

Preliminary Assessment of Cyanobacteria Diversity and Toxic Potential in Ten Freshwater Lakes in Selangor, Malaysia

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Abstract Toxic cyanobacteria blooms are increasing in magnitude and frequency worldwide. However, this issue has not been adequately addressed in Malaysia. Therefore, this study aims to better understand eutrophication levels, cyanobacteria diversity, and microcystin concentrations in ten Malaysian freshwater lakes. The results revealed that most lakes were eutrophic, with total phosphorus and total chlorophyll-*a* concentrations ranging from 15 to 4270 $\mu\text{g L}^{-1}$ and 1.1 to 903.1 $\mu\text{g L}^{-1}$, respectively. Cyanobacteria were detected in all lakes, and identified as *Microcystis* spp., *Planktothrix* spp., *Phormidium* spp., *Oscillatoria* spp., and *Lyngbya* spp. *Microcystis* spp. was the most commonly observed and most abundant cyanobacteria recorded. Semi-quantitative microcystin analysis indicated the presence of microcystin in all lakes. These findings illustrate the potential health risk of cyanobacteria in Malaysia freshwater lakes, thus magnifying the importance of cyanobacteria monitoring and management in Malaysian waterways.

Keywords Cyanobacteria · Microcystin · Freshwater lakes · Eutrophication

Similar to many other countries, Malaysia is facing a great challenge to sustain the quality of the available water resources. The increase in human activities, along with rising global temperatures and changes in the hydrological cycle, has increased nutrient loading into water bodies

(IPCC 2008; Paerl et al. 2011). In water bodies, particularly freshwater lakes, high nutrient loading is known to promote eutrophication. Subsequently, eutrophication triggers a response in phytoplankton communities that can increase biomass accumulation in the water column (Jeppesen et al. 2015; Newcombe et al. 2011). The most common phytoplankton biomass accumulation in freshwater systems is associated with blue-green algae, which is also known as cyanobacteria blooms (Chorus and Bartram 1999; Waajen et al. 2014).

The presence of high cyanobacterial biomass in water bodies, either used for drinking or recreational purposes, may pose serious health risks for human and animal populations due to toxin contamination (Rastogi et al. 2014; Romo et al. 2012; Sukenik et al. 2015). Humans may be exposed to cyanobacterial toxins through direct contact and ingestion of toxic cyanobacteria from drinking or recreational water (Chorus et al. 2000; Rastogi et al. 2014). Of all cyanobacterial toxins, hepatotoxin microcystin is the most commonly encountered, and thus most likely to pose a risk to the consumers of drinking and recreational water (WHO 1998, 2003). Toxigenic genera including *Microcystis*, *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Nodularia*, *Planktothrix*, *Nostoc*, and *Cylindrospermopsis* are known to produce hepatotoxin microcystin (Chorus and Bartram 1999).

Unlike many developed countries, issues associated with cyanobacteria blooms have only received minimal attention in Malaysia, where only a limited number of cyanobacterial studies have been conducted. Moreover, these studies were focused mostly on the occurrence of harmful algal blooms in coastal areas, rather than in freshwater ecosystems such as freshwater lakes. This may be due to inadequate attention in understanding the importance of assessing the risks of toxic cyanobacterial

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blooms in freshwater ecosystems in Malaysia. This emerging issue could pose serious health risks to the local communities, and all water consumers, in general. Therefore, research focusing on the occurrence of toxic cyanobacterial blooms in freshwater ecosystems within Malaysia is urgently needed. With regards to this knowledge gap, this study aimed to provide preliminary information on the levels of eutrophication, cyanobacteria diversity, and its toxic potential in ten Malaysian freshwater lakes. The objectives were to: (1) assess levels of eutrophication in ten Malaysian freshwater lakes; (2) identify the presence of potentially toxic cyanobacterial genera; and (3) estimate microcystin concentrations in the ten freshwater lakes using semi-quantitative analysis method. This study is important as more scientific data on cyanobacterial occurrence, diversity and toxicity are needed to attract more attention from Malaysia's scientific community on this alarming issue.

Methods and Materials

Ten urban lakes located in Selangor state were randomly selected for this study. The lakes were located at Kepong, Rawang, Kuala Kubu Bharu, Kota Damansara, Petaling

Jaya, Ulu Yam and Bukit Sentosa (Fig. 1). In general, these lakes are artificial lakes and surrounded by residential and industrial areas. These lakes receive high nutrient inputs mainly from urban runoff. They also serve as recreational sites for local communities, where the main water contact activities include kayaking, swimming and fishing.

Each lake was sampled once between the dry months of January and February 2013. Grab water samples for total chlorophyll-*a*, microcystin, total phosphorus and cyanobacteria biovolume analysis were taken from 15 cm below the surface to avoid surface scum and debris. Water samples for total chlorophyll-*a*, microcystin, and total phosphorus analysis were stored immediately in glass bottles in the dark on ice after the collection. Water samples for cyanobacteria biovolume analysis were preserved with Lugol's solution and kept in the dark (Chorus and Bartram 1999). Phytoplankton samples were also taken from each lake using a plankton net (mesh size 37 μm). The net was lowered to about 15 cm below the surface and manually retrieved using a hand-over-hand technique at a rate of 0.5 m/s. This procedure was repeated several times to ensure enough phytoplankton were collected. Subsequently, the net was rinsed with lake water, and the collected plankton samples were preserved in Lugol's solution.



Fig. 1 Map showing the locations of ten study lakes around Selangor, Malaysia (Source: Google map). TRR Tasik Rimba Riang, TKKB1 Tasik Kuala Kubu 1, TKKB2 Tasik Kuala Kubu Baru 2, TS

Tasik Sentosa, THY Tasik Hulu Yam, TBK1 Tasik Bandaran Kelana 1, TBK2 Tasik Bandaran Kelana 2, TBK3 Tasik Bandaran Kelana 3, TB Tasik Biru, TMK Tasik Metropolitan Kepong

Total phosphorus and total chlorophyll-*a* were analyzed according to standard methods (Clesceri et al. 1998). Samples for total phosphorus quantification were pre-digested using the persulfate digestion method before further analysis using the ascorbic acid method. The ascorbic acid method can quantify total phosphorus with concentration from 2 to 200 $\mu\text{g L}^{-1}$. For total chlorophyll-*a* analysis, 300 to 500 mL of water samples collected from lakes were filtered through glass fiber filter papers. Filter papers containing algal biomass were frozen and thawed prior to the extraction. During the extraction, each filter paper was placed in a test tube containing 10 mL 90 % acetone and a sonicator was used to break up the algal cells. Next, the chlorophyll-*a* extracts were centrifuged for 5 min at 5000 rpm to eliminate the remaining cell materials and filter paper. Total chlorophyll-*a* was quantified using a spectrophotometer with wavelength set at 750 nm (E750o) and 665 nm (E665o) against 90 % acetone blank. Total chlorophyll-*a* was calculated according to the equation provided in Clesceri et al. (1998). Total chlorophyll-*a* concentrations were used to calculate Carlson's trophic state index TSI (chlorophyll-*a*) to determine the levels of eutrophication in the lakes (Carlson 1977; Carlson and Simpson 1996).

The prefixed phytoplankton samples were used to assess the presence of cyanobacterial biomass, particularly from toxic cyanobacterial genera. Cyanobacteria identification was done only to the taxonomic level of genera. The plankton samples were centrifuged for 5 min at 3000 rpm and the concentrated algal cells were transferred onto glass slides prior to analysis using a compound microscope (LEICA, Model DME1395).

The relative abundance of each cyanobacterial genera (cells or colonies mL^{-1}) was determined from 10 to 50 mL of sample using an inverse microscope (Utermöhl 1958), and converted into biovolume ($\mu\text{m}^3 \text{mL}^{-1}$) using the method by Hillebrand et al. (1999). This involved multiplying the mean cell or colony biovolume (μm^3) with the total cells or colonies per mL (cells or colonies mL^{-1}).

Microcystin was assessed by using the algal toxin (microcystin) strip test kit for recreational water (Abraxis PN 520022). This test provides semi-quantitative results with categorical microcystin concentration data of <1, 1, 2.5, 5 and >10 $\mu\text{g L}^{-1}$. Test strips, reagents and the collected water samples were left at room temperature for 1 h before the analysis. During the extraction, 1.0 mL of water sample from each lake was placed in a lysis vial, and reagent paper was added to break up cyanobacterial cells. Then, the extracted samples were added to the flip-top tube containing dried reagents and microcystin test strip was inserted. After 10 min, the microcystin test strip was removed, and color change was observed after 5 min. The maximum detection limit of the test kits used was

10 $\mu\text{g L}^{-1}$. An additional test was performed using distilled water to determine the reference value of microcystin in uncontaminated water.

Bivariate correlation analysis was carried out on cyanobacteria biovolume, chlorophyll-*a* concentration, and microcystin concentration data using Statistical Package for Social Sciences (SPSS) software (version 17). This analysis was performed in order to ascertain which cyanobacteria genera contributes to high chlorophyll-*a* and categorical microcystin. In this analysis, Spearman's Rank correlation coefficient was used to determine the strength and the significance of the correlation between cyanobacteria biovolume with chlorophyll-*a* and cyanobacteria biovolume with microcystin concentration. All correlations are considered as significant at $p < 0.05$.

Results and Discussion

Phosphorus is a primary factor to limit or trigger cyanobacteria growth in freshwater environments (Chorus and Bartram 1999). In this study, total phosphorus concentrations quantified in the study lakes ranged from 15 $\mu\text{g L}^{-1}$ in TKKB1 and TRR, through 1250 $\mu\text{g L}^{-1}$ in TBK1, to 4270 $\mu\text{g L}^{-1}$ in TMK (Table 1). As suggested by Chorus and Bartram (1999), even total phosphorus concentrations less than 100 $\mu\text{g L}^{-1}$ are sufficient to induce the presence and accumulation of high cyanobacteria biomass.

The severity of nutrient enrichment or eutrophication in water bodies can be indicated by the Trophic State Index (TSI). As phytoplankton biomass, particularly cyanobacteria, is the matter of concern in this study, total chlorophyll-*a* concentration was used to calculate TSI, through an equation $\text{TSI (chlorophyll-}a) = 9.81 \ln (\text{chlorophyll-}a) + 30.6$. The total chlorophyll-*a* concentrations, TSI (chlorophyll-*a*) and trophic state for each lake are shown in Table 1. As indicated in Table 1, the total chlorophyll-*a* concentrations in the study lakes ranged over three orders of magnitudes from 1.1 to 903.1 $\mu\text{g L}^{-1}$. Total chlorophyll-*a* concentrations <10 $\mu\text{g L}^{-1}$ were detected in TKKB1, TS and TRR. Meanwhile, in TBK2, TBK3, TB, TKKB2, and THY, total chlorophyll-*a* concentrations varied from 12 to 30 $\mu\text{g L}^{-1}$. Total chlorophyll-*a* exceeding 100 $\mu\text{g L}^{-1}$ was only detected in TMK and TBK1. Subsequently, TSI (chlorophyll-*a*) in these lakes varied from 31.6 to 97.4.

Based on the calculated TSI (chlorophyll-*a*), only two lakes (TKKB1 and TRR) showed no sign of eutrophication. These lakes were classified as oligotrophic, thus considered as systems with low nutrients and low primary productivity (Havens 2007). Meanwhile, TS was classified as mesotrophic which is characterized as having intermediate level of nutrients, fairly productive and showing emerging signs of water quality problems (Havens 2007). Five other lakes

Table 1 Total phosphorus, chlorophyll-*a* concentrations and the Trophic State Index in the study lakes

	TMK	TBK1	TBK2	TBK3	TB	TKKB1	TKKB2	TS	THY	TRR
Total phosphorus ($\mu\text{g L}^{-1}$)	4270	1250	51	58	44	15	46	20	43	15
Chlorophyll- <i>a</i> ($\mu\text{g L}^{-1}$)	903.1	182.8	30.1	20.5	21.4	1.7	13.3	3.3	12.7	1.1
TSI (chlorophyll- <i>a</i>)	97.4	81.7	64.0	60.2	60.6	35.6	56.0	42.4	55.5	31.6
Trophic State based on TSI (chlorophyll- <i>a</i>)	H	H	E	E	E	O	E	M	E	O

H hypereutrophic, *E* eutrophic, *M* mesotrophic, *O* oligotrophic

(TBK2, TBK3, TB, TKKB2 and THY) were classified as eutrophic, that is, rich in nutrients, very productive and showing increasing signs of water quality problems (Havens 2007). Two lakes (TMK and TBK1) were classified as hypereutrophic, which is the extreme of eutrophication, which is characterized as high nutrients and organic matter with potential severe oxygen depletion in the water column (Havens 2007). These results indicate that eutrophication is common for lakes in the study area and also Malaysia, in general. This is consistent with findings of the National Hydraulics Research Institute of Malaysia that classified 49 out of 90 natural and artificial lakes in Malaysia as eutrophic (NAHRIM 2009). This is in line with the fact that the majority of Malaysian water bodies are experiencing elevated phosphorus levels due to massive discharges from various sources of pollution. For instance, surface runoff from the total agricultural area may introduce up to 95,669 tonnes of phosphorus into Malaysian waterways per year (Eisakhani et al. 2009). Further eutrophication is expected in the local lakes as changes in land use patterns are expected to sustain the future human population and food production.

As eutrophication of lakes is common, it is important to realize that this condition is commonly associated with the presence of toxic cyanobacteria in the water column (Jacquet et al. 2014). The microscopic examination of the collected phytoplankton samples revealed that cyanobacteria were present in all study lakes, even TKKB1 and TRR which were classified as oligotrophic with low total phosphorus (Table 2). This phenomena is possible as cyanobacteria are known to have high internal storage and high affinity for phosphorus, thus enabling cyanobacteria to bloom in oligotrophic waters (Chorus and Bartram 1999; de Figueiredo et al. 2004).

Cyanobacterial communities in the study lakes were composed of five toxin-producing cyanobacteria including *Microcystis* spp., *Planktothrix* spp., *Phormidium* spp., *Oscillatoria* spp., and *Lyngbya* spp. (Table 2). *Microcystis* spp. was the most common, as it was found in all lakes except in TRR. Additionally, *Microcystis* spp. was also the most dominant cyanobacteria based on the calculated biovolume (Fig. 2). *Microcystis* spp. biovolume ranged

from 110,489 to 4,276,503 $\mu\text{m}^3 \text{mL}^{-1}$ across the lakes. Meanwhile, the biovolume of other cyanobacterial genera ranged between 10,587 and 3,529,720 $\mu\text{m}^3 \text{mL}^{-1}$.

As indicated by Spearman's rank correlation coefficients presented Table 3, the biovolumes of *Microcystis* spp., *Phormidium* spp., and *Lyngbya* spp. are significantly correlated with total chlorophyll-*a*. These positive and strong correlations suggest that high abundance of these three cyanobacteria genera maybe the results of the elevated total chlorophyll-*a* or eutrophication in the study lakes.

Detection of Cyanobacterial Toxin Microcystin in the Study Lakes

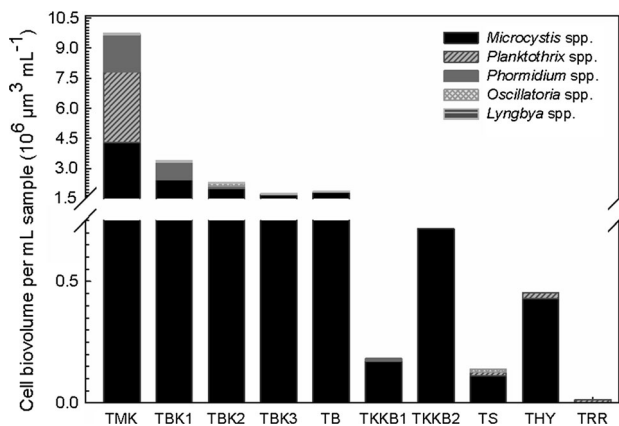
The Spearman's correlation coefficients presented in Table 3 also indicated that the biovolumes of *Microcystis* spp., *Phormidium* spp., and *Lyngbya* spp. are significantly correlated with categorical microcystin recorded in this study. The positive correlations suggest that the high abundance of these cyanobacteria biomass could result in high microcystin concentration in the water column. Such results are possible as all of the detected cyanobacteria genera are known to have species that are capable of producing various cyanotoxins, including microcystin (Chorus et al. 2000). Insignificant correlations between microcystin and *Planktothrix* spp. and *Oscillatoria* spp. could be explained through the observation that these cyanobacteria were only detected in four and two lakes, respectively. Moreover, the biovolume of *Planktothrix* spp. was only high in one lake and the biovolume of *Oscillatoria* spp. was low in both lakes.

A reference test using Abraxis Microcystin Kits was performed using distilled water and yielded two bands with similar color intensity. This result indicates that microcystin was either not present or present with concentration below $1 \mu\text{g L}^{-1}$ in distilled water. Therefore, microcystin concentrations recorded from semi-quantitative tests performed on the collected lake water samples are considered valid. In general, microcystin was present in all of the study lakes with potential concentrations ranging from 1 to more than $10 \mu\text{g L}^{-1}$. Water samples obtained from TMK

Table 2 Types of cyanobacteria detected in the study lakes

	TMK	TBK1	TBK2	TBK3	TB	TKKB1	TKKB2	TS	THY	TRR
<i>Microcystis</i> spp.	+	+	+	+	+	+	+	+	+	
<i>Planktothrix</i> spp.	+								+	+
<i>Phormidium</i> spp.	+	+	+	+		+				
<i>Oscillatoria</i> spp.			+					+		
<i>Lyngbya</i> spp.	+	+	+	+	+					

+ Indicates the cyanobacteria genera present in the collected sample

**Fig. 2** Biovolume of each cyanobacteria genera present in the study lakes

contained the highest microcystin concentration, which exceeded $10 \mu\text{g L}^{-1}$. High microcystin concentration recorded in TMK was possible as this lake was dominated by *Microcystis* spp. For this sample, the exact microcystin concentration could not be determined as it exceeded the maximum detection limit of the Abraxis test kit.

The encountered categorical microcystin concentrations in nine other lakes were relatively low compared to values published in the literature, which ranged over five orders of magnitude (Sinang et al. 2013). Based on the recorded categorical microcystin concentration, the water sample collected from TBK1 contained $5 \mu\text{g L}^{-1}$ microcystin. Meanwhile, water samples collected from TBK2, TBK3, TKKB2, TS, THY and TRR contained $2.5 \mu\text{g L}^{-1}$ microcystin. Water samples collected from TB and TKKB1 contained only $1 \mu\text{g L}^{-1}$ microcystin, consistent with the lack of obvious blooms during the sampling event.

Microcystin concentrations quantified in these lakes were under the safe level ($20 \mu\text{g L}^{-1}$) recommended for microcystin contamination in recreational waters by the World Health Organization (WHO 2003).

This finding illustrates the potential health risk associated with the presence of cyanobacteria and microcystin in Malaysian freshwater lakes, as many are used for water supply and recreational activities (NAHRIM 2009). Even though microcystin concentrations in the study lakes were potentially below the provisional guideline value of $20 \mu\text{g L}^{-1}$, serious contamination remains possible. Microcystin could be present at high concentrations as its production can be highly variable on the temporal basis (Ahmed and Wanganeo 2015), depending on various environmental factors and viable cyanobacterial biomass (Ait Hammou et al. 2014; Sinang et al. 2013). Therefore, the presence of microcystin in lakes in this region need to be seriously addressed by water authorities as it can pose a significant public health risk. Moreover, illness from accidental swallowing and inhalation of water contaminated with microcystin can occur (Chorus and Bartram 1999). Direct contact with cyanobacteria cell materials or microcystin is known to cause allergic, severe dermatitis and resembling skin burns to the recreational water users (WHO 2003).

In summary, the results obtained in this study showed eutrophication is a common feature in ten Malaysian lakes. As assessed through total chlorophyll-*a*, eutrophication, the lakes varied from the lowest, to the extreme high end of eutrophication. Moreover, cyanobacteria were present in all lakes in which the cyanobacteria communities were composed of *Microcystis* spp., *Planktothrix* spp., *Phormidium* spp., *Oscillatoria* spp., and *Lyngbya* spp. The statistical analysis performed suggests that high abundance of *Microcystis* spp., *Phormidium* spp., and *Lyngbya* spp.

Table 3 Spearman's rank correlation coefficients between biovolume of the detected cyanobacteria genera with total chlorophyll-*a* and categorical microcystin concentration

	<i>Microcystis</i> spp.	<i>Planktothrix</i> spp.	<i>Phormidium</i> spp.	<i>Oscillatoria</i> spp.	<i>Lyngbya</i> spp.
Total chlorophyll- <i>a</i>	0.988*	-0.082	0.731*	0.043	0.925*
Microcystin	0.600*	0.452	0.592*	0.002	0.592*

* Indicate significant correlation at $p < 0.05$

indicate the increased in eutrophication and categorical microcystin.

Microcystin was detected in all lakes with categorical concentrations ranging from $1 \mu\text{g L}^{-1}$ to more than $10 \mu\text{g L}^{-1}$. Even though microcystin was detected in relatively low concentrations in most lakes, the presence of cyanobacteria in these lakes should not be neglected as serious public health risk remain possible due to highly dynamic microcystin production. The findings of this study highlight the need to address the issue associated with the presence of toxic cyanobacteria in many other important Malaysia's water bodies currently in use for drinking water supply, irrigation and recreational purposes. The awareness on this issue is crucial to avoid significant economic loss and adverse impact on public health.

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