Temporal Dynamics of Microcystins In Two Reservoirs With Different Trophic Status During The Early Growth Stage of Cyanobacteria

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Abstract

Harmful cyanobacterial blooms are increasing in frequency and severity, which makes their toxic secondary metabolites of microcystins (MCs) have been widely studied, especially in their distribution and influence factors in different habitats. However, the distribution of MCs on the early growth stage of harmful cyanobacteria and its influence factors and risks are still largely unknown. Thus, in the present study, two reservoirs (Lutian Reservoir and Lake Haitang) with different trophic status in China have been studied weekly from March to May in 2018, when the cyanobacteria communities were just in the early growth stage, to investigate the variation of MCs concentration and the relationships between MCs and environmental parameters. During the investigation, Lutian Reservoir and Lake Haitang were found to be mesotrophic and light eutrophic, respectively. In Lutian Reservoir, the concentration of EMCs was obviously higher than that of IMCs with a mean value of 0.323 and 0.264 $\mu\text{g/L}$, respectively. Meanwhile, the concentration of EMCs also fluctuated more sharply than that of IMCs. Congeners of IMC-YR and EMC-LR were respectively dominant in total concentrations of IMCs and EMCs. Unsurprisingly, in Lake Haitang, the concentrations of IMC and EMC were both significantly higher than that in Lutian Reservoir with a mean concentration of 0.482 and 0.472 $\mu\text{g/L}$, respectively. Differently, the concentration of MC-YR was dominant in both IMCs and EMCs, followed by MC-LR. In correlation analysis, the IMCs were significantly and positively correlated with the density and biomass of phytoplankton phyla and potential MCs-producing cyanobacteria and the parameters of water temperature (WT), nutrients and organic matters. Similar results were also observed for EMCs. While the different variation of MCs in two reservoirs might be primarily caused by the differences in WT, nutrients (especially phosphorus), organic matters and the composition of MCs-producing cyanobacteria. In addition, the coexistence of the dominant species of *Pseudoanabaena* sp., which can produce taste-and-odor compound of 2-methylisoborneol (2-MIB), might have significant impacts on the concentration and toxicity of MCs. Our results suggested that the risks posed by MCs at the early growth stage of cyanobacteria should also deserve our attentions, especially in mesotrophic water bodies.

Introduction

Microcystins (MCs) are a family of potent hepatotoxins with nearly 300 congeners have been identified (Massey et al., 2020). They are secondary metabolites of certain genera of cyanobacteria, including *Microcystis*, *Anabaena* (*Dolichospermum*), *Oscillatoria*, *Nostoc* and *Planktothrix*, etc. (Buratti et al., 2017). Although the biological and ecological functions of MCs are still controversial, various studies have proved that it can protect the MC-producing algae from predators and oxidative stress, acting as allelochemicals, and promote the colony formation, etc. (Gan et al., 2012; Hu and Rzymiski, 2019). In addition, MCs can also be transferred to other organisms of higher trophic levels through food chain (Díez-Quijada et al., 2019). Due to its high toxicity, it can cause liver damage, promoting tumor, and affect reproduction (Chen et al., 2016; Huisman et al., 2018). What's worse, under the strong influences of both global climate change and anthropogenic activities, the increased MCs pollution has resulted in the occurrence of biological deaths of fish and mammals, even including humans. More recently, the deaths

of more than 330 African elephants in Botswana were considered to be primarily caused by MCs ingestion (Wang et al., 2021). Thus, given the high toxicity and health threat, the dynamics and potential influence factors of MCs distribution in various water bodies around the world has become a current research hotspot.

To date, at least 108 countries, which distribute in all continents except Antarctica, have been detected the distribution of MCs (Benegas et al., 2021; Chaffin et al., 2018). MCs are primarily intracellular and released to the dissolved water when the cell lysis and death occurred (Rohrlack and Hyenstrand, 2007; Sivonen and Jones, 1999). So, the concentration of intracellular MCs is usually significantly higher than that of extracellular MCs. In a small, shallow and closed-basin lake in Ontario, Canada, the maximum concentration of intracellular MCs was above 4.2 mg/L, while the concentration of extracellular MCs was extremely lower, but also exceeded 0.203 mg/L (Zastepa et al., 2014). The similar results were discovered in two freshwater bodies in China (Li et al., 2010; Zhang et al., 2018). In addition, MCs in one freshwater body usually consists of various MC congeners, and MC-LR and MC-RR are the dominant ones in most studies (Díez-Quijada et al., 2019; Tilahun et al., 2019). However, other MC congeners are also widely spread throughout the world and record relatively high concentrations in many lakes and reservoirs (Gurbuz et al., 2016; Simiyu et al., 2018). Therefore, further researches are needed to assess and prevent the risks caused by MCs pollution, and to better understand the mechanisms involved in the MCs production.

Due to the complicated interactions between various environmental variables and potential MCs-producing species, MCs in different water bodies usually exhibits obviously different variation characteristics. Several previous studies have found that the different composition of toxigenic genes played a dominant role in MCs production, congener composition and intracellular content (Okello et al., 2010a; Okello et al., 2010b; Shishido et al., 2019). Meanwhile, several studies suggested the rate of MCs-producing positively correlated with the growth rate of the cyanobacteria (Orr and Jones, 1998; Sivonen and Jones, 1999). The environmental parameters, which primarily refer to the physicochemical parameters of water bodies, also played an important role in MCs production (Xue et al., 2018). Among the various environmental parameters, water temperature is mainly positively correlated with MCs concentration (Walls et al., 2018), whereas the correlations between the nutrients and MCs varied from study to study (Wagner et al., 2021; Wang et al., 2018). In addition, the concentration of MCs can also be affected by other pollutants, such as antibiotics (Wan et al., 2021a), nanoplastics (Feng et al., 2020) and herbicides (Brêda-Alves et al., 2021). However, despite lots of studies have focused on this area, the mechanisms of how certain environmental parameters regulate the MCs production and variation are still poorly understood. What's more, most studies are concentrated in the results obtained during cyanobacterial blooms or monthly sampling, while few studies concerned on the early growth stage of cyanobacteria or higher frequency sampling.

Compared with natural lakes, reservoirs are characterized by larger fluctuations in water levels and more complex and variable hydrological conditions (Ford, 1990; Nowlin et al., 2004). Simultaneously, connections between reservoirs and human beings are more closely (Noori et al., 2018), and a slight

water pollution may lead to a potentially high threat to human health. However, there are few researches focused on reservoirs. Thus, in the present study, two reservoirs (Lutian reservoir and Lake Haitang) with different trophic status were selected and sampled during the early growth period of cyanobacteria. Both the variations of three MC congeners (MC-LR, -RR, and -YR) and the associated environmental parameters were investigated to (1) analyze the differences of physicochemical parameters and phytoplankton composition between the two reservoirs; (2) compare the temporal variation characteristics of three MC congeners in two reservoirs during the early growth stage of cyanobacteria; and (3) explore the potential driving factors that causing the different distribution patterns of MCs in two reservoirs.

Materials And Methods

Sites and sampling

Lutian Reservoir and Lake Haitang are two typical reservoirs located in Jinhua City, Zhejiang Province, China (Fig. 1). Both reservoirs are small, and cover an area of 0.11 and 1.46 km², respectively. Lutian Reservoir is located in the northern suburb of the city (Luodian County Shuanglong scenic area) at the altitude of 550~600 m (29.198° N, 119.630° E), which is about 20 km away from the downtown and rarely affected by human activities. As an important part of Shuanglong scenic spot, it provides the water source for the irrigation, aquaculture and recreation for surrounding citizens. While Lake Haitang (29.056° N, 119.645° E) is located in the downtown of Jinhua City, which attracts many citizens and tourists, with a lot of pollutants and interventions brought to it. Lake Haitang was built as core park green space of the city and was designed to have the function of driving the development of residential land, attracting commercial investment, and guiding residents of the leisure and entertainment. Due to the absolutely different functional positioning and pollution conditions, the two reservoirs are in different trophic states.

Two sites distributed in the center of two reservoirs were sampled weekly from March 11th to May 5th, 2018 (Fig. 1). All water samples were collected from the water surface (approximately at the depth of 0.5 m) using a 2.5-L Plexiglas water sampler. The samples used for phytoplankton identification were preserved with 1% acidic Lugol's solution, other samples for physiochemical parameters (0.5 L) and MCs (1 L) analysis were all stored in a portable refrigerator (0°C) and then transported to the laboratory. Water depth and Secchi disk depth (SD) was respectively measured by a sonar fathometer (SM-5A, Japan) and a Secchi disk in situ. Water temperature was measured using the water temperature meter equipped in the water sampler.

Environmental parameters and MCs analysis

Water samples were analyzed for chl-*a*, total nitrogen (TN), ammonium (NH₄⁺-N), nitrate (NO₃⁻-N), total phosphorus (TP), orthophosphate (PO₄³⁻-P), chemical oxygen demand (COD) and dissolved organic carbon (DOC), and all the analyzes were following the standard methods described in Jin and Tu (1990). Phytoplankton samples which preserved with 1% acidic Lugol's solution were concentrated to 50 mL after sedimentation for 48h. Then 0.1 mL concentrated samples were counted on a stereo microscope under ×

400 magnification after mixing. Phytoplankton species were identified and calculated according to Niu et al. (2011) and Xue et al. (2018).

Intracellular MCs (IMCs) and extracellular MCs (EMCs) were both analyzed, and the methods were according to the references of Su et al. (2015) and Xue et al. (2016), respectively. Briefly, after water sample filtration, the filter paper and filtrate were respectively used for IMCs and EMCs determination. Then, the filter paper was lyophilized and extracted. After the above treatments, the following procedures were nearly the same for IMCs and EMCs analysis. The supernatant and filtrate were both added to a pre-activated HLB cartridge (200 mg, Oasis®, Waters, Milford, MA, USA). After concentration, washing, elution, drying and redissolution, the concentrated samples were all analyzed by High-Performance Liquid Chromatography (HPLC, Agilent 1200 series, Palo Alto, CA, USA). The HPLC system was equipped with a DAD detector (Agilent, Palo Alto, CA, USA) and an ODS column (Agilent Eclipse XDB-C18, 5 µm, 4.6 mm × 150 mm). Three dominant MC congeners (MC-LR, MC-RR and MC-YR) were analyzed for all the samples. Standards (three MC congeners) used for MCs analysis were bought from Sigma-Aldrich (München, Germany).

Data analysis

The correlation analysis between different variables was conducted with “correlation plot” app in OriginPro 2021b (version 9.8.5.204, OriginLab, Northampton, MA, USA) founded on the correlation type of “spearman”, and the plot was also created by this app. One-way ANOVA and non-parametric tests were used to examine the difference of various variables between the two reservoirs, and these analyses were performed by IBM SPSS version 25 (USA). The Trophic Level Index (TLI) was calculated according to the method described in Jin et al. (1995), which based on the calculations of TLI(Chl_a), TLI(TP), TLI(TN), TLI(SD) and TLI(COD_{Mn}). In addition, the sum concentration of the three MC congeners was represented by total MC concentration (TIMCs and TEMCs) in the present study.

Results

Physicochemical parameters variation

During the survey, almost all the physicochemical parameters (except NO₃⁻-N) exhibited significantly difference between the two reservoirs (Table 1). The water depth in Lutian Reservoir was significantly higher than that in Lake Haitang with higher values both detected in the late period of the survey. Although the sampling time and reservoir location were similar, the WT of Lutian Reservoir was significantly lower than that of Lake Haitang. And the WT of the two reservoirs both showed an increasing trend during the investigation.

Table 1. The mean values and ranges of various physicochemical parameters of the two reservoirs.

Parameters	Lutian Reservoir		Lake Haitang	
	mean $\pm$ SD	range	mean $\pm$ SD	range
Water Depth ^{**} (m)	2.47 $\pm$ 0.26	2.25-3.09	1.61 $\pm$ 0.15	1.44-1.80
SD ^{**} (m)	1.83 $\pm$ 0.37	1.10-2.35	0.76 $\pm$ 0.08	0.65-0.86
WT [*] (°C)	15.07 $\pm$ 3.38	11.00-19.50	19.36 $\pm$ 3.27	14.30-23.00
TN ^{**} (mg/L)	1.07 $\pm$ 0.05	0.97-1.16	2.07 $\pm$ 0.25	1.76-2.44
NH ₄ ⁺ -N ^{**} (mg/L)	0.18 $\pm$ 0.36	0.01-1.12	0.47 $\pm$ 0.27	0.26-1.12
NO ₃ ⁻ -N(mg/L)	0.82 $\pm$ 0.07	0.70-0.93	0.88 $\pm$ 0.16	0.71-1.18
TP ^{**} (ug/L)	8.59 $\pm$ 0.95	7.06-10.23	82.23 $\pm$ 16.48	54.51-101.10
PO ₄ ³⁻ -P [*] (ug/L)	1.28 $\pm$ 1.06	0.16-3.76	8.09 $\pm$ 5.95	1.28-17.94
Chla ^{**} (ug/L)	2.78 $\pm$ 1.03	1.67-4.36	52.45 $\pm$ 24.69	21.76-89.37
COD _{Mn} ^{**} (mg/L)	1.57 $\pm$ 0.23	1.13-1.85	4.32 $\pm$ 0.80	3.48-5.81
DOC ^{**} (mg/L)	1.42 $\pm$ 0.11	1.32-1.67	2.77 $\pm$ 0.30	2.42-3.21

* and ** indicate a significant difference between the two reservoirs at $p<0.05$ and $p<0.01$, respectively.

The two reservoirs also exhibited significant differences in trophic state. The nutrient parameters of TN, NH₄⁺-N, TP and PO₄³⁻-P in Lake Haitang were all significantly higher than that in Lutian Reservoir. In Lutian reservoir, the concentrations of TN, NH₄⁺-N and NO₃⁻-N all showed an increasing trend, while TP and PO₄³⁻-P showed a downward tendency. However, in Lake Haitang, the concentrations of TN, NO₃⁻-N and TP showed a downward trend, whereas NH₄⁺-N and PO₄³⁻-P showed an increasing trend. The significant difference was also found in the concentration of chla with that in Lake Haitang and was nearly 20 times higher than that in Lutian Reservoir. In addition, the average TN/TP in Lutian Reservoir (125.23) was much higher and was nearly 5 folds of that in Lake Haitang Reservoir.

The organic pollution level in two reservoirs was both at a low level. Similarly, the concentration of COD_{Mn} in Lutian Reservoir was much lower than that in Lake Haitang. The mean concentration of COD_{Mn} in Lake Haitang was nearly 3 folds of that in Lutian Reservoir. And the COD_{Mn} in two reservoirs were both increased in the early March and reached the peak value on 18th March. Similar to the dynamics of COD_{Mn}, the DOC also showed a downward trend and was significantly higher in Lake Haitang. The significant difference of the trophic state in two reservoirs was reflected by the different TLI values. TLI values of Lutian Reservoir ranged from 29.60 to 36.91 with a mean value of 32.25, which mainly

indicated mesotrophic level of Lutian Reservoir during the survey. However, TLI values of Lake Haitang ranged between 52.98 and 62.05 with a mean value of 57.45, which represented light eutrophic of Lake Haitang.

Phytoplankton composition

The phytoplankton in both reservoirs consisted of eight phyla. The density of phytoplankton in Lutian Reservoir ranged from 1.36×10^5 to 1.19×10^6 ind./L with a mean value of 3.60×10^5 ind./L. And the maximum density was recorded on 30th March. As a whole, the density of phytoplankton in Lutian Reservoir exhibited a downward trend. Among the eight phyla, the bacillariophyta accounted for the highest proportion of 43.24%, while chlorophyta and cyanophyta respectively accounted for the second and third highest proportion of 28.50% and 20.55% in Lutian Reservoir (Fig. 2). Significantly, the density of cyanobacteria in Lutian Reservoir accounted for the highest proportion on 30th March of 75.55%. In Lake Haitang, the density of phytoplankton ranged from 1.81×10^7 to 6.12×10^7 ind./L with a mean value of 4.10×10^7 ind./L. The phytoplankton density sharply decreased in the early period and then increased to the maximum on 6th May. Different from Lutian Reservoir, the density of cyanophyta accounted for the highest proportion of 64.51% during the survey in Lake Haitang, followed by bacillariophyta and chlorophyta. Meanwhile, the proportion of cyanophyta accounted for the highest proportion during the whole investigation period except on the day of 30th March.

Variation of cyanobacteria density in both reservoirs was similar to that of the proportion of cyanobacteria in phytoplankton. Similarity, the density of cyanobacteria in both reservoirs exhibited extremely different variation trends. In Lutian Reservoir, the density of cyanobacteria ranged from 0.54×10^4 to 9.02×10^5 ind./L with a mean value of 1.37×10^5 ind./L. While the cyanobacteria density in Lake Haitang ranged from 6.34×10^6 to 5.03×10^7 ind./L with a mean value of 2.80×10^7 ind./L. The potential MC-producing algae in two reservoirs were mainly composed of *Microcystis*, *Anabaena* and *Oscillatoria* with the density of *Anabaena* accounted for the highest proportion during most of the time (Fig. 3). The density of *Anabaena* in Lake Haitang ranged from 4.63×10^6 to 3.67×10^7 ind./L with a mean value of 1.52×10^7 ind./L, while the density of *Anabaena* in Lutian reservoir was very low with a mean value of 9.35×10^4 ind./L. It's worth nothing that one species of cyanobacteria of *Pseudoanabaena* sp. was both identified in two reservoirs with a mean density of 1.05×10^4 in Lutian Reservoir and 6.58×10^6 ind./L in Lake Haitang.

Microcystin dynamics

The concentration of TIMCs in Lutian Reservoir changed gently and ranged from 0.228 to 0.33 $\mu\text{g/L}$ with a mean value of 0.264 $\mu\text{g/L}$ (Fig. 4a). The concentration of three MCs congeners of IMC-LR, IMC-RR and IMC-YR respectively accounted for 37.44%, 13.54% and 49.02% of the concentration of TIMCs. Among the three MC congeners, the concentration of IMC-YR dominated most of the time, while IMC-LR just dominated on 11th March, 21st April and 29th April. The concentration of IMC-YR ranged from 0.069 to 0.192 $\mu\text{g/L}$ with a mean value of 0.128 $\mu\text{g/L}$. The variation pattern of IMC-LR was similar to that of

TIMCs with the concentration ranged from 0.06 to 0.157 µg/L and a mean value of 0.10 µg/L. The concentration of IMC-RR was extremely low during the survey with a mean value of 0.037 µg/L.

Different from the variation of TIMCs, the concentration of TEMCs in Lutian Reservoir exhibited larger fluctuation (Fig. 4b). The concentration of TEMCs reached two peaks on 11th March and 14th April respectively with the value of 0.697 and 0.352 µg/L. The mean concentration of TEMCs was 0.323 µg/L, which was obviously higher than that of TIMCs. The dynamic of three extracellular MC congeners also varied significantly different from intracellular congeners. The mean proportions of EMC-LR, EMC-RR and EMC-YR were 49.80%, 14.60% and 35.60%, respectively. The concentration of EMC-RR dominated in the first two sampling times, and EMC-LR dominated the next three times and the last time, while EMC-YR only dominated the rest three times. The concentrations of EMC-LR and EMC-YR ranged from 0.051 to 0.231 µg/L and from 0.022 to 0.191 µg/L, respectively, with a mean value of 0.122 and 0.105 µg/L. However, the EMC-RR only detected for three times, and the maximum concentration was 0.528 µg/L, followed by 0.305 and 0.031 µg/L.

Unsurprisingly, the concentration of TIMCs in Lake Haitang was significantly higher than that in Lutian Reservoir ($p < 0.01$), with the concentration ranged from 0.331 to 0.746 µg/L and a mean value of 0.482 µg/L (Fig. 4c). The concentration of TIMCs was extremely higher on 11th March and 6th April with a mean value of 0.737 µg/L and varied gently in the other sampling times. Compared to Lutian Reservoir, MC-YR was more dominant in Lake Haitang and accounted for the highest proportion during the survey except on 11th March. Concentrations of IMC-LR, IMC-RR and IMC-YR respectively accounted for 33.49%, 12.32% and 54.19% of the TIMCs during the survey. The concentrations of IMC-LR, IMC-RR and IMC-YR ranged from 0.053 to 0.452 µg/L, from 0 to 0.143 µg/L, and from 0.134 to 0.495 µg/L, respectively, with a mean value of 0.17, 0.062, and 0.25 µg/L. The IMC-LR recorded the maximum on 11th March and sharply decreased in the other times, but it exhibited an increasing trend during the survey except the sampling on 11th March. The variation pattern of IMC-RR was similar to that of IMC-LR. Different from the above two congeners, the concentrations of IMC-YR exhibited obviously increasing trend and recorded the maximum on the last sampling.

The mean concentration of TEMCs in Lake Haitang was 0.472 µg/L, which was a little lower than that of TIMCs, with the concentrations ranged from 0.311 to 0.649 µg/L and exhibited a downward trend (Fig. 4d). Similarly, the proportion of EMC-YR was the highest, and the concentrations of EMC-LR, EMC-RR and EMC-YR respectively accounted for 36.63%, 22.98% and 40.40% of the TEMCs. Concentrations of EMC-LR, EMC-RR and EMC-YR ranged from 0 to 0.316 µg/L, from 0.029 to 0.378 µg/L, and 0.039 to 0.336 µg/L, respectively. And the mean values of EMC-LR, EMC-RR and EMC-YR were 0.176, 0.109, and 1.494 µg/L, respectively. Although extracellular and intracellular MC congeners had a similar variation trend, the concentrations of EMC-YR changed from increasing to a continuous decline after 15th April until the end of the survey.

Correlation analysis

In both reservoirs, the TIMCs and intracellular MC congeners were all positively and significantly correlated with most of the phytoplankton phyla and potential MC-producing species, including both of their density and biomass (Fig. 5). The positive correlations were also detected between extracellular MCs and phytoplankton phyla, but the number of significant correlations was much lesser. Similarly, intracellular MCs was also significantly correlated with more physicochemical parameters than extracellular MCs. The intracellular MCs positively and significantly correlated with WT, TN, $\text{NH}_4^+\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, COD_{Mn} and DOC, while only negatively and significantly correlated with WD, SD and TN/TP. The similar results were also found in extracellular MCs. There were few significant correlations obtained between any pair of the MCs. In addition, significant correlations were also found between various phytoplankton phyla and physicochemical parameters.

In Lutian Reservoir, the significant correlations were relatively rare. The positive and significant correlations were only found between TIMCs and chlorophyta, IMC-YR and *Microcystis*, and TEMCs (EMC-RR) and *chl a*, while the negative and significant correlation were only found between IMC-LR and *Pseudoanabaena* sp., TIMCs and WD, EMC-LR and Cryptophyta, TEMCs (EMC-RR) and *Pseudoanabaena* sp., and EMC-LR and TN. However, in Lake Haitang, intracellular MCs and extracellular MCs were occasionally correlated with different variables that belong to the same class. Intracellular MCs was significantly correlated with *Oscillatoria* and $\text{NO}_3^-\text{-N}$, while extracellular MCs was significantly correlated with *Microcystis* and $\text{NH}_4^+\text{-N}$. In addition, IMC-RR showed significantly correlated with COD_{Mn} ($r=0.731$) and DOC ($r=0.802$), and IMC-YR was significantly correlated with $\text{NO}_3^-\text{-N}$ ($r=-0.874$), while EMC-RR was significantly correlated with DOC ($r=0.786$), and EMC-YR was significantly correlated with $\text{PO}_4^{3-}\text{-P}$ ($r=0.711$) and COD_{Mn} ($r=-0.833$).

Discussion

In recent decades, MCs are increasing in detection frequency and severity with the global expansion of cyanobacterial blooms (Harke et al., 2016; Huisman et al., 2018). Previous studies indicated that MCs usually exhibits very different distribution characteristics in different water bodies due to the diversity of congener composition and the complexity of toxigenic mechanism (Khomutovska et al., 2020; Tilahun et al., 2019; Wood et al., 2006). In certain freshwater habitats, the concentration of MCs can reach extremely high levels. For instance, MCs in southern part of Africa reached a concentration of 124 mg/L, and the contents of MCs reached 7300 $\mu\text{g/g}$ dry weight (dw) in a fish pond in Wuhan, China and 7100 $\mu\text{g/g}$ dw in Portugal (Vasconcelos et al., 1996; Wang et al., 2021; Zhang et al., 1991). In the present study, the concentrations of total intracellular and extracellular MCs in two reservoirs were both below 1 $\mu\text{g/L}$, which were similar to the results of several other water bodies also located in subtropical areas (Cunha et al., 2018; Wang et al., 2018; Zhang et al., 2018). Although MC concentrations showed great differences in different water bodies, the intracellular MCs concentration is usually higher than that of extracellular MCs (Zastepa et al., 2014; Zhang et al., 2018). On the contrary, the concentration of extracellular MCs in Lutian Reservoir was obviously higher than that of intracellular MCs in this study, which was consistent with the

results of that on spring of Lake Chaohu in China (Shang et al., 2018). The conflicting results may be primarily related to the massive lysis of bloom or active release of intracellular MCs (Cordeiro-Araújo and Bittencourt-Oliveira, 2013; Díez-Quijada et al., 2019).

Due to the variability of molecular structure, MCs are usually recorded with multiple congeners coexistence in most water bodies (Graham et al., 2010). However, among the various MC congeners, MC-LR and MC-RR usually dominated in most of the studies, while the dominance of MC-YR was relatively scarce (Major et al., 2018; Simiyu et al., 2018). In the present study, IMC-YR were dominant in both reservoirs, and EMC-LR and EMC-YR were respectively dominant in Lutian Reservoir and Lake Haitang. This was quite different from the results of several other lakes, even though they were almost locating in the same area. For example, in Poyang Lake, the most dominant MC congener in the whole year was MC-RR, followed by MC-LR (Zhang et al., 2018), which was similarly to the results of Lake Taihu and Lake Yanghe (Wang et al., 2018). In addition, the dominate congener usually varied with time, as in many studies, MC-LR just dominant in a period and decreased its dominance or replaced by other congeners in other times (Tilahun et al., 2019; Xue et al., 2018; Xue et al., 2016). Nevertheless, although a lot of studies in natural freshwater ecosystems have focused on the relationships between various environmental factors and the composition of MC congeners, the exact regulating mechanisms are still in debate.

Among the various influencing factors, the characteristics of toxin-producing cyanobacteria species can directly affect the distribution of MCs (Wu et al., 2006). Different composition of MC-producing genera can not only lead to different concentrations but also congener compositions of MCs (Monchamp et al., 2014). In the current study, the dominant congener of IMC-YR was respectively correlated with the density of *Microcystis* and *Oscillatoria* in Lutian Reservoir and Lake Haitang, which might be one reason for the difference in MCs between the two reservoirs. Moreover, several previous studies have suggested that the growth rate of cyanobacteria cells is positively correlated with MCs production (Kaebernick et al., 2000; Orr and Jones, 1998). Thus, MCs can also reach a high concentration during the early growth period of cyanobacteria, but few studies have focused on this. Besides above biotic factors, programmed cell death, proportion of toxin-producing algae and colony formation are all reported correlating with MCs production (Harke et al., 2016; Hu and Rzymiski, 2019; Wu et al., 2006). Meanwhile, the production of MCs also correlates with the existence of other aquatic organisms, and the concentrations of MCs usually be detected to significantly correlate with non-MC-producing species (Hu and Rzymiski, 2019; Rzymiski et al., 2020; Xue et al., 2018). Similarly, the concentrations of most congeners and total MCs in both reservoirs were significantly and positively correlated with the density of non-MC-producing algae.

The abiotic parameters of water temperature, nutrients, and turbidity are the main factors involved in various studies that can regulate MCs production (Harke et al., 2016; Xue et al., 2018). The elevated water temperature not only leads to the increase of cyanobacteria biomass but also the release of MCs (Walls et al., 2018). In the present study, water temperature was positively and significantly correlated with MCs concentration, which might be one of the reasons to explain the higher concentration in Lake Haitang. In contrast, water depth and SD were both negatively correlated with the concentration of MCs. The fluctuation of water depth can affect MCs through its influences on nutrient concentrations and water

temperature, which can significantly influence the density of cyanobacteria (Bakker and Hilt, 2016). In addition, both water depth and SD can impact the structure of communities of aquatic organisms, such as zooplankton and aquatic plants (Bakker and Hilt, 2016; Jacoby et al., 2000), and thus the distribution of cyanobacteria and MCs production. The light condition in water column, which is closely related to water depth and SD, is also very important in MCs production (Harke et al., 2016).

Different forms of nutrients (except NO_3^- -N) in the present study were all positively and significantly correlated with MCs concentration, while negatively correlation was only detected between the ratio of TN/TP and MCs concentration. Likewise, nutrient concentrations are positively correlated with MC concentrations in most other studies (Yuan and Pollard, 2017). However, the dynamic of MC may show a different trend when the nutrients are in limiting condition (Harke et al., 2016; Wagner et al., 2021; Xue et al., 2018). In addition, supported by several previous studies, nitrogen may have more significant effects on MCs production when compared with phosphorus (Downing et al., 2005; Harke and Gobler, 2013). Under nitrogen limited condition, the MC synthetic gene was downregulated and the content of MCs per cell was decreased. The COD_{Mn} and DOC were also found to positively correlated with MCs concentration in the present study. The value of COD_{Mn} is usually used to indicate the levels of organic pollution, which was very important in the absorption of MCs on to suspended particles or sediment (Liu et al., 2019; Pang et al., 2021). Whereas DOC from different sources can affect cyanobacteria communities in different ways (Zhao et al., 2019). Although the above two parameters play an important role in the life history of cyanobacteria, there are still few relevant researches, especially on their effects on MCs production.

Recently, the coexistence of emerging contaminants and MCs in freshwater ecosystems has attracted more attention. Wan et al. (2021a) studied the effects of two antibiotics of moxifloxacin and gatifloxacin on MC release and found the appropriate concentration of fluoroquinolone antibiotics could increase MC release. Another contaminant of nanoplastics was also found to promote MC synthesis and release from *Microcystis* (Feng et al., 2020). The coexistence of MCs and other pollutants makes the biotoxicity more complex, with different concentrations exhibited different effects (Wan et al., 2021b). In the present study, IMC-YR and TIMCs were positively correlated with the density of *Pseudoanabaena* sp., which can produce taste-and-odor compound of 2-methylisoborneol (2-MIB) (Li et al., 2018), and different reservoirs and congeners exhibited different correlations. Although several studies have focused on the coexistence of MCs and taste-and-odor compounds (Graham et al., 2010; Shang et al., 2018), the interactions and toxicology between them are still unclear.

Summarily, during the early growth stage of cyanobacteria, the mean concentrations of intracellular and extracellular MCs were both in low levels ($< 1 \mu\text{g/L}$) in two reservoirs, which suggested the risks of MCs posed to humans were also weak. However, several studies indicated the toxicity of MC-YR was similar to that of MC-LR (Gurbuz et al., 2016). Thus, the dominant of MC-YR in two reservoirs may also pose high potential risk to various organisms and humans, especially in Lutian Reservoir which was still in mesotrophic level and obtained little attention. Meanwhile, the fluctuation of MCs was sharp, monthly sampling in most studies certainly cannot assess the potential risk of MCs completely. Moreover, the

coexistence of emerging contaminants and MCs should be paid more attention, especially the combined toxicity of them was largely unclear.

Conclusion

Although the concentrations of intracellular and extracellular MCs in both reservoirs were still below the value of the WHO drinking water guideline ($< 1 \mu\text{g/L}$) during the early growth stage of cyanobacteria, the relatively high concentration of MCs and their associated risks in Lutian Reservoir may be overlooked. The concentration of MCs in two reservoirs both showed a sharply fluctuation, so the monthly sampling cannot completely assess their risks. In both reservoirs, the intracellular and extracellular MC congeners were all dominated by MC-YR most of the time, but its toxicity remains unclear. In addition, it was found that the concentration of extracellular MCs in both reservoirs was sometimes higher than that of intracellular MCs, which was contrary to the results of most studies, while the reasons for this are needed further studies.

The dynamic of MCs in two reservoirs were significantly and positively correlated with the density and biomass of phytoplankton phyla and potential MCs-producing cyanobacteria, WT, TN, $\text{NH}_4^+\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, COD_{Mn} and DOC, while were only negatively and significantly correlated with WD, SD and TN/TP. The differences of MCs between the two reservoirs might be primarily caused by the differences in WT, nutrients (especially phosphorus), organic matters and the composition of MCs-producing cyanobacteria. In addition, MCs in both reservoirs, especially in Lutian Reservoir, were significantly correlated with the density of *Pseudoanabaena* sp., which can produce taste-and-odor compound of 2-methylisoborneol (2-MIB), might have significantly impacts on the concentration and toxicity of MCs.

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Authors' contributions

Qingju Xue: formal analysis, writing, and original draft preparation. Ming Kong: sample analysis and manuscript review. Liqiang Xie: methodology and manuscript review. Tong Li, Mengna Liao and Zebin Yan: sample sampling, data acquisition and analysis. Yanyan Zhao: methodology, manuscript review and editing. All authors read and approved the final manuscript.

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Figures

Figure 1

Reservoirs and sampling sites locations.

Figure 2

Temporal variations of relative abundance of the phytoplankton in Lutian Reservoir (a) and Lake Haitang (b).

Figure 3

Dynamics of potential MC-producing algae density in Lutian Reservoir (a) and Lake Haitang (b).

Figure 4

Temporal variations of IMCs and EMCs in Lutian Reservoir (a, b) and Lake Haitang (c, d).

Figure 5

The correlation between various MCs and different environmental parameters in Lutian Reservoir and Lake Haitang.