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Comprehensive analysis of cyanotoxins distribution and toxicity in Taihu Lake of China

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Abstract

This study addresses the growing concern regarding cyanotoxin contamination which is not limited to microcystin in Taihu Lake, highlighting the diversity of cyanotoxins and their potential health implications. Currently, most existing research focuses primarily on well-known cyanotoxins. However, recent technological advancements have revealed numerous previously unknown cyanotoxins that were not covered in prior studies on cyanotoxins in Taihu Lake, indicating the need to strengthen the assessment of multiple toxin categories in this lake. Regarding the types of cyanotoxins in Taihu Lake, the discussion was conducted from both qualitative and quantitative perspectives. Water samples collected from the vicinity of Taihu Lake were analyzed using quadrupole time-of-flight mass spectrometry to identify the types of cyanotoxins. Subsequently, a home-made mixed solid-phase extraction method combined triple quadrupole mass spectrometry showed the recovery rates of cyanotoxins including anatoxin, cylindrospermopsin, microcystins, and other peptide-cyanotoxins all reached over 70%. The quantitative analysis also indicated the concentration of microcystins varied from 1.2 ng/L to 23.8 ng/L from different locations of Taihu Lake, and the concentration of non-microcystins varied from 0.1 ng/L to 57.6 ng/L. In addition, the toxicity of cyanotoxins in the water samples was evaluated using the T.E.S.T model, based on the median lethal concentration (LC₅₀). The calculated hazard quotient indicated that the MCs account for only 24.82% of the total cyanotoxin-related risks in Taihu Lake.

Keywords: Cyanobacteria, Cyanotoxins, qualitative, quantitative

1. Introduction

Cyanobacterial blooms remain a critical and unavoidable subject in research concerning aquatic ecosystem health. This significance stems from two primary factors: firstly, rapid economic and industrial development has exceeded the self-purification capacity of many water bodies, leading to increasingly frequent algal blooms that can suppress the growth of aquatic organisms; secondly, cyanobacteria produce toxic metabolic byproducts during proliferation. These harmful substances, commonly referred to as cyanotoxins in the literature, pose substantial risks to living organisms(Onstad et al. 2007a). In fact, cyanotoxins often represent a greater threat than the cyanobacteria themselves and decrease the quality of drinking water(Mohamed and Al Shehri 2007). Existing studies have documented that cyanotoxins exhibit multiple toxicities, including hepatotoxic, neurotoxic, and nephrotoxic effects, capable of causing injury to both animals and humans, and in severe cases, leading to fatality(Gkelis et al. 2019). Therefore, further research and thorough discussion on cyanotoxins are not only warranted but essential.

In studies concerning cyanotoxins, a fundamental step has been to identify their variants, followed by investigations into their nomenclature, chemical structures, molecular characteristics, and toxicological profiles. Based on existing literature, cyanotoxins are commonly classified into two major groups according to molecular structure: alkaloids and cyanopeptides(Beversdorf et al. 2017). Among alkaloids, anatoxin (ANTX) and cylindrospermopsin (CYN) are widely recognized, with a substantial body of reported research. For instance, in 2007, ANTX was detected in Scottish waters, and ozonation was identified as an effective method for its degradation(Onstad et al. 2007b). Similarly, Yan and Song documented a purification protocol for CYN and examined the roles of $\cdot\text{OH}$ and O_3 in its breakdown, using a human hepatoma cell line to demonstrate its pronounced toxicity(Song et al. 2012, Yan et al. 2016). Regarding cyanopeptides, microcystins (MCs) are the most extensively studied. The World Health Organization (WHO) has set a provisional guideline value of $1\text{ }\mu\text{g L}^{-1}$ for MCs in drinking water to

mitigate associated health risks(WHO 2004).

With advances in analytical techniques, an increasing variety of cyanopeptides beyond MCs have been identified. These are generally categorized into five groups: cyanopeptolins, anabaenopeptins, aerucyclamides, aeruginosines, and microginins(Christophoridis et al. 2018, Gkelis et al. 2019, Janssen 2019). Despite over 2,000 distinct cyanopeptides being reported to date (Janssen et al. 2024), relatively limited attention has been paid to these non-MCs groups. This underscores the need to recognize that the ecological and health risks of cyanopeptides extend well beyond MCs. Unlike MCs, the identification and quantification of other cyanopeptides in aquatic environments remain challenging, largely due to the lack of commercial reference standards. To address this, high-resolution mass spectrometry coupled with suspect screening has been employed for their qualitative and semi-quantitative analysis(Bouaïcha et al. 2019, Le Manach et al. 2019). However, the effectiveness of this approach depends heavily on comprehensive cyanobacterial metabolite databases, with data richness directly influencing identification accuracy (Lian et al. 2020). Fortunately, ongoing efforts by researchers have gradually expanded these databases. Our research group has integrated and summarized available information to establish a preliminary database (Level 3)(Schymanski et al. 2014, Shang et al. 2024). Moreover, the application of frontier fragmentation techniques can enhance identification confidence from Level 3 to Level 2. Nevertheless, accurate concentration measurement ultimately requires authentic chemical standards. To this end, our group has initiated the purification of cyanopeptides and has successfully isolated Aerucyclamide A (ACA)(Sha et al. 2021). Concurrently, several cyanopeptide standards were acquired from Biotech GmbH for use in subsequent studies(Shang et al. 2024).

The concentration of cyanotoxins in environmental waters is often too low to be detected directly. While solid-phase extraction is commonly used to preconcentrate samples and bring analyte levels above the detection limit, this offline approach can be labor-intensive and time-consuming. In contrast, online

solid-phase extraction coupled with liquid chromatography-tandem mass spectrometry (online SPE-LC-MS/MS) offers a viable alternative to enhance methodological sensitivity while significantly reducing both pretreatment and analysis time. This technique has been widely adopted for monitoring trace organic contaminants in aquatic systems(Feng et al. 2026a, Yao et al. 2016, Yao et al. 2018). The selection of sorbent is critical in online SPE, as it determines the efficiency of capturing and enriching target analytes. However, cyanotoxins comprise compounds of varying polarity, such as alkaloids and cyanopeptides, making it difficult for a single sorbent to effectively extract all subtypes simultaneously within the same analytical run(Zou et al. 2022). To address this limitation, researchers have explored the use of mixed sorbents within a single SPE cartridge, an approach previously applied to the analysis of pesticides and other trace organic pollutants(Feng et al. 2026b, Huntscha et al. 2012, Kovalova et al. 2012).

During the past studies, most of articles on cyanotoxins in Taihu Lake focused on MCs and CYN, and did not cover the other major kinds of cyanotoxins mentioned above(Li et al. 2022, Shi et al. 2015). In this work, we selected Taihu Lake in China as a real-world case to investigate the distribution and concentration of cyanotoxins. Nineteen water samples were collected from various locations in the lake, and cyanobacterial biomass was also collected from Taihu Lake. Following solid-phase extraction pretreatment, the samples were qualitatively screened using an optimized version of the CyanoMetDB database. Subsequently, a quantitative detection method was established for cyanotoxins for which reference standards were available, employing liquid chromatography coupled with triple quadrupole mass spectrometry. To enable the simultaneous determination of alkaloids and cyanopeptides, we prepared new online solid-phase extraction columns incorporating different sorbent types, and further evaluated their performance in capturing cyanotoxins. Finally, T.E.S.T software was applied to asses to the cyanotoxins toxicity in water samples.

2. Experimental section

2.1 Chemicals

Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma-Aldrich (Shanghai, China) and used without further purification. Methanol (MeOH) and acetonitrile (ACN) were purchased from *J&T Baker* (US). Formic acid, acetic acid and ammonium fluoride for mobile phase modification were obtained from Sigma-Aldrich (St. Louis, US). *Microcystis aeruginosa* (cat #: 905) was obtained from Wuhan Institute of Hydrobiology, Chinese Academy of Science. A total 20 cyanotoxins (as shown in Table S1 of the Supporting information (SI)) were purchased in this study, where Microcystin-LR (MC-LR), CYN and ACA were purified in our previous studies (Sha et al. 2021, Yan et al. 2016, Yan et al. 2014). All solutions were prepared by using 18.2 M Ω cm deionized water ($\text{Di-H}_2\text{O}$).

2.2 Collection and pre-treatment of samples

Samples were collected in August 2025 from various sites in Taihu Lake, as shown in Figure S1 of the SI. Given the differing polarities of cyanotoxins, a single solid-phase extraction (SPE) column was insufficient to ensure comprehensive recovery. Therefore, three types of SPE columns-HLB, PSA, and CBA-were employed to enrich the cyanotoxins (Shi et al. 2020, Zhang et al. 2020, Zhang et al. 2021). Prior to extraction, each column was sequentially conditioned with methanol and water at a flow rate of 10 mL min⁻¹. The samples were then passed successively through the three columns. During elution, different solvents were used according to column chemistry: pure methanol for HLB, methanol containing 5% (v/v) ammonia solution for SCX, and methanol containing 5% (v/v) formic acid for CBA. Finally, the eluates from the three columns were combined, dried under a gentle stream of nitrogen, and reconstituted in 1 mL of methanol for subsequent analysis.

2.3 Mass spectrum analysis

In this study, two mass spectrometry platforms were utilized: a triple quadrupole (QQQ) MS and a

quadrupole time-of-flight (Q-TOF) MS. For qualitative analysis, a high-performance liquid chromatography quadrupole time-of-flight system (HPLC-Q-TOF-MS) was employed. The system consisted of an Agilent G1290 HPLC coupled with an Agilent G6540 Q-TOF mass spectrometer. Separation was performed on a C₁₈ column (150 × 2.1 mm, 3.0 μm, Waters, US) eluting with a gradient of ACN and acidified H₂O (0.1% v/v formic acid) at a flow rate of 0.3 mL min⁻¹. The detailed gradient information is shown in Table S2 of the SI. The Q-TOF mass spectrometer was operated in positive ionization mode with Auto MS/MS acquisition. Key operating parameters were as follows: sheath gas (11.0 L min⁻¹), drying gas (8.0 L min⁻¹ at 300 °C), nebulizer (40 psi), capillary voltage (3000 V) and fragmentor (160 V). The obtained Q-TOF MS data was analyzed by the CyanoMetDB cyanobacterial database (Shang et al. 2024). For quantitative analysis, a high-performance liquid chromatography triple quadrupole system (HPLC-QQQ-MS) was used. An HPLC (G1290, Agilent, US) was equipped with a C₁₈ column (50 × 2.1 mm, 1.8 μm, Agilent, US) eluting with a gradient of ACN and acidified H₂O (0.1% v/v formic acid) at a flow rate of 0.3 mL min⁻¹. The detailed gradient information is shown in Table S3 of the SI. The QQQ MS/MS of cyanotoxins with standards was shown in Table S4 of the SI.

2.4 Online SPE LC system

Online solid-phase extraction (SPE) was employed to capture target compounds from the samples for qualitative screening of various cyanotoxins. To overcome the cumbersome and time-consuming nature of offline pretreatment and to enable efficient quantitative analysis, an automated online SPE coupled to a LC-QQQ-MS/MS (Agilent 1290–6430 with Flex Cube module, Santa Clara, CA) was used in this work. Basically, online SPE contained a 2-position-10-port valve and two trapping columns. Briefly, 900 μL of sample was loaded onto one of the two online SPE columns together with 5.7 mL of water delivered by an LC pump. Subsequently, the valve was switched to connect the SPE column to the analytical column (50 × 2.1 mm, 1.8 μm, Agilent, US). Notably, one of the SPE columns was custom-

packed in-house and contained five sorbent materials (as shown in Figure S2 of the SI): HLB (Waters, US), X-CW (Phenomenex, US), X-AW (Phenomenex, US), ENV⁺ (Biotage, Sweden), Envi carb (Sigma-Aldrich, US). The other is a commercial SPE cartridge (PLRP, US). After extraction, the retained analytes were eluted from the SPE column in backflush mode using 100% methanol and then transferred to the analytical column for separation. The detailed parameters of flexible cube were presented in Table S5 of the SI.

2.5 Toxicity of cyanotoxins

Research on cyanotoxins that fails to consider their toxicity levels is neither rigorous nor necessary. However, the lack of standard samples has hindered the implementation of routine toxicity tests. Therefore, based on the structures of cyanotoxins identified from water samples, the Toxicity Estimation Software Tool (T.E.S.T), which was a quantitative structure–activity relationship (QSAR) computational toxicology program officially developed and maintained by the United States Environmental Protection Agency (US EPA)(EPA and Exposure Division Cincinnati O 2020, Martin et al. 2008) and established quantitative correlations between molecular structural descriptors and toxicological endpoints, was employed to predict their toxicity.

3. Results and discussion

3.1 The qualitative analysis of cyanotoxins for water samples

The types of cyanotoxins in Taihu Lake were determined by Q-TOF Mass Spectrometry, where only substances with a matching score greater than 95 were identified as cyanotoxins. The water samples were pretreated as described above to capture cyanotoxins as comprehensively as possible. The types of cyanotoxins at different locations in Taihu Lake are listed in Table S6 of the SI, and the summary results are presented in Figure 1. It was evident that the types of cyanotoxins varied significantly across different sampling locations. The number of detected cyanotoxin types in Samples 1-7 was higher than that in other

185 samples, with the majority being newly identified cyanotoxins that have not been previously reported (as
186 listed in Table S6 of the SI). This finding is attributed to the rapid development and severe pollution in the
187 towns located on the west bank of Taihu Lake. The water in this area is also abundant in inorganic salts
188 (e.g., ammonia nitrogen) and other aromatic chemical substances, which contribute to greater diversity
189 and complexity in cyanobacterial growth(Natumi and Janssen 2020). Consequently, changes in
190 cyanobacterial species composition resulted in the production of a wider variety of cyanotoxins. From
191 Sample 7 to Sample 16, the corresponding sampling locations gradually shifted from the southwestern
192 part of Taihu Lake to the east bank, and a gradual decrease in the types of cyanotoxins was observed. This
193 decrease is due to the self-purification effect of the river, which improves water quality, coupled with the
194 relatively low pollution levels of nearby water bodies, resulting in a less pronounced promotional effect
195 on cyanotoxin production compared to the west bank. However, the types of cyanotoxins in Sample 18
196 were highly unusual. Although Sample 18 is situated on the east coast, the number of detected cyanotoxin
197 types was higher than that in adjacent samples, as this site serves as a local water intake point. The weak
198 water flow in the vicinity of water intake areas results in a higher cyanobacterial concentration. Overall,
199 the types of cyanotoxins observed in the present study were far more diverse than microcystins alone.
200 Hence, the irregular distribution of cyanotoxin types in Taihu Lake highlights the inadequacy of research
201 focusing exclusively on microcystins.

202 (Insert Figure 1)

203 **3.2 The quantitative analysis of cyanotoxins for water samples**

204 3.2.1 The optimization of online SPE

205 Quadrupole time-of-flight mass spectrometry was employed for the identification of cyanotoxin
206 types. However, for cyanotoxins exhibiting biological toxicity, the assessment of cyanotoxin-related risks
207 without considering their concentrations is scientifically irrelevant. Consequently, triple quadrupole mass

spectrometry (QQQ-MS) was utilized to quantify cyanotoxin concentrations in Samples 1-19. In contrast to conventional solid-phase extraction, an online solid-phase extraction mode coupled with QQQ-MS was implemented in the present study. Furthermore, to ensure the comprehensive capture of all cyanotoxin types-including both polar and non-polar species-five distinct sorbent types (as depicted in Scheme 1) were mixed into a single extraction cartridge.

(Insert Scheme 1)

Among the chosen adsorbents, HLB (Waters Corporation) is widely employed in SPE procedures, whereas ENV⁺ from Biotage is a hypercross-linked polymer sorbent extensively utilized for the recovery of trace organic compounds (TrOCs) with moderate to high polarity(Anumol and Snyder 2015). Cyanotoxins containing amino or carboxylic functional groups exhibit positive or negative charges in neutral aqueous solutions, accordingly, cation and anion-exchange resins (X-AW and X-CW from Phenomenex) were used to capture these molecules through ionic exchange or π - π bonding effects(Fontanals et al. 2008). Envi Carb is a graphitized nonporous carbon material that demonstrates strong interactions with both polar and nonpolar compounds, thereby serving as a supplementary adsorbent for the effective capture of a broad spectrum of cyanotoxins with diverse polarity profiles. In this work, the packing ratios of five adsorbents were conducted as 3:1:1:3:2 for HLB/X-CW/X-AW/ENV⁺/Envi carb, and the total weight of packing material is about 15 mg (as shown in Figure S2 of the SI).

To evaluate the performance of the home-made mixed solid-phase extraction column, cyanotoxins with available standard reference materials (as listed in Table S4 of the SI) were utilized to determine recovery efficiencies, and the recovery performance of the PLRP cartridge was also investigated for comparative purposes. As illustrated in Figure 2, the recovery efficiencies of the mixed-mode cartridge were consistently higher than those of the PLRP cartridge. Notably, the recoveries of ANTX and CYN

were significantly enhanced from 0.8% and 11% to 69% and 72%, respectively. This observation indicates that conventional SPE cartridges are not suitable for the simultaneous extraction of substances with varying polarities. Additionally, the recovery of cyanopeptides within the cyanotoxin fraction was further improved when the mixed-mode cartridges were employed. Collectively, these findings highlight the necessity of the novel mixed online extraction column for the simultaneous detection of two major classes of algal toxins, namely alkaloids and peptides.

(Insert Figure 2)

3.2.2 The concentration of cyanotoxins in water samples

Based on the methods obtained using the mixed cartridges, the concentrations of cyanotoxins in Samples 1-19 are presented in Table 1. In the present work, cyanotoxins listed in Table 1 other than MCs were designated as “others”. Owing to the lack of standard reference materials, these “others” just included anabaenopeptins, microginins, aeruginosins, aerucyclamides, CYN, and ANTX. As previously described, peptide cyanotoxins were classified into six groups based on their structural characteristics: microcystins, cyanopeptolins, anabaenopeptins, aerucyclamides, aeruginosins, and microginins (Janssen 2019, Natumi et al. 2021). It should be noted that cyanopeptolins were not detected in this study, and only two types of alkaloid cyanotoxins (CYN and ANTX) were identified.

(Insert Table 1)

Table 1 details the occurrence and concentrations of cyanotoxins in each sampling area. Additionally, the distributions of MCs and others are illustrated in Figure 3. From a regional distribution perspective, the cyanotoxin content on the west bank of Taihu Lake was higher than that on the south and east banks, which is consistent with the distribution pattern of cyanotoxin types. In general, cyanotoxins are metabolic products of cyanobacteria, and cyanotoxin concentrations are directly proportional to cyanobacterial concentrations. Besides, from the perspective of cyanotoxin concentrations, MCs were not the most

abundant cyanotoxin type in the samples; instead, the concentration of anabaenopeptins was one order of magnitude higher than that of MCs. However, research regarding the toxicity, distribution, and degradation of anabaenopeptins remains scarce. Existing studies, as well as guidelines established by the World Health Organization (WHO), focus exclusively on MCs. Furthermore, CYN and ANTX were also detected in the samples, demonstrating that the home-made mixed cartridge can be effectively utilized for the simultaneous detection of both polar and non-polar substances in real aquatic environments. Meanwhile, a comparison of MCs concentrations with those of others (Figure 3) revealed that non-microcystin toxins accounted for 0%–95% of the total cyanotoxin content. High proportions of non-microcystin toxins were more frequently observed in the southwest and west banks of Taihu Lake, which is also consistent with the aforementioned distribution pattern of cyanotoxin types. These results confirm that non-microcystin toxins are indeed present in the environment, with contents not lower than-and in some cases higher than-those of MCs. Therefore, it is crucial and urgent to expand research on non-microcystin toxins.

(Insert Figure 3)

3.3 The qualitative analysis of cyanotoxins from cyanobacterial

Owing to the extensive scale of the Taihu Lake basin and the inherent fluidity of riverine water bodies, inherent limitations were associated with sampling procedures, and uncertainties were introduced by the complex hydrological environment. To address these constraints, cyanobacterial suspensions obtained from the Taihu Lake Cyanobacteria Analysis and Monitoring Station were subjected to cell lysis, followed by the analysis of cyanotoxin types and concentrations therein. Simultaneously, laboratory-cultivated *Microcystis aeruginosa* 905 (MC 905) was compared with cyanobacterial isolates obtained from the aforementioned actual water bodies. Following matching against the primary mass spectrometry database, only data with a matching score exceeding 95 for both Taihu Lake cyanobacteria and MC 905 were recorded, as detailed in Table S7 of the SI. It was observed that the number of cyanotoxin types in Taihu

277 Lake cyanobacteria was significantly higher than that in MC 905 cultures. This discrepancy is attributed
278 to the fact that growth conditions in natural aquatic environments are substantially more complex than
279 those under controlled laboratory conditions, thereby facilitating the production of a broader diversity of
280 cyanotoxins.

281 Furthermore, consistent with findings from previous studies, MC-LR, the most abundant cyanotoxin
282 in cyanobacterial metabolic profiles, was detected in both Taihu Lake cyanobacteria and MC 905 cultures.
283 In addition to MC-LR, Aerucyclamide A was also identified in both cyanobacterial suspensions, which
284 confirms the symbiotic association between MCs and other cyanotoxin species and highlights a critical
285 limitation of prior cyanotoxin research that focused exclusively on MCs. Moreover, a substantially greater
286 diversity of cyanotoxins was observed in Taihu Lake cyanobacteria, including Cyanopeptolin C,
287 Anabaenopeptin MM823, Microcystbiopterin A, Aeruginosin 865, and Aerucyclamide D, all of which fall
288 within the six major categories of polypeptide-type algal toxins. This observation indicates that the algal
289 community composition in actual aquatic environments is more diverse, and correspondingly, the types
290 of algal toxins produced are more complex.

291 **3.4 The quantitative analysis of cyanotoxins from cyanobacterial**

292 Using the QQQ quantitative analysis method described in the preceding section, the concentrations
293 of cyanotoxins in both Taihu Lake cyanobacterial isolates and laboratory-cultivated MC 905 cultures are
294 illustrated in Figure 4. Notably, the number of cyanotoxin types detected in the Taihu Lake cyanobacterial
295 samples was significantly lower than that identified in the environmental water samples collected earlier,
296 with only two specific cyanotoxin species, Aeruginosin 98A (Aer 98A) and Anabaenopeptin B (Ana B),
297 successfully identified. This notable discrepancy can be primarily attributed to the relatively limited
298 geographic range of the cyanobacterial sampling site and the inherently poor mobility of the
299 cyanobacterial isolates themselves, which restricted the representation of cyanotoxin diversity in this

particular subset of samples. Furthermore, quantitative analysis revealed that MCs accounted for approximately 98% of the total cyanotoxin concentration in the laboratory cultivated MC 905 strain, whereas in the Taihu Lake cyanobacterial isolates, MCs only represented 46% of the total cyanotoxin concentration. These findings clearly demonstrate that the types and concentration profiles of algal toxins in natural aquatic environments are substantially more diverse compared to laboratory cultivated counterparts, thereby rendering them of far greater research significance for understanding real world cyanotoxin risks. Meanwhile, it should be acknowledged that the quantitative detection methods employed in this study were constrained by the limited availability of cyanotoxin standard reference materials, which restricted the scope of toxins that could be accurately quantified. Nevertheless, even with these limitations, the present study provides compelling evidence that the presence of non-microcystin algal toxins in aquatic environments is widespread and cannot be neglected in future research and risk assessment efforts.

(Insert Figure 4)

3.5 The assessment of toxicity for cyanotoxins

With the increasing in-depth research on cyanotoxins and the improvement of cyanotoxins detection methods, there is growing interest in utilizing cyanotoxins as indicators of water quality. As a typical type of cyanotoxin, MCs have a tolerable daily intake set by the WHO. Meanwhile, the latest drinking water quality hygiene code (GB 5749-2006) in China also focused on MC-LR but lacks explicit requirements for other cyanotoxins. Hence, it is crucial to conduct and understand the potential toxicity of non-MCs cyanotoxins.

To comprehensively assess the toxicity of cyanotoxins, it is necessary to consider both the inherent toxicity of the toxins themselves and their environmental concentrations. Therefore, this study focuses primarily on estimating the toxicity of cyanotoxins listed in Table 1. Additionally, due to the scarcity of cyanotoxin standard samples, the T.E.S.T model was applied to assess the toxicity of each cyanotoxin to

evaluate the acute toxicity of cyanotoxins. As shown in Figure S3 of the SI, the LC₅₀ values of MCs were generally higher than that of other cyanotoxins, but the values of LC₅₀ were not equivalent to toxicity on the exposure concentration.

$$HQ = \frac{\text{Exposure concentration}}{LC_{50}} \quad (1)$$

Hence, to eliminate the influence of inherent toxicity differences among various cyanotoxins and enable direct comparison of risk levels across substances with different structures and toxicities, the hazard quotient (HQ) was used to assess the acute toxicity of cyanotoxins from Taihu Lake. The HQ is expressed as the ratio of exposure concentration to LC₅₀, calculated using Equation (1). The application of HQ can provide a quantitative basis for classifying acute toxic risks and identifying the dominant risk contributors in complex environmental systems containing multiple cyanotoxins (Carlson et al. 2022, Chidya et al. 2022, Landis et al. 2017). As shown in Figure 5, Anabaenopeptin A was the most significant contributor to the acute toxicity of cyanotoxins (24.18%). The total contribution of MCs accounted for only 24.82%. This result indicates that MCs do not play a dominant role in the acute toxicity assessment of *Daphnia magna*, whereas non-MC cyanotoxins exert an overall decisive effect.

(Insert Figure 5)

4. Conclusions

In this work, we conducted a comprehensive analysis of cyanotoxins in water samples collected from various location in Taihu Lake using high-resolution mass spectrometry. Through solid phase extraction using multiple extraction columns and screening of mass spectrometry, the types of cyanotoxins from water samples were determined. The results indicated that the types of cyanotoxins in Taihu Lake are far more than just MCs, also include other polypeptide-type cyanotoxins.

In addition, a feasible and commercially available detection method for cyanotoxins was established using automated online SPE coupled to high-performance liquid chromatography triple quadrupole. We

replaced the PLRP cartridge with a home-made mixed cartridge, which can simultaneously enrich both polar and non-polar cyanotoxins. The obtained results indicated that the recovery rates of ANTX and CYN by the home-made mixed cartridge respectively increased from 8% and 11% to 88% and 86% compared to that via PLRP cartridge, and the recovery rates of peptide-cyanotoxins also improved. Based on the concentrations of cyanotoxins, we emphasized the environment risk assessment of the detected cyanotoxins. The LC_{50} values predicated from T.E.S.T model and the calculation of HQ indicated the toxicity was primarily associated with the total cyanotoxins concentration rather than MCs alone, with MCs accounting for only 24.82% of the total cyanotoxin-related risks. In conclusion, our study conducted a comprehensive qualitative analysis of cyanotoxins, as well as quantitative analysis and toxicity assessment of cyanotoxins in Taihu Lake. With advancements in cyanotoxin research, more efforts should be directed towards optimizing detection methods, purifying more cyanotoxin standard samples, and understanding the broader ecological effects of cyanobacterial blooms on aquatic ecosystems.

Supporting Information

Additional text with experimental details is provided in the Supporting Information.

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Authorship contribution

Haitao Sha: Writing – review & editing, Writing–original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. Jinjin Qiu: Writing – original draft, Methodology, Investigation, Data curation. Shuping Zhang: Writing – review & editing, Investigation, Data curation, Conceptualization. Jianguo Zhang: Writing – original draft, Data curation, Conceptualization.

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Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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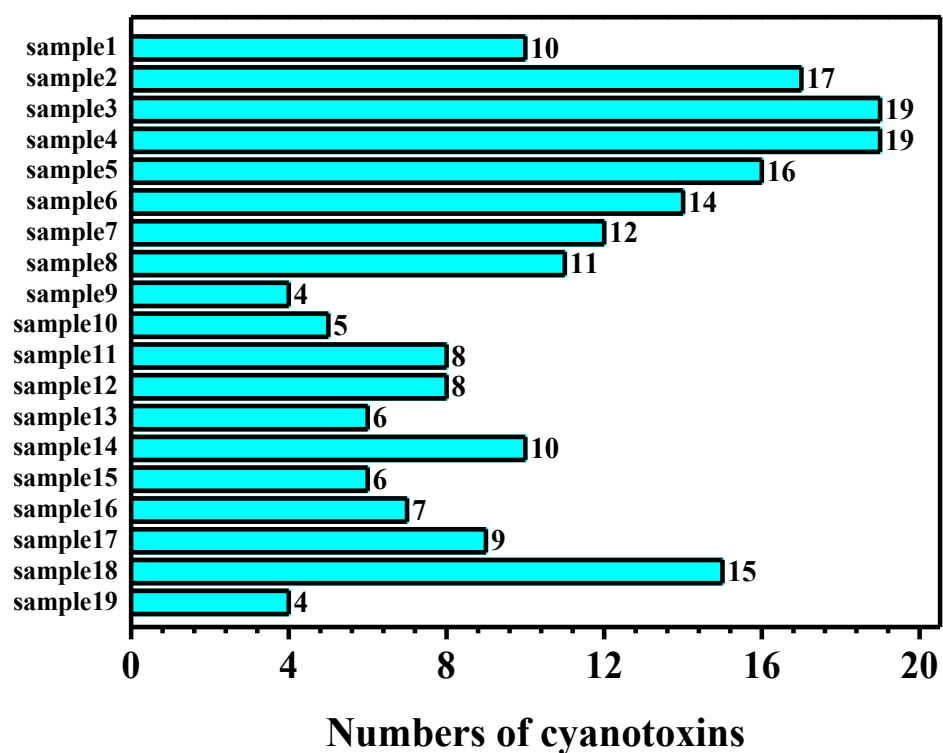
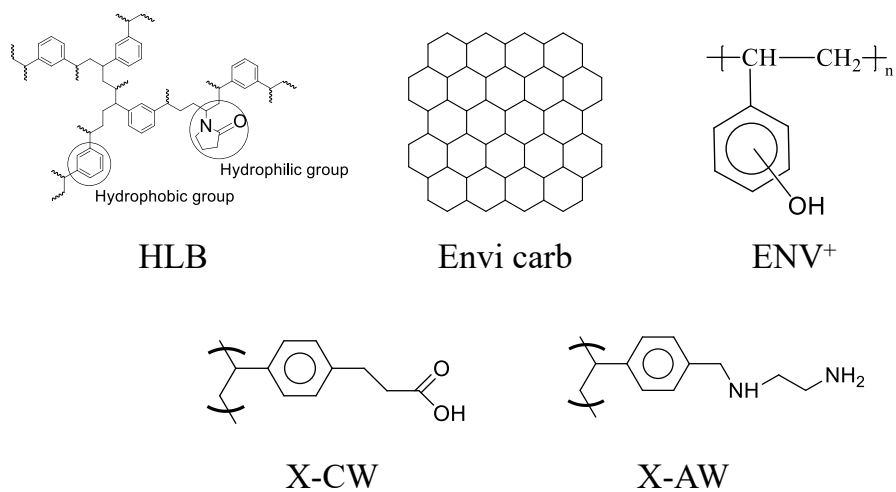


Figure 1. The numbers of the types of cyanotoxins at different samples via mass screen



Scheme 1. Structural information of each sorbent in the mixed mode online SPE cartridge

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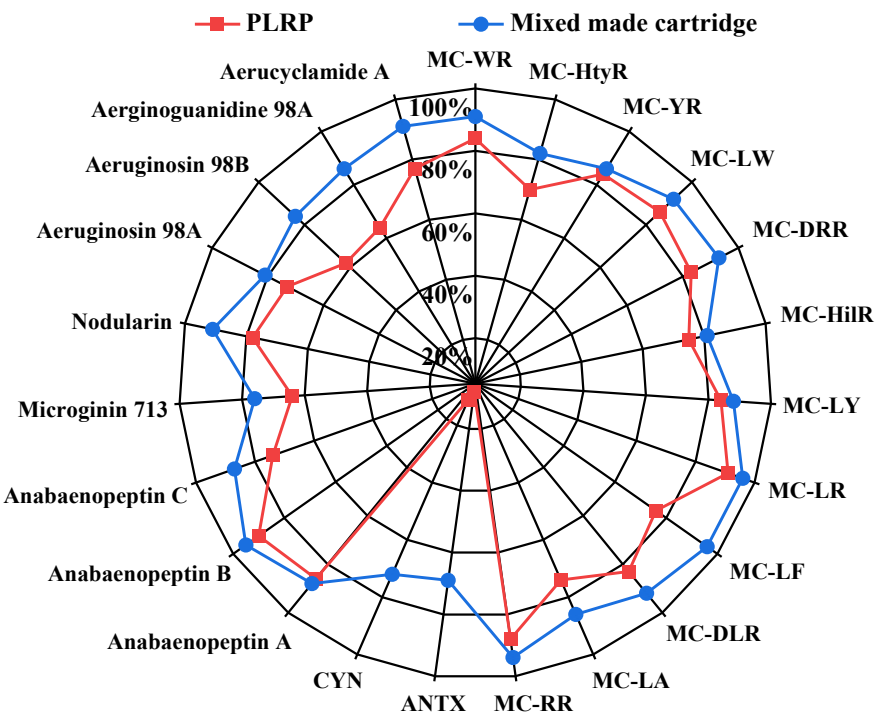


Figure 2. The recovery test for 23 selected cyanotoxins with two online cartridges.

Table 1 The concentration of cyanotoxins in water samples collected from Taihu Lake during August 2025

NO. Con.(ng L ⁻¹)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
MC-WR	-	-	-	-	-	-	-	2.76	-	-	-	-	-	-	-	0.63	-	-	-
MC-HtyR	-	-	-	-	-	-	-	0.88	-	-	-	-	-	-	-	-	-	-	-
MC-YR	-	0.24	-	0.27	-	-	-	3.12	-	-	-	-	-	-	-	-	-	-	-
MC-LW	-	-	-	-	-	-	-	1.33	0.64	-	-	-	-	-	-	-	-	-	-
MC-DRR	-	-	-	-	-	-	-	-	-	-	-	-	-	0.87	-	-	-	-	-
MC-HilR	-	1.65	-	-	-	-	-	7.34	3.12	-	-	-	-	1.56	-	-	-	-	-
MC-LY	-	-	-	1.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MC-LR	-	-	-	-	-	-	5.48	5.78	9.63	0.43	0.47	1.02	3.17	0.75	-	1.92	0.78	1.31	0.84
MC-LF	-	-	-	-	-	-	-	-	-	-	-	-	-	0.67	-	-	-	-	-
MC-DLR	1.24	-	-	-	-	-	-	0.66	-	-	3.24	-	-	-	-	-	-	-	-
MC-LA	-	-	-	2.85	2.07	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-
MC-RR	-	-	-	-	-	-	-	3.26	5.34	0.67	-	0.89	2.61	-	-	0.58	0.54	-	-
CYN	-	-	-	0.12	-	-	-	0.12	-	-	-	-	-	-	-	0.23	-	-	-
ANTX	-	-	1.13	0.77	0.89	4.91	-	-	-	-	-	5.43	-	-	-	3.25	-	-	1.31
Anabaenopeptin A	1.44	1.61	0.17	1.05	1.92	1.25	-	2.72	-	1.01	-	-	-	1.27	0.85	-	-	-	-
Anabaenopeptin B	1.18	-	0.22	0.66	1.85	-	-	-	-	2.06	-	-	-	1.68	1.88	-	-	-	-
Anabaenopeptin C	-	2.23	-	-	-	2.13	-	3.43	-	-	-	-	-	-	-	-	-	-	-

Nodularin	-	-	-	-	0.41	-	-	-	-	-	-	-	-	-	-	-	-	0.47	-
Microginin 713	-	-	-	-	-	-	-	5.72	-	-	-	-	-	-	-	-	-	-	-
Aeruginosin 98A	-	-	-	-	-	-	-	0.67	-	-	-	-	-	-	-	-	-	-	-
Aeruginosin 98B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aeruginoguanidine 98A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aerucyclamide A	-	-	3.65	-	5.77	-	2.13	4.22	-	-	-	-	4.33		3.15	-	-	-	-

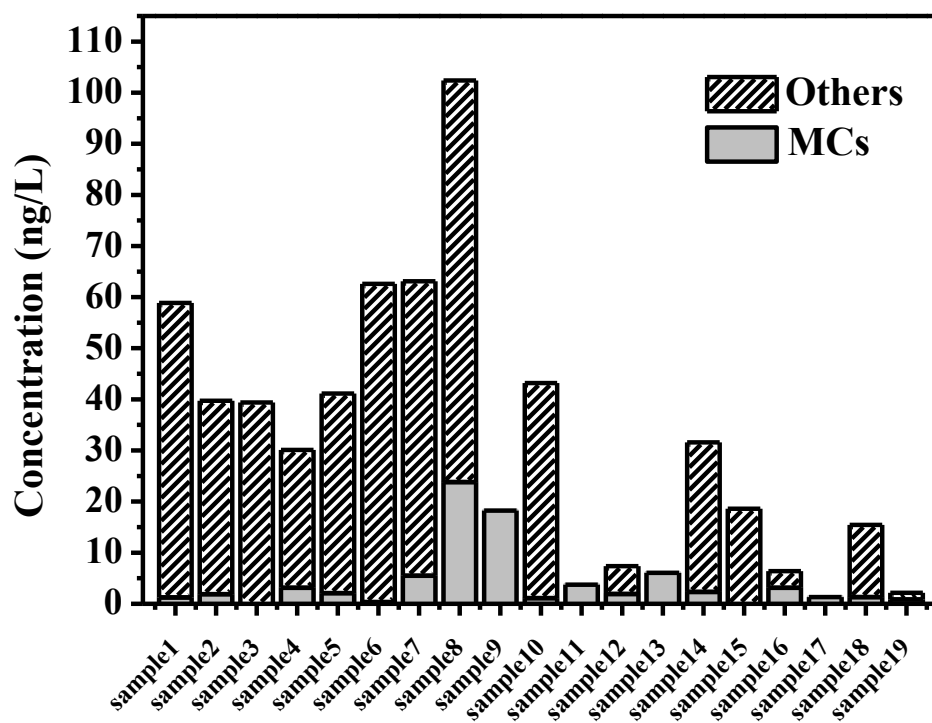


Figure 3. The concentrations of MCs and non-microcystin cyanotoxins of different samples

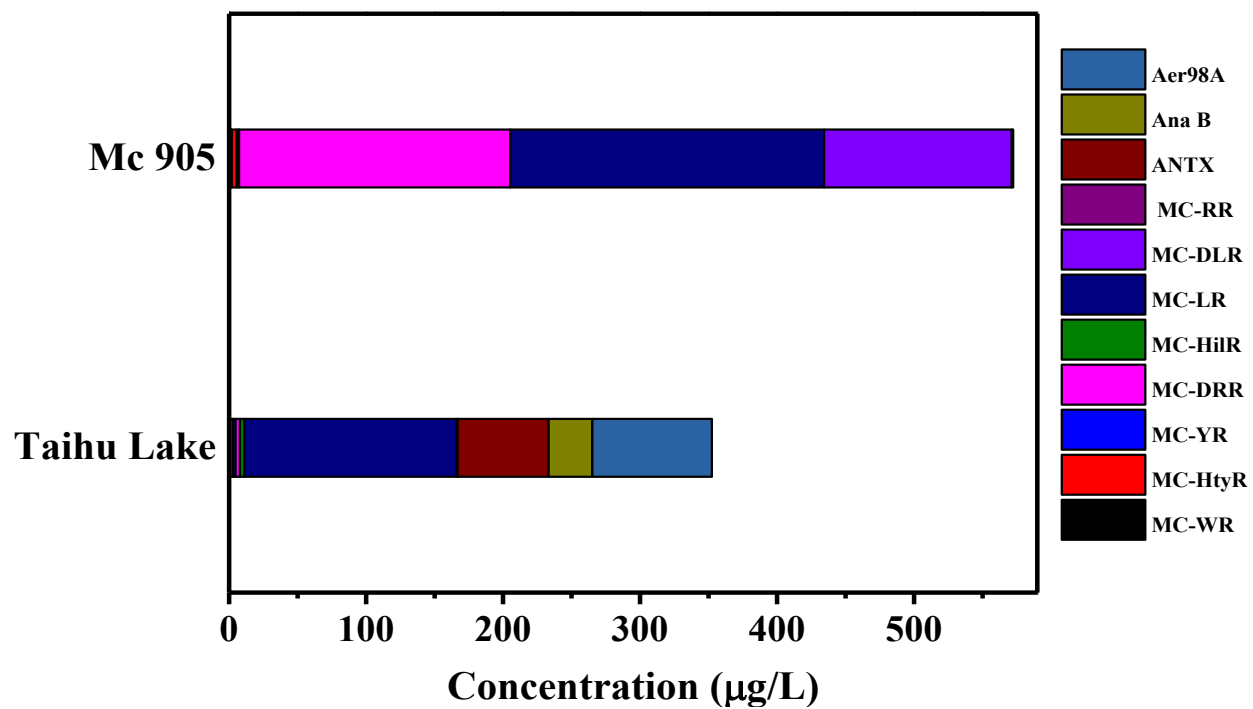


Figure 4. The concentrations of cyanotoxins from cyanobacterial of Taihu Lake and *Microcystis aeruginosa* 905

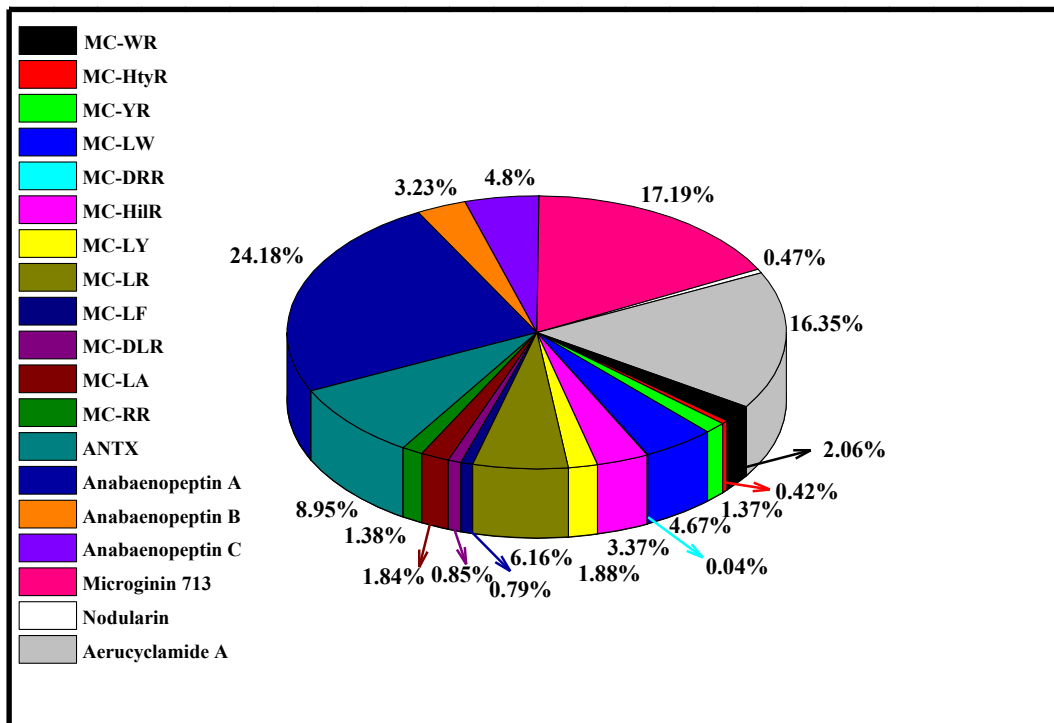


Figure 5. The contributions of the detected cyanotoxins from Taihu Lake for environment risk

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