used fluorophores. Journal of Applied Phycology 1994; 6: 105-112.

Gophen M. Climate change-enhanced cyanobacteria domination in Lake Kinneret: A retrospective overview. Water 2021; 13(2): 163.

Ho JC, Michalak AM. Exploring temperature and precipitation impacts on harmful algal blooms across continental US lakes. Limnology and Oceanography 2020; 65(5): 992-1009.

Hu L, Shan K, Huang L, Li Y, Zhao L, Zhou Q, Song L. Environmental factors associated with cyanobacterial assemblages in a mesotrophic subtropical plateau lake: A focus on bloom toxicity. Science of the Total Environment 2021; 777: 146052.

Huo D, Gan N, Geng R, Cao Q, Song L, Yu G, Li R. Cyanobacterial blooms in China: Diversity, distribution, and cyanotoxins. Harmful Algae 2021; 109: 102106.

Kazmi SS, Yapa N, Karunarathna SC, Suwannarach N. Perceived intensification in harmful algal blooms is a wave of cumulative threat to the aquatic ecosystems. Biology 2022; 11(6): 852.

Kimambo ON, Gumbo JR, Chikoore H. The occurrence of cyanobacteria blooms in freshwater ecosystems and their link with hydro-meteorological and environmental variations in Tanzania. Heliyon 2019; 5(3).

Kubickova B, Babica P, Hilscherová K, Šindlerová L. Effects of cyanobacterial toxins on the human gastrointestinal tract and the mucosal innate immune system. Environmental Sciences Europe 2019; 31(1): 1–27.

Lintern A, Webb JA, Ryu D, Liu S, Waters D, Leahy P, Bende-Michl U, Western AW. What are the key catchment characteristics affecting spatial differences in riverine water quality? Water Resources Research 2018; (10): 7252-7272.

Mainali J, Chang H. Environmental and spatial factors affecting surface water quality in a Himalayan watershed, Central Nepal. Environmental and Sustainability Indicators 2021; 9: 100096.

Massey IY, Al Osman M, Yang F. An overview on cyanobacterial blooms and toxins production: their occurrence and influencing factors. Toxin Reviews 2022; 41(1): 326-46.

Matej-Łukowicz K, Wojciechowska E, Kolerski T, Nawrot N, Kuliński K, Winogradow A. Sources of contamination in sediments of retention tanks and the influence of precipitation type on the size of pollution load. Scientific Reports 2023; 13(1): 8884.

Méresse S, Larrigaldie V, Asma Oummadi, Vidian de Concini, Séverine Morisset-Lopez, Reverchon F, Menuet A, Montecot-Dubourg C, Mortaud S. β-N-Methyl-Amino-L-Alanine cyanotoxin promotes modification of undifferentiated cells population and disrupts the inflammatory status in primary cultures of neural stem cells. Toxicology 2022; 482:153358–8.

Michalak AM, Anderson EJ, Beletsky D, Boland S, Bosch NS, Bridgeman TB, Chaffin JD, Cho K, Confesor R, Daloğlu I, DePinto JV. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proceedings of the National Academy of Sciences. 2013; 110(16): 6448-6452.

Mullin CA, Kirchhoff CJ, Wang G, Vlahos P. Future projections of water temperature and thermal stratification in Connecticut reservoirs and possible implications for cyanobacteria. Water Resources Research 2020; 56(11): e2020WR027185.

Nalley JO, O'Donnell DR, Litchman E. Temperature effects on growth rates and fatty acid content in freshwater algae and cyanobacteria. Algal Research 2018; 35:500-507.

Ninio S, Lupu A, Viner-Mozzini Y, Zohary T, Sukenik A. Multiannual variations in Microcystis bloom episodes–Temperature drives shift in species composition. Harmful Algae 2020; 92: 101710.

Noori R, Ansari E, Bhattarai R, Tang Q, Aradpour S, Maghrebi M, Haghighi AT, Bengtsson L, Kløve B. Complex dynamics of water quality mixing in a warm monomictic reservoir. Science of the Total Environment 2021; 777:146097.

Omar R, Ibrahim Z, Zahid Z, Ismail E, Hisham I, Omar SI, Mustafar S. Algae Disintegration in Raw Water at Semberong Barat Dam Johor Malaysia Using Sonification Wave. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2017; 8(1): 1931-1944.

Padovan A, Kennedy K, Gibb K. A microcystin synthesis mcyE/ndaF gene assay enables early detection of microcystin production in a tropical wastewater pond. Harmful Algae 2023; 127: 102476.

Palinkas LA, Wong M. Global climate change and mental health. Current Opinion in Psychology 2020; 32:12-16.

Park HK, Lee HJ, Heo J, Yun JH, Kim YJ, Kim HM, Hong DG, Lee IJ. Deciphering the key factors determining spatio-temporal heterogeneity of cyanobacterial bloom dynamics in the Nakdong River with consecutive large weirs. Science of the Total Environment 2021; 755: 143079.

Perello MM, Kane DD, Golnick P, Hughes MC, Thomas MA, Conroy JD. Effects of local weather variation on water-column stratification and hypoxia in the western, Sandusky, and central basins of Lake Erie. Water 2017; 9(4): 279.

Pham TL, Tran TH, Shimizu K, Li Q, Utsumi M. Toxic cyanobacteria and microcystin dynamics in a tropical reservoir: assessing the influence of environmental variables. Environmental Science and Pollution Research 2021; 28: 63544-63557.

Pham TL, Utsumi M. An overview of the accumulation of microcystins in aquatic ecosystems. Journal of Environmental Management 2018; 213: 520-529.

Pound HL, Martin RM, Sheik CS, Steffen MM, Newell SE, Dick GJ, McKay RM, Bullerjahn GS, Wilhelm SW. Environmental studies of cyanobacterial harmful algal blooms should include interactions with the dynamic microbiome. Environmental Science and Technology 2021; 55(19): 12776-12779.

Prakash S. Impact of climate change on aquatic ecosystem and its biodiversity: An overview. International Journal of Biological Innovations 2021; 3(2).

Rantala A, Rajaniemi-Wacklin P, Lyra C, Lepistö L, Rintala J, Mankiewicz-Boczek J, Sivonen K. Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (mcyE) PCR and associations with environmental factors. Applied and Environmental Microbiology 2006; 72(9): 6101-6110.

Rao K, Zhang X, Wang M, Liu J, Guo W, Huang G, Xu J. The relative importance of environmental factors in predicting phytoplankton shifting and cyanobacteria abundance in regulated shallow lakes. Environmental Pollution 2021; 286: 117555.

Reichwaldt ES, Ghadouani A. Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: between simplistic scenarios and complex dynamics. Water Research 2012; 46(5): 1372-93.

Ren X, Sun J, Zhang Q, Zuo Y, Liu J, Liu J, Li L, Song L. The Emergent Integrated Constructed Wetland-Reservoir (CW-R) Is Being Challenged by 2-Methylisoborneol Episode A Case Study in Yanlonghu CW-R. Water (Switzerland) 2022; 14(17).

Richardson J, Feuchtmayr H, Miller C, Hunter PD, Maberly SC, Carvalho L. Response of cyanobacteria and phytoplankton abundance to warming, extreme rainfall events and nutrient enrichment. Global Change Biology 2019; 25(10): 3365-3380.

Rinta-Kanto JM, Ouellette AJ, Boyer GL, Twiss MR, Bridgeman TB, Wilhelm SW. Quantification of toxic Microcystis spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environmental Science and Technology 2005; 39(11): 4198-4205.

Rohaslinda M. Detection and identification of cyanobacterial toxin encoding gene in Malaysia. Master of Science, Universiti Sains Malaysia. 2017.

Simkin AJ, Kapoor L, Doss CG, Hofmann TA, Lawson T, Ramamoorthy S. The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. Photosynthesis Research 2022; 152(1): 23-42.

Smucker NJ, Beaulieu JJ, Nietch CT, Young JL. Increasingly severe cyanobacterial blooms and deep water hypoxia coincide with warming water temperatures in reservoirs. Global Change Biology 2021; 27(11): 2507-2519.

Stoyneva-Gärtner M, Stefanova K, Descy JP, Uzunov B, Radkova M, Pavlova V, Mitreva M, Gärtner G. Microcystis aeruginosa and M. wesenbergii were the primary planktonic microcystin producers in several Bulgarian waterbodies (August 2019). Applied Sciences 2020; 11(1): 357.

Sukchinda S, Phewnil O, Pattamapitoon T, Srichomphu M. Effect of Solar Radiation on Cyanobacteria Bloom in Oxidation Ponds Community Wastewater Treatment at the King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Phetchaburi, Thailand. EnvironmentAsia 2019; 12(3).

Thomas MK, Litchman E. Effects of temperature and nitrogen availability on the growth of invasive and native cyanobacteria. Hydrobiologia 2016; 763(1): 357-369.

Vaitomaa J, Rantala A, Halinen K, Rouhiainen L, Tallberg P, Mokelke L, Sivonen K. Quantitative real-time PCR for determination of microcystin synthetase E copy numbers for Microcystis and Anabaena in lakes. Applied Environmental Microbiology 2003; 69(12): 7289-7297.

Visser PM, Verspagen JM, Sandrini G, Stal LJ, Matthijs HC, Davis TW, Paerl HW, Huisman J. How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 2016; 54:145-159.

Wang X, Sun J, Yu H. Distribution and environmental impact factors of phytoplankton in the Bay of Bengal during autumn. Diversity 2022;14(5):361.

Wang Z, Wang T, Liu X, Hu S, Ma L, Sun X. Water level decline in a reservoir: implications for water quality variation and pollution source identification. International Journal of Environmental Research and Public Health 2020; 17(7): 2400.

World Health Organization. Cyanobacterial toxins: Anatoxin-a and analogues; Cylindrospermopsins; Microcystins; Saxitoxins. Background documents for development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. Geneva: World Health Organization. 2020. Available from https://www.who.int/teams/environment-climate-change-and-health/water-sanitation-and-health/water-safety-and-quality/publications

Yang W, Zheng Z, Zheng C, Lu K, Ding D, Zhu J. Temporal variations in a phytoplankton community in a subtropical reservoir: an interplay of extrinsic and intrinsic community effects. Science of the Total Environment 2018; 612:720-727.

Yu Y, Song X, Zhang Y, Zheng F. Assessment of water quality using chemometrics and multivariate statistics: a case study in chaobai river replenished by reclaimed water, North China. Water 2020; 12(9): 2551.

Zhang Z, Fan X, Peijnenburg WJ, Zhang M, Sun L, Zhai Y, Yu Q, Wu J, Lu T, Qian H. Alteration of dominant cyanobacteria in different bloom periods caused by abiotic factors and species interactions. Journal of Environmental Sciences (China) 2021; 99: 1-9.

Zou W, Zhu G, Cai Y, Vilmi A, Xu H, Zhu M, Gong Z, Zhang Y, Qin B. Relationships between nutrient, chlorophyll-a and Secchi depth in lakes of the Chinese Eastern Plains ecoregion: Implications for eutrophication management. Journal of Environmental Management 2020; 260:109923.