The Monthly Variation Tendency of Microcystin-LR Levels in The Huangpu River (China) by Applications of ELISA and HPLC

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Abstract

In this study, the contents of microcystin-LR (MC-LR) of *Microcystis aeruginosa* cultures in the laboratory and natural water samples from the Huangpu River in different seasons were detected through enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC), respectively. Excellent correlation between the two methods was obtained ($R^2 > 0.99$). ELISA was a reliable and simple method with high reproducibility (coefficient of variation < 25%) and satisfactory recovery for the monitoring of low levels of MC-LR. MC-LR concentrations in Huangpu River varied with the seasonal variation, which peaked in August with the temperature over 30°C and then gradually declined with the decreasing temperature after August. The highest MC-LR concentration in the Huangpu River was below the WHO drinking water quality standard (1 µg/L). These results indicated that warm temperature accelerated the MC-LR synthesis and release, and it is necessary to regularly monitor the MC-LR levels, especially during the high algae period in summer. ELISA can be applied to detect the low levels of MC-LR in the field without complex treatment, avoiding the samples from denaturation and degradation during the transportation. Hence, ELISA is a better alternative of HPLC when HPLC is unavailable, especially when rapid testing is required in routine MC-LR analysis.

1. Introduction

The cyanobacteria bloom pollution caused by eutrophication has attracted increasing attention in the world in recent years (Budzynska et al., 2019; Jiang et al., 2008). Cyanobacterial species produce cyanotoxins which exhibit toxicities including hepatotoxicity, neurotoxicity, cytotoxicity, dermatotoxicity, and lipopolysaccharide toxicity (Harke et al., 2016; Jasionek et al., 2010). Microcystins (MCs) are the most widespread cyanotoxins in water bodies (Merel et al., 2013), and approximately 70 MC variants have been isolated (Zurawell et al., 2005). MC-LR is one of the most common and most hepatotoxic MCs. It has been reported that sixty patients at a haemodialysis unit in Caruaru, north-east Brazil died for the use of inadequately treated water from a local reservoir with blooms of cyanobacteria, developed a toxic illness of varying severity (Pouria et al., 1998). MC-LR also posed adverse effects on animals and plants (Corbel et al., 2014; de Figueiredo et al., 2004). For example, MC-LR causes liver damage and death of animals (Gupta et al., 2003; Li et al., 2003; Zimba et al., 2001), and the growth of plants including the roots and the leaves were damaged (Mathe et al., 2009; McElhiney et al., 2001; Mitrovic et al., 2005). Specifically, the photosynthetic apparatus of plants was damaged (Abe et al., 1996).

MCs have been detected in natural waters around the world. For example, Hepatotoxic MCs were widely distributed in the San Francisco Bay Estuary and the North American Great Lakes (Carmichael and Boyer, 2016; Imai et al., 2009). The dissolved MC concentrations in Lake Kovada was quantified by ELISA and ranged from 0.7 to 48.5 µg MC-LR equivalents L⁻¹ (Gurbuz et al., 2009). MC concentrations ranging from 0 mg/g to 0.23 mg/g (freeze-dried cells) were observed in Grand-Lieu Lake, France (Vezie et al., 1998). In East Asia and South Korea, MCs varied from 20 µg/g to 1500 µg/g freeze-dried bloom material in more than a dozen cyanobacteria polluted lakes (Park et al., 1998). And in Japan, Approximately 64.5 kg of MCs was discharged into the bay with 371 million tons of effluent in a year (Umehara et al., 2012). In

addition, MCs were detected in 28 of 30 subtropical lakes in China and MC-LR was the primary variant observed (Wan et al., 2020).

Temperature is a main factor leading to the toxin production by the cyanobacteria (Davis et al., 2009; Mantzouki et al., 2018). Summer heatwaves can enhance *M. aeruginosa* bloom (Johnk et al., 2008), and most MCs were produced and released when the water temperature ranged from 24.7°C to 33.9°C (Imai et al., 2009). Cyanobacterial bloom dominated by *Microcystis aeruginosa* at Taihu Lake was caused by human activities and seasonal warming trends (Qin et al., 2010). In our previous study, we also found that the synthesis and release of MC-LR reached peak at 25°C (Ye et al., 2020). In Shanghai, the temperature in summer often exceeds 28°C, thereby likely promoting the synthesis of MCs and posing a risk to those who use polluted water resources for drinking, entertainments, and aquaculture (Harke et al., 2016). Thus, comprehensively evaluating the MC-LR distribution and exploring the relationship between temperature and MC-LR content in the Huangpu River, the mother river of Shanghai City, are necessary.

Many analysis methods have been developed for detecting a broad variety of MCs (Foss et al., 2018). High-performance liquid chromatography (HPLC) has been used widely for the detection of MCs in which quantitative and qualitative analyses can be achieved (Lawton et al., 1994). However, the operation of HPLC is relatively complicated and time consuming in terms of sample analysis and system cleaning (Rapala et al., 2002). Enzyme-linked immunosorbent assay (ELISA) is an ideal and reliable method with high efficiency, sensitivity, cost-effective (Merel et al., 2013). ELISA kits are available easily and performed simply with high specificity (Rivasseau et al., 1999). Surface water containing MC-LR and MC-YR in the range of 0.2–4 µg/L can be detected by ELISA rapidly and directly without any pretreatment (Rivasseau et al., 1999). A previous study (Samdal et al., 2014) showed that the results obtained by liquid chromatograph-mass spectrometry and ELISA in the detection of MCs are extremely very close. ELISA gives a reliable correlation with HPLC for the detection of MCs in the water extracts of natural blooms and cultured cyanobacterial cells ($R^2 = 0.98$) (Nagata et al., 1997). ELISA was used to detect MC-LR in tap and lake water with good recovery (94–110%) (Liu et al., 2014). However, the quantification of actual toxicity by ELISA may be overestimated. This condition maybe because the conjugated forms of MC-LR can cross-react with commercially available ELISA kits (Campas and Marty, 2007; McElhiney and Lawton, 2005). It is not yet clear what variations among results are due to difference between methodologies (Chik et al., 2021). Therefore, the accuracy and stability of ELISA and the correlation between ELISA and HPLC require further investigation. Under the conditions where HPLC is unavailable or where rapid test results are required in field investigations, the results are helpful to justify whether ELISA can replace HPLC.

In the current study, the MC-LR concentrations in the Huangpu River over a year were investigated to explore the effects of temperature on the synthesis and release of MC-LR in *Microcystis* bloom water body. To simulate the contaminated water environment and characterize variability among results when using HPLC and ELISA, aliquots from *M. aeruginosa* cultures in the laboratory were spiked with 10 mg/L glyphosate (the glyphosate group). Those cultures without glyphosate treatment were set as the control group. This approach was designed to capture the collective experience and capacity of ELISA and HPLC,

and to allow for border inferences to be drawn related differences in data generation and handling approaches. The aim of this study was (1) to establish the correlation between ELISA and HPLC in detecting low levels of MC-LR; (2) to prove that sample preparation methods did not have a clear, systematic impact on the results; (3) to explore the relationship between temperature and MC-LR content in the Huangpu River, China.

2. Materials And Methods

2.1 Cyanobacterial strain and cultivation

M. aeruginosa (Code: 905) used in our experiment was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (Wuhan, China) and cultured in BG11 medium. The Cyanobacterial growth tests were conducted following the Organization for Economic Cooperation and Development (OECD) guidelines 201-Freshwater Alga and Cyanobacteria with 0 and 10 mg/L of glyphosate (purity $\geq 99\%$) obtained from Ao Yali Plant Protection Technology Service Co., Ltd. (Hefei, China). The correlation between ELISA and HPLC was established by detecting the MC-LR concentration in *M. aeruginosa* cultures exposed to 10 mg/L glyphosate at different culture periods in the laboratory. Standard MC-LR (purity $\geq 95\%$) was purchased from Express Technology Co., Ltd. (Beijing, China). *M. aeruginosa* samples were cultured in an RXZ-380A artificial intelligence climate chamber with a 12/12 h light/dark cycle at $28 \pm 1^\circ\text{C}$, illuminance of $40 \mu\text{mol}/\text{m}^2 \text{ s}$, and humidity of 60%. The flasks were shaken three times a day and rearranged randomly to minimize the effects caused by space in illumination and temperature.

2.2 Analysis of extracellular MC-LR from *M. aeruginosa* cultivated in the laboratory

2.2.1 Analysis Of Mc-Lr By Elisa

After freezeing-thrawing five times, *M. aeruginosa* cultivation samples (15 mL) were centrifuged at 8000 r/min under 0°C , and the supernatant was reserved. All operations were performed at room temperature.

MC-LR in supernatants was determined using microcystin diagnostic kits obtained from Puhuashi Technology Development Co., LTD. (Beijing, China). The ELISA kits were made of polyclonal antibodies that could conjugate MC-LR and MC-LR horse radish peroxidase. MC-LR in the samples competed with MC-LR horse radish peroxidase to conjugated limit antibody nodes. The test wells were coated with goat anti-rabbit IgG, which was used to capture the added rabbit anti-MC-LR antibody.

Horse radish peroxidase (HRP, 50 μL) was added in the wells of a 96-well microplate containing goat anti rabbit IgG. MC-LR standards (50 μL) of different concentrations (0, 100, 300, 800, and 2000 ng/L) and 50 μL processed samples were added in the remaining wells. Rabbit anti-MC-LR antibody (50 μL) was added in each well. The plate was incubated with thin film for 0.5 h at 37°C . The solution in each well was then removed. The plates were washed with ELISA washing buffer for five times and blotted dry before

addition of the HRP substrate (100 µL/well). The reaction was stopped by adding 100 µL of 1.0 mol/L HCL. All samples were performed in triplicate. The optical density was measured at 450 nm with a microplate reader within 30 min. The MC-LR concentration was calculated by referring to a standard curve constructed with MC-LR ($R^2 = 0.9899$) through ELISA. The limit of detection (LOD) was 50 ng/L.

2.2.2 Analysis Of Mc-Lr By Hplc

After freezeing-thrawing five times, the MC-LR of 15 mL cultures was extracted by centrifugation and concentrated on a Promosil C18 column with methanol. Extractions for analysis were filtered through a filter (0.45 µm). The MC-LR of 15 mL cultures was concentrated in 1 mL methanol by the nitrogen blowing concentrator and stored at -20°C until analysis using HPLC. The elution profile of the MC-LR from the HPLC analysis is shown in Fig. 1.

The MC-LR content was detected on a Prominence Series LC-20AT HPLC system (Shimadzu, Japan). Toxins were detected using a Promosil C18 column (4.6 mm × 250 mm, i.d., 5 µm) at a flow rate of 1.0 mL/min, a mobile phase of 65: 35 methanol/water (v: v), a detection wavelength of 238 nm, and an injection volume of 10 µL at room temperature. The retention times for MC-LR was approximately 6 min. The MC-LR concentrations were then calculated by referring to a standard curve constructed with MC-LR standard sample ($R^2 = 0.9999$) by HPLC. Its LOD was 0.1 mg/L.

2.3 MC-LR detection in the Huangpu River analyzed by ELISA and HPLC

Water samples were collected from four locations nearby the Huangpu River. These locations were Jiangchuan Road in Minhang District, Huajing Road in Xuhui District, the Bund in Yangpu District, and Danyang Road in Yangpu District (Fig. 2). The sampling period from the Huangpu River lasted from January to December, 2018. Sampling was performed twice a month at each site. Water samples (1.5 L) were collected from a depth of 0.5–1 m in polyethylene bottles at each site, and then frozen in a refrigerator at -4°C in the laboratory. They were freeze-thawed for five times to tear cells apart. The solutions were analyzed through ELISA and HPLC with the previously mentioned steps.

2.4 Statistical Analysis

Statistical analysis was performed on SPSS 14.0 (SPSS, USA) and Origin 2018 (Microcal Software, Northampton, MA, USA) to determine the significance between different treatments. A value of $p < 0.05$ was considered statistically significant.

3. Results And Discussion

Comparison between ELISA and HPLC on MC-LR detection produced by *M. aeruginosa* cultivated in the laboratory

3.1.1 Comparison Between Elisa And Hplc In Mc-Ir Concentration

The results of MC-LR concentration at different culture periods measured by ELISA and HPLC in the control group and those in the glyphosate group are shown in Fig. 3. All *M. aeruginosa* samples cultivated in the laboratory were treated in triplicate. Compared HPLC, the average relative errors of MC-LR content from ELISA method in the control group after 24, 48, 72, and 96 h cultivation were 9.11%, 7.60%, 12.49%, and 3.30%, respectively. In the glyphosate group, the average relative errors were 18.51%, 17.19%, 11.03%, and 3.14%. The MC-LR concentration increased over time in cultures and the MC-LR concentrations found in HPLC and ELISA are similar. After 72 h cultivation, the MC-LR concentration in glyphosate was higher than the control group detected by both methods. The lowest average relative error between ELISA and HPLC was observed after 96 h cultivation in the control and glyphosate groups, indicating that the MC-LR content detected by ELISA was the closest to that detected by HPLC. The results showed that total MC-LR concentrations were generally consistent mostly within an order of magnitude for a given glyphosate-spike condition. Little relative errors were generally observed in the spike condition compared to that of non-spike condition. This condition may be attributed to the effect of glyphosate on ELISA kits that may disturb the sensitivity of ELISA. The commercially available ELISA kits showed variable cross-reactivity (McElhiney and Lawton, 2005). In addition to free MC-LR, ELISA cross-reacted strongly with conjugated MCs such as cysteine and glutathione conjugates (Samdal et al., 2014), and glyphosate affected the reaction of the antibody, thereby overestimating the MC-LR content, which should be investigated in future research.

3.1.2 Linear Relationships Between Elisa And Hplc

Linear relationships (Fig. 3) were established to explore the correlation between ELISA and HPLC. The linear equations between ELISA and HPLC were $y=0.99x+0.73$ for control groups and $y=0.95x+2.48$ for glyphosate groups, where y represents the concentration of MC-LR detected by ELISA, and x represents the concentration of MC-LR detected by HPLC. The correlation coefficients for the control and glyphosate groups were 0.998 and 0.994, respectively. The correlation between ELISA and HPLC performed well with obvious linearity (Fig. 3). This finding demonstrated that ELISA was an effective alternative to HPLC in the detection of MC-LR.

3.1.3 Comparison between ELISA and HPLC in terms of reproducibility and recovery

The samples cultivated after 72 and 96 h of the control group (Table. 1) were measured by two methods in octuplicate to verify the reproducibility of the experiment. The coefficients of variation of ELISA were less than 1.5%, indicating high reproducibility. The higher coefficients of variation of ELISA than HPLC reflects the ELISA assay is easily affected by many factors such as sample composition and various experimental conditions (Babica et al., 2006). Overall, highly reproducible results between ELISA and

HPLC were indicated to estimate MC-LR concentrations produced by *M. aeruginosa* cultivated in the laboratory.

Table 1
Reproducibility of ELISA detecting MC-LR after 72 h and 96 h cultivation of *M. aeruginosa* in the control group

Method	Time (h)	Mean (mg/L)	SD	CV ^a (%)
ELISA	72	18.838	0.283	1.5
	96	39.957	0.401	1.0
HPLC	72	18.361	0.053	0.3
	96	38.349	0.045	0.1
^a CV means the coefficient of variation.				
The MC-LR content after 96 h cultivation detected by ELISA in the control and glyphosate groups are shown in Fig. 4. The average recovery is 112.6% and 92.2%. The average recoveries of MC-LR content detected by HPLC in the control and glyphosate groups were 92% and 89.6%, respectively. The recoveries of ELISA and HPLC fell into the standard range (80–120%) to ensure their accuracy.				

As expected, rare variability was observed in the results between HPLC and ELISA in detecting MC-LR contents. Detections did not appear link to methods. Sample preparation methods did not have a clear, systematic impact on results. ELISA is simpler than HPLC in sample preparation, thereby reducing operation time and improving working efficiency. Overall, both HPLC and ELISA yielded comparable results in present study. No matter HPLC or ELISA was used to explore the MC-LR temporal trends in environmental water system, with appropriate quality control protocols and documented in adequate detail should succeed. The results justify the feasibility to replace HPLC with ELISA under certain conditions. In addition, ELISA can detect the MC-LR concentration in the field without complex treatment, avoiding samples from denaturation and degradation during the transportation. Hence, ELISA is a better alternative of HPLC when HPLC is unavailable, especially when rapid testing is required in routine MC-LR analysis.

3.2 Spatial and Temporal distribution of MC-LR in the Huangpu River detected by ELISA and HPLC

The MC-LR contents in the water samples detected by ELISA and HPLC at four locations are shown in Fig. 5 (A–D). The MC-LR contents increased most rapidly from June to August in a year and decreased from September at all locations. The MC-LR content detected by ELISA was absent in some months, especially in winter, owing to the lower concentration than LOD. At Huajing Road (Fig. 5A) and Jiangchuan Road (Fig. 5B), the MC-LR concentration was not detected by ELISA from November to April. At Danyang Road (Fig. 5C), the MC-LR content detected by ELISA was absent from January to March. This condition was because the MC-LR content at Danyang Road (Fig. 5C) was highest among the four locations and higher than LOD in most months. Danyang Road was located nearby the industrial area

and had a considerable impact on the highest level of MC-LR in water body. The trend of MC-LR content variation at the Bund (Fig. 5D) was similar to that at Jiangchuan Road. The MC-LR content in the Bund was the lowest in all locations may due to its high flow rate.

The MC-LR concentrations at the four sites showed similar seasonal variation trends with the change in water temperature (Fig. 5). They peaked in the summer and then decreased with the decrease in temperature. Low MC-LR concentration can be detected by ELISA in the water without complicated preparation with its low LOD (50 ng/L). It is important to monitor the MC-LR concentration in the natural water body when not exceeding 1 µg/L, which is the World Health Organization guideline value of total MC-LR, and to ensure the safety of drinking water.

The MC-LR concentrations in four sites of the Huangpu River in Shanghai were measured by two methods. They all reached their peak in summer when the water temperature ranged from 22°C–34°C, which is approximately consistent with our previous study (Ye et al., 2020). MC-LR concentrations in summer were higher than those in winter, which was consistent with the seasonal trend of algal biomass. This temperature relationship infers that the MC-LR pollution can occur easily in summer in the Huangpu River. The MC-LR concentration levels of surface water bodies should be controlled and regularly monitored, especially during the high algae period in summer. Other studies have found that increasing temperature promotes the growth rate of cyanobacteria cells and the cell density reaches the maximum in summer (Gkelis et al., 2014; Mohamed et al., 2015). The concentrations of MCs produced by the cyanobacterial community, the spatial distribution of MCs, and the MCs quota per algal cell were reported to be driven by temperature. In Dianchi Lake, high MC concentrations occurred from April to December, which were warm months, and the maximum concentration was observed in the middle of the lake (Wu et al., 2014). In Lake Taihu, the toxin concentrations in northern parts were significantly higher than in the eastern part in summer (Su et al., 2015). Mowe et al.(2015) reported that the MCs quota per algal cell varies from the species under warm temperature. Increasing water temperature caused by warming climate potentially motivates the selection of a few highly toxic species or strains (Mantzouki et al., 2018). Under high temperature of 15°C–30°C, the abundance of *Microcystis* species producing many MCs was observed; this condition may be because more toxin-related genes per cell are presented copied (Davis et al., 2009).

According to Tsuji et al. (Tsuji et al., 1994), photocatalytic degradation of pigments is the main natural degradation pathway of MC-LR in water. The water temperature at 39°C contributed to the removal of dissolved MC-LR in the presence of sediment, thereby affecting adsorption and biodegradation (Santos et al., 2020). However, the MC-LR content in Huangpu River was found increasing with high temperature and strong light intensity in summer according to our study. It may be attributed to the other factors effected by temperature and spatial distribution such as bacterial strains and microbial community in the Huangpu River which need further study.

4. Conclusion

Considering the environmental safety of drinking water, it is necessary to detect low levels of MC-LR in surface water. This study justified that HPLC and ELISA can reliably detect the low levels of MC-LR concentration, and the two methods can yield comparable results for both laboratory cultures and environmental water samples. Given that ELISA can detect the low levels of MC-LR in the field, and can obtain the results immediately without complicated pretreatments and long-distance transportation, it is reliable to monitor the MC-LR concentration routinely in the environmental water body, especially MC-LR is the mainly MCs variant.

This study assessed the temporal and spatial distribution of MC-LR in the Huangpu River. The results show that the MC-LR content increases with the increasing temperature in the Huangpu River. The highest MC-LR content is observed in August at approximately 30°C. The highest MC-LR concentration was 282.03 ± 8.07 ng/L at Danyang Road where is nearby the industrial area. Hence, climate variation, especially the warming trend, can promote the release of MC-LR in eutrophic freshwater. It is necessary to regularly monitor the MC-LR concentration levels at the pollution area, especially during the high algae period in summer.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions SH carried out the HPLC and ELISA detection and drafted the manuscript. JC took the water samples from Huangpu River and conducted the sample pre-treatment. LW participated in the HPLC detection. XY participated in the design of the study and participated in the ELISA detection. JY conceived of the study, design the study, and helped to draft the manuscript. YL developed the concept of this study in discussion. YZ and FT performed the statistical analysis. All authors read and approved the final manuscript.

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References

1. Abe T, Lawson T, Weyers JDB et al (1996) Microcystin-LR inhibits photosynthesis of *Phaseolus vulgaris* primary leaves: Implications for current spray irrigation practice. *New Phytologist* 133: 651-658. <https://doi.org/10.1111/j.1469-8137.1996.tb01934.x>
2. Babica P, Kohoutek J, Bláha L et al (2006) Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic material contents. *Anal Bioanal Chem* 385: 1545-1551. <https://doi.org/10.1007/s00216-006-0545-8>
3. Budzyńska A, Rosińska J, Pełechata A et al (2019) Environmental factors driving the occurrence of the invasive cyanobacterium *Sphaerospermopsis aphanizomenoides* (Nostocales) in temperate lakes. *Science of the Total Environment* 650: 1338-1347. <https://doi.org/10.1016/j.scitotenv.2018.09.144>
4. Campas M, Marty JL (2007) Highly sensitive amperometric immunosensors for microcystin detection in algae. *Biosens Bioelectron* 22: 1034-1040. <https://doi.org/10.1016/j.bios.2006.04.025>
5. Carmichael WW, Boyer GL (2016) Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae* 54: 194-212. <https://doi.org/10.1016/j.hal.2016.02.002>
6. Corbel S, Mougin C, Bouaïcha N et al (2014) Cyanobacterial toxins: modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. *Chemosphere* 96: 1-15. <https://doi.org/10.1016/j.chemosphere.2013.07.056>
7. Chik, AHS, Giler MB, Servos M et al (2021) Comparison of approaches to quantify SARS-CoV-2 in wastewater using RT-qPCR: Results and implications from a collaborative inter-laboratory study in Canada. *Journal of Environmental Sciences*, 107: 218-229.
8. Davis TW, Berry DL, Boyer GL et al (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae* 8: 715-725. <https://doi.org/10.1016/j.hal.2009.02.004>
9. de Figueiredo, DR, Azeiteiro UM, Esteves SM et al (2004) Microcystin-producing blooms - a serious global public health issue. *Ecotoxicology and Environmental Safety* 59: 151-163. <https://doi.org/10.1016/j.ecoenv.2004.04.006>
10. Foss AJ, Miles CO, Samdal IA et al (2018) Analysis of free and metabolized microcystins in samples following a bird mortality event. *Harmful Algae* 80: 117-129. <https://doi.org/10.1016/j.hal.2018.10.006>
11. Gkelis S, Papadimitriou T, Zaoutsos N et al (2014) Anthropogenic and climate-induced change favors toxic cyanobacteria blooms: Evidence from monitoring a highly eutrophic, urban Mediterranean lake. *Harmful Algae* 39: 322-333. <https://doi.org/10.1016/j.hal.2014.09.002>
12. Gupta N, Pant SC, Vijayaraghavan R et al (2003) Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology* 188: 285-296. [https://doi.org/10.1016/s0300-483x\(03\)00112-4](https://doi.org/10.1016/s0300-483x(03)00112-4)
13. Gurbuz F, Metcalf JS, Karahan AG et al (2009) Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey. *Sci Total Environ* 407: 4038-4046.

<https://doi.org/10.1016/j.scitotenv.2009.02.039>

14. Harke MJ, Steffen MM, Gobler CJ et al (2016) A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae* 54: 4-20.
<https://doi.org/10.1016/j.hal.2015.12.007>
15. Imai H, Chang KH, Kusaba M et al (2009) Temperature-dependent dominance of *Microcystis* (Cyanophyceae) species: *M. aeruginosa* and *M. wesenbergii*. *Journal of Plankton Research* 31: 171-178. <https://doi.org/10.1093/plankt/fbn110>
16. Jasione G, Zhdanov A, Davenport John et al (2010) Mitochondrial Toxicity of Microcystin-LR on Cultured Cells: Application to the Analysis of Contaminated Water Samples. *Environmental Science & Technology* 44: 2535-2541. <https://doi.org/10.1021/es903157h>
17. Jiang Y, Ji B, Wong RNS et al (2008) Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium - *Microcystis aeruginosa*. *Harmful Algae* 7: 127-136. <https://doi.org/10.1016/j.hal.2007.05.012>
18. Li X, Liu Y, Song L et al (2003) Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon* 42: 85-89.
[https://doi.org/10.1016/s0041-0101\(03\)00104-1](https://doi.org/10.1016/s0041-0101(03)00104-1)
19. Liu L, Xing C, Yan H et al (2014) Development of an ELISA and Immunochromatographic Strip for Highly Sensitive Detection of Microcystin-LR. *Sensors* 14: 14672-14685.
<https://doi.org/10.3390/s140814672>
20. Mantzouki E, Lüring M, Fastner J et al (2018) Temperature Effects Explain Continental Scale Distribution of Cyanobacterial Toxins. *Toxins* 10: 24. <https://doi.org/10.3390/toxins10040156>
21. Máthé C, Beyer D, Erdodi F et al (2009) Microcystin-LR induces abnormal root development by altering microtubule organization in tissue-cultured common reed (*Phragmites australis*) plantlets. *Aquatic Toxicology* 92: 122-130. <https://doi.org/10.1016/j.aquatox.2009.02.005>
22. McElhiney J, Lawton LA (2005) Detection of the cyanobacterial hepatotoxins microcystins. *Toxicology and Applied Pharmacology* 203: 219-230. <https://doi.org/10.1016/j.taap.2004.06.002>
23. McElhiney J, Lawton LA, Leifert Carlo (2001) Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. *Toxicon* 39: 1411-1420.
[https://doi.org/10.1016/s0041-0101\(01\)00100-3](https://doi.org/10.1016/s0041-0101(01)00100-3)
24. Merel S, Walker D, Chicana R et al (2013) State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International* 59: 303-327.
<https://doi.org/10.1016/j.envint.2013.06.013>
25. Mitrovic SM, Allis O, Furey A (2005) Bioaccumulation and harmful effects of microcystin-LR in the aquatic plants *Lemna minor* and *Wolffia arrhiza* and the filamentous alga *Chladophora fracta*. *Ecotoxicology and Environmental Safety* 61: 345-352. <https://doi.org/10.1016/j.ecoenv.2004.11.003>
26. Mohamed ZA, Deyab MA, Abou-Dobara MI et al (2015) Occurrence of cyanobacteria and microcystin toxins in raw and treated waters of the Nile River, Egypt: implication for water treatment and human

- health. *Environmental Science and Pollution Research* 22: 11716-11727.
<https://doi.org/10.1007/s11356-015-4420-z>
27. Mowe MAD, Porojan C, Abbas F et al (2015) Rising temperatures may increase growth rates and microcystin production in tropical *Microcystis* species. *Harmful Algae* 50: 88-98.
<https://doi.org/10.1016/j.hal.2015.10.011>
28. Nagata S, Tsutsumi T, Hasegawa A et al (1997) Enzyme immunoassay for direct determination of microcystins in environmental water. *Journal of Aoac International* 80: 408-417.
29. Pouria S, de Andrade A, Barbosa J et al (1998) Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *Lancet* 352: 21-26. [https://doi.org/10.1016/s0140-6736\(97\)12285-1](https://doi.org/10.1016/s0140-6736(97)12285-1)
30. Qin B, Zhu G, Gao G et al (2010) A Drinking Water Crisis in Lake Taihu, China: Linkage to Climatic Variability and Lake Management. *Environmental Management* 45: 105-112.
<https://doi.org/10.1007/s00267-009-9393-6>
31. Rapala J, Erkomaa K, Kukkonen J et al (2002) Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography-UV detection and enzyme-linked immunosorbent assay - Comparison of methods. *Analytica Chimica Acta* 466: 213-231.
[https://doi.org/10.1016/s0003-2670\(02\)00588-3](https://doi.org/10.1016/s0003-2670(02)00588-3)
32. Rivasseau C, Racaud P, Deguin A et al (1999) Evaluation of an ELISA kit for the monitoring of microcystins (cyanobacterial toxins) in water and algae environmental samples. *Environmental Science & Technology* 33: 1520-1527. <https://doi.org/10.1021/es980460g>
33. Samdal IA, Ballot A, Løvberg KE et al (2014) Multihapten Approach Leading to a Sensitive ELISA with Broad Cross-Reactivity to Microcystins and Nodularin. *Environmental Science & Technology* 48: 8035-8043. <https://doi.org/10.1021/es5012675>
34. Santos A, Rachid C, Pacheco AB et al (2020) Biotic and abiotic factors affect microcystin-LR concentrations in water/sediment interface. *Microbiol Res* 236: 126452.
<https://doi.org/10.1016/j.micres.2020.126452>
35. Su X, Xue Q, Steinman AD et al (2015) Spatiotemporal Dynamics of Microcystin Variants and Relationships with Environmental Parameters in Lake Taihu, China. *Toxins* 7: 3224-3244.
<https://doi.org/10.3390/toxins7083224>
36. Tsuji K, Watanuki T, Kondo F et al (1994) Stability of microcystins from cyanobacteria - effect of light on decomposition and isomerization. *Environmental Science & Technology* 28: 173-177.
<https://doi.org/10.1021/es00050a024>
37. Umehara A, Tsutsumi H, Takahashi T (2012) Blooming of *Microcystis aeruginosa* in the reservoir of the reclaimed land and discharge of microcystins to Isahaya Bay (Japan). *Environmental Science and Pollution Research* 19: 3257-3267. <https://doi.org/10.1007/s11356-012-0835-y>
38. Vezie C, Brient L, Sivonen K et al (1998) Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). *Microbial Ecology* 35: 126-135.
<https://doi.org/10.1007/s002489900067>

39. Wu Y, Li L, Gan N et al (2014) Seasonal dynamics of water bloom-forming *Microcystis* morphospecies and the associated extracellular microcystin concentrations in large, shallow, eutrophic Dianchi Lake. *Journal of Environmental Sciences* 26: 1921-1929.
<https://doi.org/10.1016/j.jes.2014.06.031>
40. Wan X, Steinman AD, Gu Y et al (2020) Occurrence and risk assessment of microcystin and its relationship with environmental factors in lakes of the eastern plain ecoregion, China. *Environ Sci Pollut Res Int* 27(36): 45095-45107. doi: 10.1007/s11356-020-10384-0.
41. Ye J, Guan Y, Wu L et al (2020) Effects of glyphosate on microcystin-LR production and release from *Microcystis aeruginosa* at different temperatures. *Environmental Science and Pollution Research*, 27(33):41961-41969. <https://doi.org/10.1007/s11356-020-10185-5>
42. Zurawell RW, Chen H, Burke JM et al (2005) Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 8: 1-37. <https://doi.org/10.1080/10937400590889412>

Figures

Figure 1

The elution profile of the MC-LR from the HPLC analysis.

Figure 2

The locations in the Huangpu River where the water samples were collected.

Figure 3

Linear comparison in (A) control group and (B) glyphosate group. The results are presented as mean $\pm$ SD of three independent assays.

Figure 4

Reproducibility of ELISA and HPLC detecting MC-LR after 24, 48, 72, and 96 h cultivation of *M. aeruginosa* in the control group (A) and glyphosate (B) group. The results are presented as mean $\pm$ SD of eight independent assay.

Figure 5

MC-LR concentration detected by HPLC and ELISA and the water temperature variation in the Huangpu River: (A) Huajing Road, Xuhui District, (B) Jiangchuan Road, Minhang District, (C) Danyang Road, Yangpu District, (D) the Bund, Huangpu District. The results are presented as $\pm$ SD of two independent assays. N means Not detected.