

Environmental Toxicology

A spatiotemporal study of a recreational freshwater lake: ultraperformance liquid chromatography–tandem mass spectrometry profiling of cyanotoxins and the dermal toxicity assessment

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Abstract

Cyanotoxins are toxic secondary metabolites produced by cyanobacteria with significant health risks to aquatic ecosystems and humans. In this study, spatiotemporal profiling of cyanotoxins from a recreational freshwater lake were determined by ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) across 6 months at three study sites. Their toxicity on human skin keratinocytes were examined because dermal exposure to recreational freshwater is an understudied route of exposure as compared with ingestion. A network toxicology framework was constructed to predict and reveal the possible mechanistic insights of how the cyanotoxins induce dermal toxicity. Our findings showed that significant levels of cyanotoxins were identified through UPLC-MS/MS, with microcystin-RR being the most prevalent cyanotoxin present in the freshwater. Microcystin-enriched water samples significantly reduced the viability of human skin keratinocytes; nonetheless, membrane permeabilization may not be the primary mechanisms of dermal toxicity. Network toxicology predicted significant enrichment of pathways involved in skin homeostasis and development. The findings suggest potential risks of dermal toxicity upon exposure to cyanotoxin-contaminated recreational freshwater, indicating the necessity for ongoing monitoring and immediate actions in evidence-based water management policies to protect the ecosystem and public health.

Keywords: microcystin, skin toxicity, membrane permeabilization, skin homeostasis

Introduction

Cyanobacteria, commonly known as blue-green algae, is a phylum of photosynthetic bacteria that are found in a range of terrestrial and aquatic environments, including oceans, freshwater bodies, and moist soils (Pandey, 2015; Sathyananth and Raj, 2024). Cyanobacterial blooms alter the physicochemical properties of water and release cyanotoxins that can bioaccumulate through the food web, posing risks to aquatic organisms and humans (Zhang et al., 2022). The decay of these blooms exacerbates eutrophication by releasing additional nutrients and creating anoxic conditions in water bodies (Zhang et al., 2022).

Cyanobacteria thrive in a range of aquatic environments and adapt to extreme conditions, enabling them to proliferate in diverse habitats. In recent decades, studies have shown a correlation between the rise in global temperatures and an increased prevalence of cyanobacterial blooms. This is amplified by the stagnation of waters as a consequence of flooding—both of which are impacts of climate change (Journey et al., 2013). Rising global temperatures accelerate cyanobacterial metabolism, leading to an increase in cyanotoxin production (Manning and Nobles, 2017). In shallow water bodies with limited water movement,

these conditions create an ideal environment for the proliferation of harmful cyanobacterial blooms.

Cyanotoxins are toxic secondary metabolites produced by cyanobacteria (Thirunavukkarasu et al., 2023) that pose significant adverse impacts to the aquatic ecosystems and human health (Malik et al., 2020). These toxins are primarily produced by the algal blooms, which occur under favorable conditions such as high nutrient levels (primarily nitrogen and phosphorus), elevated temperatures (>30 °C), and low salinity levels (Sahoo et al., 2024). They are released during cell death or lysis, increasing the overall toxicity of the affected water body (Svrcek and Smith, 2004). Generally, cyanobacteria play an essential role in aquatic ecosystems by fixing nitrogen and contributing to the food web (De Almeida et al., 2024; Lalić, 2024). However, excessive growth of cyanobacteria can lead to harmful algal blooms, resulting in water quality deterioration through oxygen depletion and the release of cyanotoxins (Blackall and Burrell 1998; Lalić, 2024).

Microcystin (MC) is the most commonly found cyanotoxin globally. It is a potent hepatotoxin that inhibits protein phosphatases, induces oxidative stress, and causes liver damage (Humbert, 2009). Nodularin (NOD), structurally similar to MC, is

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a cyclic pentapeptide hepatotoxin produced by *Nodularia spumigena* (Humbert, 2009). Nodularin also inhibits protein phosphatases and induces oxidative stress, resulting in genotoxic effects and liver cancer (Štern et al., 2019). Cylindrospermopsin (CYL) is another notable cyanotoxin that inhibits protein and RNA synthesis, leading to toxic effects on multiple organs and contributing to oxidative stress (Beasley, 2019).

This study assesses the water health of a recreational freshwater lake in Petaling Jaya, Malaysia. This freshwater lake was a tin-mining pool that has now been transformed into a recreational urban park for the community, serving the dual function of flood retention while providing a recreational value that residents enjoy due to its convenient location within the urban area. While the park offers various amenities, it has significant water quality concerns that threaten its ecosystem and recreational values among the residents and visitors of this lake (Yap, 2024), which received considerable media attention. This issue is particularly acute as a freshwater body is critical to human and ecological health—sustaining diverse biodiversity and regulating climate. The recreational freshwater of the study site suffers from poor water circulation and water quality deterioration due to runoff from surrounding catchments, exacerbated by extensive waste dumping into the lake. Preliminary water quality assessment showed exceptionally high inputs of nitrogen and phosphorus from the runoffs, leading to uncontrolled algal blooms and high chemical and biochemical oxygen demands (Chang et al., 2024). Recent reports have indicated that the recreational freshwater resource is dominated with uncontrolled algal blooms, and based on microscopic examination, these blooms were identified as *Microcystis aeruginosa* (Kützing) Kützing, a colonial cyanobacteria known for its potential to produce toxins such as MCs (Chang et al., 2024). In this study, the multiple reaction monitoring method was used in ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) because it allows for selective and accurate quantification of cyanotoxins, even at trace levels, making it an ideal analytical method for monitoring aquatic samples (Jacinavicius et al., 2023). Furthermore, the multiple reaction monitoring method allows simultaneous quantification of different cyanotoxins in a single UPLC run; it also offers high reproducibility and interlaboratory standardization with reference standards for reliable quantification and monitoring of cyanotoxins in aquatic samples.

The cyanotoxins profiled in this recreational freshwater and their potential risks to visitors of the recreational site remain unknown, hindering effective management strategies and policies. This gap poses risks to public health and ecological balance. The toxicity of cyanotoxins has been extensively studied via oral and systemic exposure. However, there are limited studies on the dermal toxicity effects of cyanotoxins, an underestimated route of exposure during recreational activities. In a recent study, MC was shown to reduce cell viability in human skin keratinocytes, but only a limited number of congeners were investigated (Hughes et al., 2025). Meanwhile, CYL could induce skin sensitization in mice, suggesting potential dermal effects, although this study did not examine its cytotoxicity in human skin keratinocytes (Stewart et al., 2006). Additionally, MC and NOD were found to inhibit protein phosphatase activity in mouse skin (Matsushima et al., 1990). Altogether, these findings highlight critical gaps in understanding direct cytotoxicity and permeability capacity of different cyanotoxins in human skin, especially from system toxicology perspectives.

This study aimed to investigate the cyanotoxin levels of the said recreational freshwater through UPLC-MS/MS and examine

their cytotoxicity effects on human skin cells. The network toxicology of the cyanotoxins will be constructed and analyzed to understand the correlation between the cyanotoxin-involved pathways in triggering dermal toxicity.

Methods

Water sampling

Our study was conducted in a recreational lake situated in Petaling Jaya, Malaysia (coordinates: 3°6'10"N 101°37'30"E), with three sites labeled Zones A, B, and C (Figure 1). These sites were selected by the presence of channel flow, which serves as influential discharge pathways and points, potentially transporting terrestrial anthropogenic pollutants. Sample collection was carried out every fortnight (March–May) to establish and ensure consistent baseline data and followed by monthly sampling (June–August). One liter of water sample was collected with a high-density polyethylene bottle once at each sampling zone and time from March to August. The water samples were immediately stored in a cooler box with ice to maintain low temperature during transport and minimize sample degradation. Upon reaching the laboratory, samples were preserved in 4°C prior to subsequent processing.

Water sample processing

Water filtration

The collected water samples were prefiltered with a cloth coffee filter to remove large debris, followed by vacuum filtration via Whatman GF/C Glass Microfiber Filters (47mm, 1.2-μm pore size).

Solid-phase extraction of water samples for cyanotoxin enrichment

Solid-phase extraction (SPE) was performed with the Waters Sep-Pak tC18 Plus Light Cartridge (37–55 μm) for MC and NOD. Since CYL is a highly polar molecule (Bogialli et al., 2006), it was analyzed directly by using the filtered water samples.

SPE enrichment for MC

Solid-phase extraction enrichment for MC was conducted with the protocol from Wang et al. (2015). The cartridge was first preconditioned with 10 mL of 100% high-performance liquid chromatography-grade methanol, followed by 10 mL of Milli-Q water. Then, 40 mL of filtered water sample was loaded into the cartridge and washed with 10 mL of Milli-Q water and then 20% methanol, respectively. Microcystin was eluted with 10 mL of 100% methanol. The eluate was aliquoted and freeze-dried. The enriched samples were reconstituted with 1 mL of 50% methanol for UPLC-MS/MS analysis and 1 mL of phosphate-buffered saline for cytotoxicity assays.

SPE enrichment for NOD

Solid-phase extraction enrichment for NOD was adapted from methods by Mazur-Marzec and Pliński (2001). The cartridge was preconditioned with 10 mL of 100% high-performance liquid chromatography-grade methanol and washed with 10 mL of Milli-Q water. Then, 40 mL of filtered water sample was loaded into the cartridge. After washing with 10 mL of Milli-Q water, NOD was eluted with 10 mL of 100% methanol. The eluate was aliquoted and freeze-dried. The enriched samples were reconstituted with 1 mL of 50% methanol for UPLC-MS/MS analysis and 1 mL of phosphate-buffered saline for cytotoxicity assays.

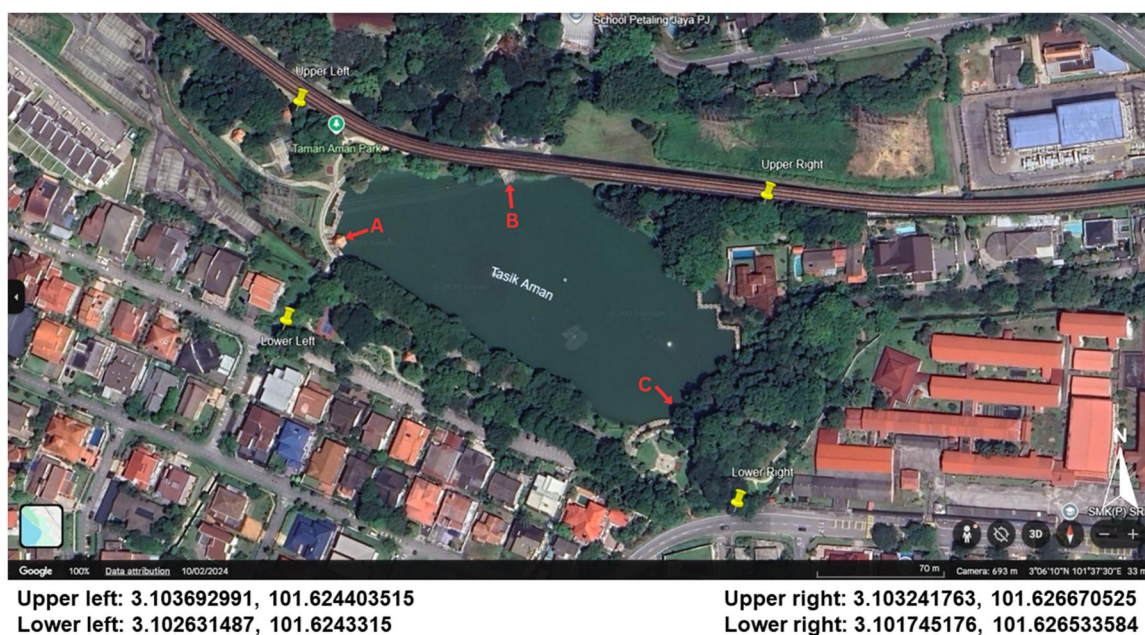


Figure 1. Google Earth image of a recreational lake at Petaling Jaya, Malaysia. Three locations are the sampling sites in this study: Zones A, B, and C.

UPLC-MS/MS analysis

Cyanotoxin identification and quantification were conducted with Waters Acquity UPLC with Xevo TQ-XS Triple Quadrupole MS, equipped with an electrospray ionization source. Chromatographic separation was carried out via a Waters Acquity UPLC BEH C₁₈ column (1.7 μm, 2.1 × 50 mm). In total, 50 μL of the filtered and enriched water samples were injected and eluted with 0.1% trifluoroacetic acid (Mobile Phase A) and acetonitrile (Mobile Phase B) at a flow rate of 0.4 mL/min. The entire chromatography separation was 7.5 min following the elution gradient of 5% B (0–0.75 min), 80% B (0.75–5 min), 100% B (5–6 min), and 5% B (6–7.5 min). The MS/MS conditions were set as follows: capillary voltage of 3 kV, desolvation temperature of 600 °C, and desolvation gas flow of 1,000 L/hr with a source temperature of 150 °C. The cone gas flow and collision gas flow were 150 L/hr and 0.15 mL/min, respectively. The nebulizer gas pressure was 7 bar. The data were acquired by MassLynx software and processed in the TargetLynx XS Application Manager based on multiple reaction monitoring transitions. Standard curves of cyanotoxins were constructed for quantification purposes. For MC-LR, MC-RR, CYL, and NOD, a concentration range of 0.05, 0.5, 5, 50, and 500 ng/L was constructed; for MC-YR and MC-LW, a concentration range of 25, 50, 100, 150, and 200 ng/L was constructed. The concentrations of cyanotoxins in the water samples were quantified from the standard curve.

Cytotoxicity assays

The cytotoxicity of the filtered and enriched water samples was assessed on human skin keratinocytes (HaCaT) cells to determine the effects of cyanotoxins on skin as a first line of exposure. Two complementary assays were used: Cell Counting Kit-8 (CCK-8) to measure cell viability and metabolic activity and propidium iodide (PI) to determine cell membrane integrity.

CCK-8 cell viability assay

Approximately 25,000 cells/mL of HaCaT cells in serum-free Dulbecco's Modified Eagle Medium were seeded on a sterile and tissue culture-treated 96-well plate. Then the cells were treated with filtered and enriched water samples for 24 hr. After

treatment, the old serum-free media was aspirated, and 100 μL of CCK-8 in serum-free Dulbecco's Modified Eagle Medium was added and incubated at room temperature for 3 hr. Absorbance at 460 nm was measured by a Tecan Sunrise Plate Reader. A positive control of 5% Triton-X was included alongside the assay.

PI quantitative measurement of cell membrane integrity

Approximately 25,000 cells/mL of HaCaT cells in serum-free Dulbecco's Modified Eagle Medium were seeded on a sterile, black, tissue culture-treated 96-well plate. Then the cells were treated with filtered and enriched water samples for 24 hr. After treatment, the plates were centrifuged twice at 1,000 rpm for 5 min to pellet the cells. Between centrifugation, the cells were washed with 1× phosphate-buffered saline to remove residual toxins or media. Later, 100 μL of 5-μg/mL PI was added to each well and incubated at 37 °C for 1 hr. The fluorescence intensity of PI-stained cells was measured with a Tecan Spark 10M Microplate Reader at excitation and emission wavelengths of 535 and 617 nm, respectively. A positive control of 5% Triton-X was included alongside the assay. The relative change in fluorescence intensity was calculated per the following equation:

$$\text{relative fluorescence intensity (RFI)} = \frac{\text{adjusted fluorescence intensity of treated cells}}{\text{adjusted fluorescence intensity of untreated cells}} \times 100\%.$$

Network toxicology

Toxicity analysis of cyanotoxins

We used ADMETsar (<https://lmmd.ecust.edu.cn/admet3/>; Gu et al., 2024) to predict the toxicity of cyanotoxins. The SMILES sequence of these cyanotoxins was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>; Kim et al., 2025) to input into ADMETsar for analysis, and the assessment of dermal toxicity was performed.

Target construction of cyanotoxins and dermal toxicity

The SMILES sequence of all cyanotoxins was submitted to Swiss Target Prediction (<http://www.swisstargetprediction.ch/>; Daina

et al., 2019) with species set to *Homo sapiens*. The outputs (i.e., targets) with probability >0 were finalized as the target's library for all cyanotoxins. The disease target libraries were constructed by using GeneCards (<http://www.genecards.org/>; Stelzer et al., 2016). The keyword "dermal toxicity" was used to search for targets. The top 5,000 targets were selected due to the substantial numbers of targets predicted by GeneCards. Then, the intersection of target libraries for cyanotoxins and dermal toxicity was determined per Bioinformatics & Evolutionary Genomics Venn analysis (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). The overlapping targets obtained were analyzed for protein-protein interaction (PPI), functional annotations, and pathway enrichment analysis.

PPIs

The PPI network was constructed to visualize the interactions of overlapping protein targets associated with dermal toxicity caused by cyanotoxins. First, the overlapping targets obtained from the Venn diagram were matched against *H. sapiens* entries and input into the STRING database (<https://cn.string-db.org/>; Szklarczyk et al., 2023).

The following parameters were used:

- Organisms: *H. sapiens*
- Network type: full STRING network
- Required score: medium confidence (0.400)
- False discovery rate stringency: medium (5%)
- Active interaction sources: text mining, experiments, databases, coexpression, neighborhood, gene fusion, and co-occurrence

The generated network was sent to Cytoscape Version 3.10.3 (Kohl et al., 2011) and analyzed by the NetworkAnalyzer plugin function for degree, clustering coefficient, closeness centrality, betweenness centrality, and neighborhood connectivity. The PPI network illustrated the affected pathways and the connection between these targets and cyanotoxins.

Functional annotation and pathway enrichment analyses

Gene Ontology terms, Kyoto Encyclopedia of Genes and Genomes pathway, and functional annotations of cyanotoxin targets were performed with the DAVID functional annotation tool (<https://davidbioinformatics.nih.gov/summary.jsp>; Sherman et al., 2022), followed by enrichment cluster-based medium stringency with a threshold count of 0.5 and EASE of 0.5. Enrichment scores, statistical parameters such as *p* value, Benjamini coefficient, and false discovery rate were also determined. Pathway enrichment analyses of the targets were performed in Gprofiler (<https://biit.cs.ut.ee/gprofiler/gost>; Raudvere et al., 2019) to illustrate the REACTOME pathways affected by cyanotoxins.

Statistical analyses

All experimental data were expressed as mean and standard error of the mean ($M \pm SEM$; $n = 3$). One-way analysis of variance was performed in Prism (Version 10.1.1; GraphPad) to compare mean differences for cyanotoxin levels at $p < 0.05$. Pearson correlation assessed variable linearity, and regression analysis examined predictive relationships among cyanotoxin levels, percentage cell viability, and relative fluorescence intensity (RFI).

Results and discussion

UPLC-MS/MS profiling of cyanotoxins

Figure 2 illustrates the temporal and spatial distributions of cyanotoxin levels in SPE-enriched water samples collected at Zones

A to C from March to August 2024. The results showed notable and significant fluctuations in the toxin levels.

The MC-RR was the predominant MC isotype in the water samples, with an average concentration of 3,890 ng/L (equivalent to 3.89 $\mu\text{g/L}$). It was also present at the highest levels in the early sampling periods. This was followed by CYL (average concentrations of 1,734.06 ng/L), MC-YR (776.19 ng/L), and MC-LR (356.56 ng/L). Cylindrospermopsin displayed relatively stable levels across all zones. Notably, NOD and MC-LW levels were comparatively lower than those of other cyanotoxins. The NOD levels increased drastically from July to August across all zones. Solid-phase extraction significantly enriched the cyanotoxins in water samples, resulting in higher detectable levels by UPLC-MS/MS.

The study began on March 12 with a notable peak in cyanotoxin levels, particularly in Zone C, primarily consisting of MC-RR with concentrations of 35,463.65 ng/L. Zone A showed moderate levels of cyanotoxins, while Zone B had the lowest levels, dominated by MC-RR as well. By March 26, a significant decrease in cyanotoxins was observed across all zones. This rapid decline suggests a possible response to changing physicochemical parameters of the lake water, such as temperature or nutrient concentration.

From April 9 to May 21, all zones showed relatively stable cyanotoxin levels, with only subtle, nonsignificant fluctuations observed from March to June, although overall levels remained low. The cyanotoxin compositions exhibited broad diversity, with a regular presence of MC-RR, MC-LR, and CYL. The CYL levels showed high variability in Zone A with a peak in May before declining significantly in June. However, Zones B and C had identical distribution patterns, with the highest levels detected in first sampling date of March before declining to relatively stable levels throughout the sampling dates. NOD levels were generally undetected for most periods at all sampling zones.

Zone A exhibited intermediate dynamics of cyanotoxins, except for MC-RR, which declined after March. It showed more consistent responses to seasonal changes. Zone B had relatively lower cyanotoxin levels (<10,000 ng/L) than other zones, representing a moderate distribution of toxin levels. Zone C demonstrated distinct characteristics as an MC-RR hub, showing the highest concentrations among all zones. Zone C was predominated by MC-RR and CYL, particularly during peak periods, and showed the most pronounced response to seasonal changes. This indicates that Zone C may be more susceptible to factors that promote cyanobacterial growth, including biogeochemical factors (temperature, dissolved oxygen) and hydrological ones (precipitation, evaporation, runoff), as well as point-source nutrient enrichment.

All zones showed overall declining trends of MC levels over time. Factors such as nutrient enrichment, water flow, and light availability may uniformly affect cyanobacterial growth across the lake, resulting in similar toxin levels despite the varying conditions of each zone (Chen et al., 2023; Wurtsbaugh et al., 2019). Eutrophication, driven by nutrient enrichment, is a primary factor to the formation of cyanobacterial blooms and release of cyanotoxins (Sinang et al., 2015). The presence of cyanotoxins should not be neglected as a public health risk, due to the high variability in the cyanotoxins produced. Exposure to cyanotoxin-contaminated water has been associated with skin allergy and irritation, particularly during recreational activities (World Health Organisation, 2003).

Weather conditions play crucial roles in influencing the cyanotoxin levels in the lake water. Variations in temperature, precipitation, humidity, and wind patterns significantly affect the

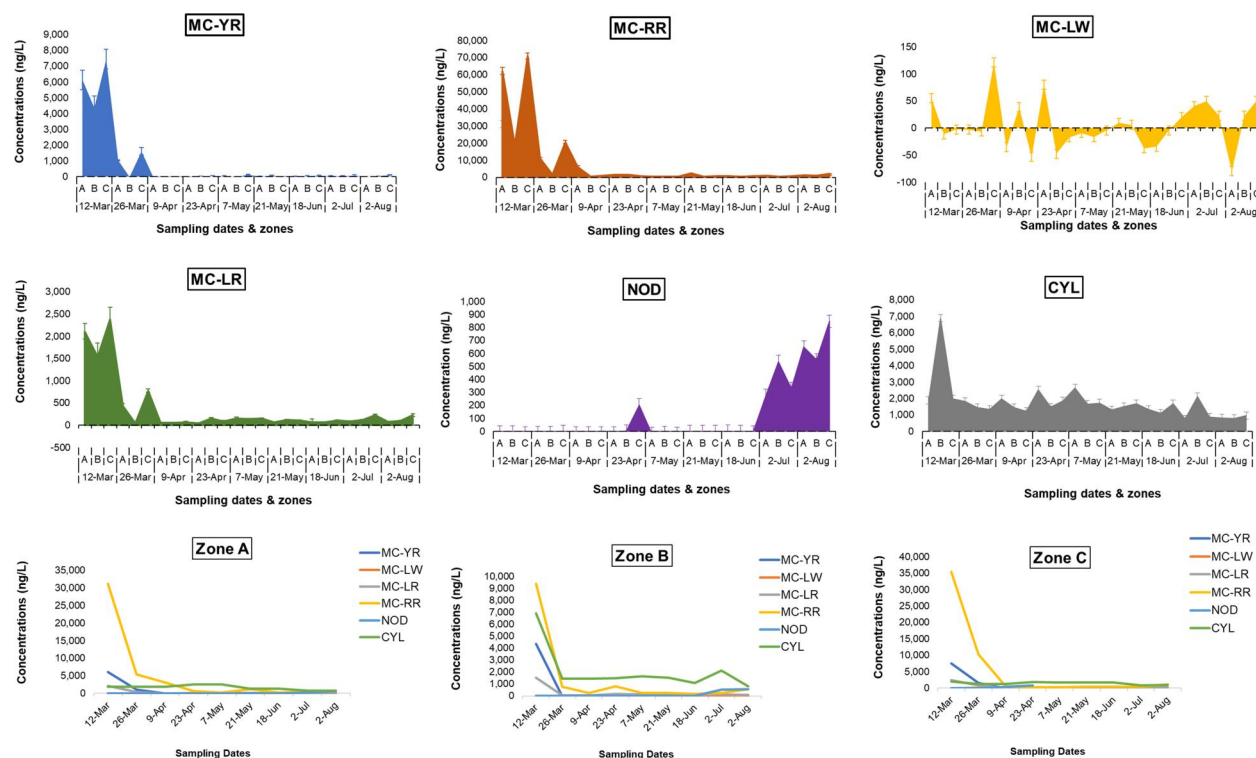


Figure 2. Temporal and spatial variations of cyanotoxins across sampling dates (March–August) and zones (A–C) for different cyanotoxins: microcystin (MC)-YR, MC-RR, MC-LW, MC-LR, nodularin, and cylindrospermopsin. There were distribution patterns of different cyanotoxins. All three zones exhibited different levels of cyanotoxins, in which Zone B had relatively lower cyanotoxin levels as compared with the other zones. Data are presented as $M \pm SEM$. CYL = cylindrospermopsin; MC = microcystin; NOD = nodularin.

proliferation of cyanobacteria, which are the primary producers of these harmful cyanotoxins. It has been suggested that climate conditions such as droughts and warmer temperatures can trigger harmful cyanobacterial blooms, leading to extensive production of cyanotoxins (Gophen, 2021). The weather data of Petaling Jaya, Malaysia, during the sampling dates were taken from Weather Underground (2024) and Weather Atlas (2024), as summarized in online supplementary material Table S1.

Baseline water quality data were collected to assess the freshwater's physicochemical properties (online supplementary material Table S2). The data were benchmarked against the National Lake Water Quality Classification System, indicating severe eutrophication that can promote algal blooms. The weather events and the cyanotoxin levels were corresponded to illustrate the major transitions in the cyanotoxin distribution patterns. For example, a decline in MC-YR during March 12 to 26 coincided with a weather shift from dry to moderate humidity. From April 23 to May 7, an increase of CYL levels was observed in conjunction with an increased rainfall period, whereas from June to July, a sharp increase of NOD levels happened in conjunction with a weather shift from persistent to reduced rainfall.

The World Health Organisation provisional guideline values for MCs and CYL for recreational purposes are 20 and 6 $\mu\text{g/L}$, respectively (World Health Organisation, 2020). Although the MCs and CYL levels in the water samples were below the World Health Organisation provisional guideline values for recreational use (MC, 0.006–4 $\mu\text{g/L}$; CYL, 1.73 $\mu\text{g/L}$), suggesting no immediate risks, such contamination cannot be neglected because UPLC-MS/MS profiling indicated that the cyanotoxin levels exceed the safe levels for edible sources (Table 1). Although there are no specific guidelines for NOD, similar precautionary measures are

recommended due to its structural and toxic similarity to MCs. Evidence suggests that bioaccumulation of cyanotoxins occurs in the aquatic food web, potentially entering the human food chain. Thus, the presence of cyanotoxins in the recreational freshwater environment should receive adequate attention for mitigation measures (Ferrão-Filho and Kozłowsky-Suzuki, 2011; Zamora-Barrios et al., 2019).

Cytotoxicity assays Cytotoxicity index

In this study, we used HaCaT, an immortalized human skin keratinocyte cell, to assess the cytotoxicity of the water samples, as skin is the first exposure line to recreational lake water. We used the cytotoxicity index as an indicator to represent the cytotoxicity of water samples collected from all zones from March to August 2024. The higher the value, the higher the cytotoxicity of the water samples. The CCK-8 assay determines intracellular nicotinamide adenine dinucleotide phosphate dehydrogenase activity in viable cells via tetrazolium salts, which reflects the metabolic activity and thus the cell viability.

The assessment of cell viability per the CCK-8 assay revealed that the cell viability (percentage) of HaCaT cells was significantly reduced after treatment by the cyanotoxin samples. The mean cytotoxicity index of HaCaT cells from MC- and NOD-enriched samples was 79.52 ± 3.16 and 79.35 ± 3.23 , respectively. Meanwhile, CYL-containing samples had a cytotoxicity index of 50.79 ± 2.81 . These findings showed that the lake water samples are highly cytotoxic, suggesting that exposure to cyanotoxins could significantly cause dermal toxicity.

Figure 3A illustrates a trendline graph depicting the cytotoxicity index all samples across Zones A, B, and C during the

Table 1. Permittable values of cyanotoxins according to different guidelines.

Cyanotoxins	WHO provisional values ^a	USEPA values ^b
MCs	Drinking water: 1 µg/L Recreational use: 20 µg/L (WHO, 2020)	Drinking water (for children <6 years): 0.3 µg/L Drinking water (for adults): 1.6 µg/L
NOD	Drinking water: 1.5 µg/L (WHO, 2017)	No available data
CYL	Drinking water: 0.7 µg/L Recreational use: 6 µg/L (WHO, 2020)	Drinking water (for children <6 years): 0.7 µg/L Drinking water (for adults): 3.0 µg/L

Note. MC = microcystin; NOD = nodularin; CYL = cylindrospermopsin; WHO = World Health Organization; USEPA = U. S. Environmental Protection Agency.

^a World Health Organisation (2017, 2020).

^b U.S. Environmental Protection Agency (2014).

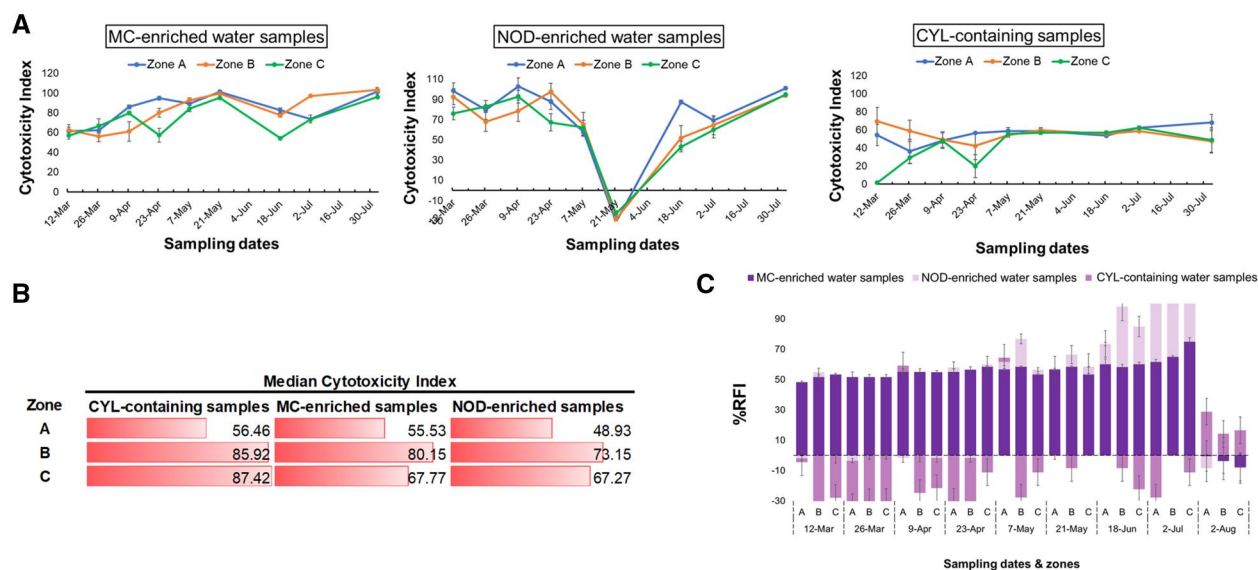


Figure 3. The cytotoxicity of water samples. (A) Cytotoxicity index of microcystin (MC)-enriched, nodularin (NOD)-enriched, and cylindrospermopsin (CYL)-containing water collected at Zones A to C from March to August. Data are presented as $M \pm SEM$. (B) Zone B had the highest median cytotoxicity index, followed by Zone C and Zone A. The variability of the cytotoxicity index may indicate dynamically changing environmental conditions. (C) Percentage relative fluorescence intensity of propidium iodide quantitative measurement of human skin keratinocytes after treatment with MC-enriched, NOD-enriched, and CYL-containing water samples. Error bars indicate SEM.

sampling duration. For CYL-containing samples, the results clearly illustrated fluctuations in the cytotoxicity index across three zones throughout the sampling duration. At the beginning of the sampling date, the zones showed remarkable trends, whereby Zone B exhibited the highest cytotoxicity index, followed by Zone A and then Zone C. However, throughout the study period, each zone demonstrated unique patterns of change. For example, Zone A showed a gradual but steady increase in the cytotoxicity index over time. After an initial decline in late March, the cytotoxicity index exhibited a consistent upward trend, ultimately achieving the highest level of all zones by August. This suggested a gradual accumulation of toxic elements in Zone A, which warrants further investigation and potential mitigation strategies.

Despite having the highest cytotoxicity index in early March, Zone B showed an overall decreasing trend throughout the monitoring period. The most significant reduction occurred between March 12 and April 23, after which the levels stabilized, fluctuating between 50 and 60. Last, Zone C demonstrated the highest dynamics in its cytotoxicity index, whereby at the beginning of the monitoring period, it showed a near zero cytotoxicity index. However, there was a significant increase within the first 2 weeks, followed by continuous fluctuations. May onward, Zone C displayed a more stable cytotoxicity index, aligning more closely with the other zones. A significant convergence occurred

between May 7 and May 21, when all three zones achieved a similar cytotoxicity index. By the end of the sampling period in August 2024, there was a divergence in the cytotoxicity index, in which Zone A showed an increase and Zones B and C showed a decline.

Similarly, for MC-enriched samples, all three sampling zones exhibited fluctuations in the cytotoxicity index over the sampling period. Zones A and B displayed similar patterns with consistently higher cytotoxicity indexes than Zone C on most sampling dates. The cytotoxicity index peaked on May 21 for all three zones, followed by a remarkable decrease in June, where Zone C had the lowest cytotoxicity index. By comparing zonal differences, Zone A maintained the highest cytotoxicity index across most sampling dates, followed by Zone B in a similar magnitude. It is interesting to note that Zone C showed a moderate cytotoxicity index with a substantial decline in June 18.

In NOD-enriched samples, the cytotoxicity index fluctuated significantly over the sampling period for all zones. A significant decline was observed in May 21, followed by an increase toward July 30. During the beginning of the sampling period, the cytotoxicity index was relatively higher for all zones, especially Zone A. Surprisingly, all zones displayed a sharp decline on May 21 before increasing again afterward.

As reflected from the median cytotoxicity index (Figure 3B), Zones B and C had a comparably higher cytotoxicity index than

Zone C. The variability in Zone A may indicate dynamically changing environmental conditions or intermittent pollution spots.

PI quantitative measurement of cell membrane integrity

Propidium iodide staining is a common method used to assess cell membrane integrity, as it is a fluorescent dye that cannot permeate intact membranes but binds to DNA when cell membranes are compromised. An increase in RFI often indicates greater PI uptake by the cells, signifying more extensive cell membrane permeabilization. The analysis of cell membrane integrity via PI staining (Figure 3C) revealed significant differences in the average full changes in RFI among the various cyanotoxins. For all MC-enriched samples, the average change of RFI was $49.9\% \pm 3.87\%$. This suggested that MC-enriched samples permeabilized the cell membranes of HaCaT cell lines. MCs are known to inhibit protein phosphatase 1 and 2A (Humbert, 2009); thus, cell membrane damage could be a secondary mechanism for dermal toxicity. In contrast, NOD-enriched samples had an average change of $11.98\% \pm 4.60\%$, suggesting that NOD had minimal effects on cell membrane permeabilization. On the contrary, CYL-containing samples showed an average RFI of $-12.25\% \pm 3.51\%$, implying that CYL did not have an impact on cell membrane integrity.

Both MC- and NOD-enriched samples exhibited a mean cytotoxicity index >79 , whereas that of CYL-containing samples was only 50.79 ± 2.81 . The difference in their cytotoxicity could be attributed to their mechanistic actions on cell membranes. Both MCs and NOD are known to inhibit protein phosphatases (Humbert, 2009; Otero and Silva, 2022), which can cause cytoskeletal disruption (Máthé et al., 2016) that impairs cellular integrity and functionality, eventually leading to cell death. The membrane-permeabilizing effect of MCs was more prominent than that of NOD, as reflected by the PI assay, presumably due to the structural difference between the cyanotoxins. Microcystins are heptapeptides while NOD is a pentapeptide; their potency in causing cytoskeletal disruption could be different. In contrast, CYL exerts its toxicity primarily by inhibiting protein synthesis, oxidative stress, and severe DNA damage (Mathe et al., 2017), which can have chronic effects. Because it does not directly trigger cytoskeletal disruption, this explains the absence of PI uptake as an indicator of direct impact on cell membrane integrity. Thus, the cytotoxicity of CYL within the assay duration (i.e., 24 hr) was less extensive than that of MCs and NOD.

Pearson's correlation analyses

Pearson's correlation analysis among concentrations (nanograms per liter), cell viability, and RFI signified the relationship between cyanotoxin levels and the cytotoxicity of MC-YR, MC-LR, and MC-RR. There was a moderate positive correlation (online supplementary material Table S3) between concentrations and cell viability, suggesting concentration-dependent cytotoxic effects on the cells. On the contrary, MC-LW and CYL showed weak correlations, while NOD displayed a negative and nonsignificant correlation. Pearson's correlation analysis revealed that the concentrations of MC-LR, MC-RR, MC-YR, and CYL can cause reduced cell viability, while MC-LW and NOD levels did not significantly affect cell viability. It is interesting that no correlation was found between cyanotoxin concentrations and RFI, suggesting that cyanotoxin concentrations did not affect the membrane permeability of HaCaT cells. Pearson's correlation analysis between cell viability (percentage) and changes in RFI was conducted to assess whether HaCaT cell viability correlated to membrane disruption. Cyindrospermopsin had a negative

correlation between cell viability and membrane permeabilization, implying that CYL did not cause membrane disruption as the PI quantitative measurement supported. Meanwhile, MC-YR, MC-LW, and MC-LR exhibited a moderate correlation between cell viability and RFI, suggesting that their cytotoxicity was moderately mediated by membrane permeabilization. Both MC-RR and NOD showed very weak correlations. The results suggested that membrane permeabilization may not be the primary mechanism for the observed cytotoxic effects.

Network toxicology

Cyanotoxins are well known for their detrimental effects on various biological systems, primarily through their interference with key biochemical pathways. Microcystins, including subtypes such as MC-LR, MC-RR, MC-YR, and MC-LW, exert their toxic effects by inhibiting protein phosphatase 1 and 2A (Humbert, 2009). This impairs normal protein phosphorylation processes, leading to uncontrolled phosphorylation of cellular proteins and subsequently cellular stress and apoptosis.

Likewise, NOD exhibits a similar mechanism of action to MC; it also targets protein phosphatases, particularly protein phosphatase 1 and 2A (Otero and Silva, 2022). Thus, it causes protein phosphorylation imbalances, disrupting essential cellular functions and eventually inducing apoptosis or necrosis. In contrast, CYL primarily affects protein synthesis by inhibiting detoxifying enzymes such as glutathione reductase and glutathione S-transferase (Mathe et al., 2017). This causes oxidative stress and severe DNA damage. It further inhibits protein and nucleic acid synthesis, significantly impairing cellular viability and proliferation.

These mechanisms of toxicity have been well documented in liver and kidney, which are known primary sites of cyanotoxin accumulation (Arman and Clarke, 2021; Beasley, 2019; Chowdhury et al., 2024). Nonetheless, there are currently no reports on the effects of cyanotoxins on skin cells. The skin, the first protective barrier, is potentially the target for these toxins upon environmental exposure, such as swimming in lakes or any form of still water bodies. The lack of studies on skin cells leaves a gap in the literature to understand the toxic effects of cyanotoxins on skins.

In this study, all enriched water samples containing cyanotoxins (MC-LR, MC-LW, MC-RR, MC-YR, NOD, and CYL) caused a reduction in skin cell viability. However, statistical and Pearson's correlation analyses suggested that the cytotoxic effects did not occur through direct membrane permeabilization, as evidenced by the lack of significant correlation between the cyanotoxin levels and RFI. Both MCs and CYL are known to cause oxidative stress, which damages proteins, lipids, and DNA (De Oliveira and Silva, 2021; Hinojosa et al., 2023). Oxidative stress could interfere with essential cellular functions that lead to apoptosis without immediately compromising membrane integrity.

The inhibition of protein phosphatases by MCs could lead to dysregulation of signaling pathways, such as the mitogen-activated protein kinase pathway, which has a crucial role in cell proliferation, differentiation, and survival (Bubik et al., 2024; Máthé et al., 2016). Dysregulation of this pathway causes apoptosis or senescence (Anerillas et al., 2020).

Nevertheless, the toxicology theory behind these cyanotoxins on human skins is yet to be understood. As such, we performed network toxicology with bioinformatics analyses to predict and comprehend the direct effects of these cyanotoxins with a focus on human skins.

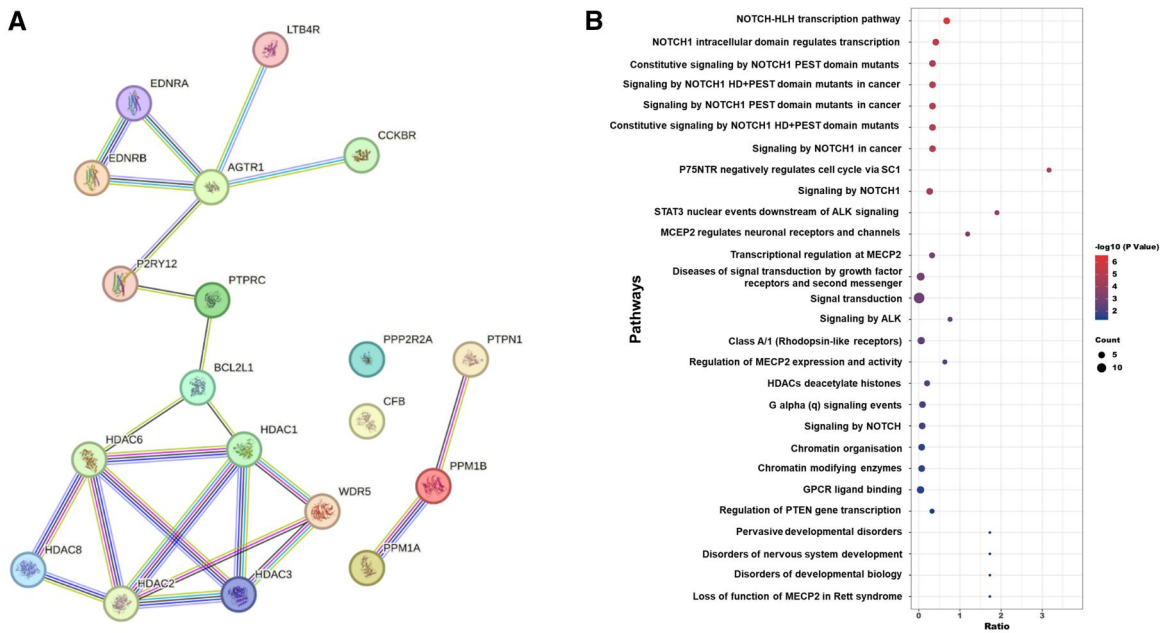


Figure 4. Network toxicology of cyanotoxins. (A) Protein-protein interaction network of overlapping target proteins of all cyanotoxins and dermal toxicity. The overlapping target proteins were annotated with different colors besides the network nodes. (B) The dot plot of pathway enrichment analysis for the overlapping target proteins of cyanotoxins and dermal toxicity. The enriched pathways are ranked by their ratios, with dot size indicating the target protein count associated with each pathway. Color intensity represents the statistical significance ($-\log_{10} p$ value) of the enriched pathways. The analysis revealed significant enrichment of NOTCH1 signaling pathways and developmental-related disorders.

(Pan et al., 2024). A protein phosphatase 1B, PTPN1, has important functions in insulin signaling and metabolic pathways, as it is a negative regulator of insulin (Meshkani et al., 2007); its interaction with PPP2R2A suggested a regulatory loop involving phosphorylation/dephosphorylation. Finally, complement factor B (CFB), which is part of the complement system, suggested that this cluster presumably connects metabolic regulation to immune responses.

From the PPI network, P2RY12 interacted with BCL2L1, forming a connection between the purinergic signaling pathways and cell survival mechanisms. This receptor is involved in platelet aggregation (Kahner et al., 2006) and inflammatory responses (Gómez Morillas et al., 2021); thus, the interactions suggest that P2RY12 signaling could influence survival pathways mediated by BCL2L1.

Pathway enrichment analyses and functional annotations: implications in dermal toxicity

We performed Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway and functional annotations for the overlapping protein targets of cyanotoxins and dermal toxicity, followed by pathway enrichment analyses based on Reactome, to comprehend the potentially affected pathways in dermal toxicity caused by cyanotoxins. The functional annotation of these proteins demonstrated their association in various biological processes, such as transcription, histone deacetylation, G protein-coupled receptor pathways, cancer-related pathways, and negative regulation of apoptosis (Table 3).

The dot plot in Figure 4B shows the pathway enrichment analysis based on the Reactome database. The ratios represent the protein targets involved in a pathway to the total number of protein targets in that pathway. A higher ratio indicates greater enrichment of that pathway in the data. The dot size indicates the number of proteins associated with the pathway, whereby the larger the dots, the higher count of the identified proteins. A $-\log_{10} p$ value indicates the significance of pathway enrichment,

whereby the lower the p value, the more significant the pathway enrichment.

The most enriched are centered on Notch signaling pathways, possibly related to transcription regulation, cancer biology, or neural development. Notch signaling is crucial for skin development, including keratinocyte differentiation and epidermal barrier formation. Dysregulation of Notch signaling is implicated in skin cancer (e.g., squamous cell carcinoma) and inflammatory skin conditions such as psoriasis and atopic dermatitis (Maner et al., 2020).

Methyl-CpG binding protein 2 (MECP2)-related pathways were enriched, albeit with lower significance as compared with Notch pathways. Methyl-CpG binding protein 2 regulates neuronal receptors and channels also showing a high ratio. Involvement indicates potential connections to cell cycle regulation and transcription factors downstream of signaling events. Methyl-CpG binding protein 2 is associated with chromatin remodeling and gene regulation. While MECP2 is primarily linked to neurological disorders such as Rett syndrome (Gonzales and LaSalle, 2010), recent research suggests that it has a role in cellular homeostasis in skin (Bajikar et al., 2025) and can influence gene expression in skin cell differentiation and function (Good et al., 2025). Methyl-CpG binding protein 2 mutations may disrupt stem cell regulation in skin. This disruption could contribute to abnormal wound healing (Saldanha et al., 2015).

The analyses displayed diverse biological functions, including chromatin organization, G protein-coupled receptor ligand binding, and G alpha (q) signaling events. Apart from that, pathways related to diseases such as pervasive developmental disorders and disorders of developmental biology are present, suggesting possible links to developmental disorders or disease states.

The pathway named p75 neurotrophin receptor (p75NTR), which negatively regulates the cell cycle via SC1, has the highest ratio (3.1667) and shows particularly strong enrichment in the dataset. A multifunctional receptor, p75NTR plays important counterbalance roles in the proliferative and survival activities of

Table 3. Functional annotation clustering of biological processes for overlapping protein targets of cyanotoxins and dermal toxicity per the DAVID functional annotation tool.

Cluster	Enrichment score	Go Term, UP, and KEGG ^a	Count	p-value	Fold change	Benjamini	FDR
1	4.94	Negative regulation of transcription by RNA polymerase II	9	9.60E-07	9.6	1.60E-04	1.50E-04
		Nucleoplasm	8	4.40E-02	2.2	6.60E-01	6.60E-01
		Chromatin regulator	6	4.60E-05	13	4.60E-04	4.60E-04
		Transcription regulation	6	4.00E-02	2.6	1.50E-01	1.50E-01
		Transcription	6	4.50E-02	2.5	1.50E-01	1.50E-01
		Enzyme binding	5	3.50E-04	14	2.60E-03	2.30E-03
		Alcoholism	5	4.70E-04	12	1.80E-02	1.70E-02
		Neutrophil extracellular trap formation	5	5.20E-04	12	1.80E-02	1.70E-02
		Negative regulation of apoptotic process	5	1.00E-03	10	5.30E-02	5.00E-02
		Repressor	5	8.90E-03	5.5	1.30E-02	1.30E-02
		Tubulin deacetylase activity	5	1.90E-11	720	2.10E-09	1.80E-09
		Histone H3K14 deacetylase activity, hydrolytic mechanism	5	1.10E-10	510	2.10E-09	1.80E-09
		Histone H4K16 deacetylase activity, hydrolytic mechanism	5	1.10E-10	510	2.10E-09	1.80E-09
		Histone H4K5 deacetylase activity, hydrolytic mechanism	5	1.10E-10	510	2.10E-09	1.80E-09
		Histone H4K8 deacetylase activity, hydrolytic mechanism	5	1.10E-10	510	2.10E-09	1.80E-09
		Histone H3K4 deacetylase activity, hydrolytic mechanism	5	1.10E-10	510	2.10E-09	1.80E-09
		Histone H3K9 deacetylase activity, hydrolytic mechanism	5	1.80E-10	460	2.50E-09	2.20E-09
		Histone H4K12 deacetylase activity, hydrolytic mechanism	5	1.80E-10	460	2.50E-09	2.20E-09
		Protein lysine deacetylase activity	5	3.80E-10	390	4.80E-09	4.10E-09
		Histone deacetylase activity	5	2.10E-09	270	2.30E-08	2.00E-08
		Histone deacetylase complex	5	4.90E-08	130	3.70E-06	3.70E-06
		Protein decrotonylase activity	5	2.80E-09	1000	2.60E-08	2.20E-08
		Histone decrotonylase activity	5	2.80E-09	1000	2.60E-08	2.20E-08
		Viral carcinogenesis	5	6.60E-04	11	1.80E-02	1.70E-02
		Negative regulation of DNA-templated transcription	4	1.50E-02	7.1	2.20E-01	2.10E-01
		Histone deacetylase binding	4	2.20E-04	31	1.80E-03	1.60E-03
		Protein lysine delactylase activity	3	2.50E-06	1000	2.20E-05	1.90E-05
		Protein deacetylation	3	4.40E-05	280	4.30E-03	4.10E-03
		Positive regulation of signaling receptor activity	3	4.40E-05	280	4.30E-03	4.10E-03
		NF-kappaB binding	3	5.40E-04	82	3.80E-03	3.30E-03
		Transcription corepressor binding	3	9.90E-04	61	6.60E-03	5.60E-03
		Epigenetic regulation of gene expression	3	1.10E-03	57	5.30E-02	5.00E-02
		Heterochromatin formation	3	1.80E-03	44	6.70E-02	6.30E-02
		Circadian regulation of gene expression	3	1.90E-03	43	6.70E-02	6.30E-02
		Regulation of protein stability	3	6.40E-03	23	1.40E-01	1.40E-01
		Biological rhythms	3	7.10E-03	21	9.20E-02	9.20E-02
		DNA-binding transcription factor binding	3	1.10E-02	18	6.30E-02	5.40E-02
		Polycomb repressive complex	3	1.20E-02	17	1.40E-01	1.40E-01
		Chromatin organization	3	2.00E-02	13	2.40E-01	2.20E-01
		Thyroid hormone signaling pathway	3	2.50E-02	11	2.30E-01	2.20E-01
		Cell cycle	3	4.00E-02	8.9	3.30E-01	3.10E-01
2	4.02	Chromatin remodeling	6	1.20E-03	6.7	5.30E-02	5.00E-02
		Protein dephosphorylation	5	8.60E-07	62	1.60E-04	1.50E-04
		Protein phosphatase	4	8.70E-04	19	2.90E-03	2.90E-03

(continued)

Table 3. (continued)

Cluster	Enrichment score	Go Term, UP, and KEGG ^a	Count	p-value	Fold change	Benjamini	FDR
3	2.65	Plasma membrane	11	9.10E-03	2.2	3.50E-01	3.50E-01
		Receptor	8	7.10E-03	3	1.20E-02	1.20E-02
		Phospholipase C-activating G protein-coupled receptor signaling pathway	6	8.90E-08	48	4.30E-05	4.10E-05
		Transducer	6	6.00E-03	4.5	1.20E-02	1.20E-02
		G protein-coupled receptor	6	4.30E-03	4.9	1.10E-02	1.10E-02
		Neuroactive ligand-receptor interaction	5	5.60E-03	6.3	9.50E-02	8.90E-02
		G protein-coupled receptor signaling pathway	5	9.60E-03	5.5	1.80E-01	1.70E-01
		Positive regulation of cytosolic calcium ion concentration	4	3.10E-04	28	2.50E-02	2.40E-02
		Calcium signaling pathway	4	1.40E-02	7.3	1.40E-01	1.40E-01
		Calcium-mediated signaling	3	5.30E-03	26	1.30E-01	1.20E-01
		cGMP-PKG signaling pathway	3	4.40E-02	8.4	3.30E-01	3.10E-01
		Pathways in cancer	6	3.40E-03	5.3	7.20E-02	6.80E-02
4	2.48	Negative regulation of apoptotic process	5	1.00E-03	10	5.30E-02	5.00E-02
		Chronic myeloid leukemia	3	1.00E-02	18	1.40E-01	1.40E-01

Note. The 4 most enriched clusters (reflected by enrichment score) are listed with their count, p value, fold change, Benjamini score, and false-discovery rate (FDR).
^a Annotated biological processes included Gene Ontology (GO) Terms, UniProt (UP), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

skin keratinocytes (Pincelli, 2017). It is known to negatively regulate the cell cycle, it is expressed in keratinocytes, and it may play a role in apoptosis and cell proliferation (Truzzi et al., 2011). In addition, p75NTR is involved in epidermal differentiation via the extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), and protein kinase B (AKT) signaling pathways (Li et al., 2014). Dysregulation of p75NTR has been linked to wound-healing disorders, ulcerative conditions, and possibly cutaneous nerve involvement in inflammatory skin diseases (Iwata et al., 2013; Raychaudhuri and Raychaudhuri, 2004). Although the interaction between cyanotoxins and p75NTR is not well characterized, this could be related to their mechanisms of action, whereby the cyanotoxins can alter the gene expression of p75NTR, which can affect skin cell survival, inflammatory responses, and skin ulcerations.

The other enriched pathway was signal transducer and activator of transcription 3 (STAT3) nuclear events downstream of anaplastic lymphoma kinase (ALK) signaling. Signal transducer and activator of transcription 3 is a transcription factor involved in cell survival, proliferation, and inflammation (Hu et al., 2024; Tošić and Frank, 2021). Hyperactivation of STAT3 is implicated in psoriasis and skin cancers (Levy and Inghirami, 2006; Xie et al., 2015). Signal transducer and activator of transcription 3 promotes keratinocyte proliferation and inflammatory cytokine production such as interleukin 22, which are hallmark features of many skin conditions (Boniface et al., 2005). As shown in the pathway enrichment chart (Figure 4B), chromatin organization and enzyme modification involve epigenetic regulation via histone modification (Bannister and Kouzarides 2011). Epigenetic changes are increasingly recognized in conditions such as psoriasis and skin aging (Mateu-Arrom and Puig, 2023; Orioli and Dellambra, 2018). Other signal transduction and growth factor pathways [epidermal growth factor (EGF), transforming growth factor beta (TGFβ)] are vital for keratinocyte function, wound healing, and skin barrier integrity (O’Kane and Ferguson, 1997; Stoll et al., 1997). Dysregulation is linked to fibrotic skin diseases, chronic ulcers, and skin aging. Inflammatory skin conditions, skin cancers, and wound-healing disorders can be associated with this pathway (Feng et al., 2022).

The PPI network and pathway enrichment analyses provided a systems-level overview of potential mechanisms of cyanotoxin-induced dermal toxicity and generated hypothetical models of key protein targets and pathways for future experimental validations, which are more targeted and conclusive. This includes the Notch, MECP2, and p75NTR pathways in skin cell homeostasis and survival.

Implications of the findings toward freshwater management policy and strategies

The significant levels of MC-RR in the study sites and the observed cytotoxic effects on human skin keratinocytes highlight the importance of reexamining current water management policy. Current guidelines mainly focus on the ingestion route via drinking water. Our findings showed that dermal exposure to cyanotoxin-contaminated recreational freshwater might pose an underestimated health risk, suggesting the necessity to include an extensive policy that considers the dermal exposure route in formulating the water quality standards.

This study is a real-world case scenario for spatiotemporal profiling to analyze the patterns and trends of cyanotoxin distribution, which recommends predictive management systems such as weather or seasonal tracking, a cyanobacterial bloom prediction model, and establishment of intervention triggers as early warning systems (Chang et al., 2024). The findings also highlight the necessity for nutrient management, which is the primary factor in bloom formation (Chang et al., 2024). Furthermore, our network toxicology analyses demonstrated the mechanistic effects of cyanotoxins on human skin, which supports exposure limits and enables health risk assessments.

Following our findings, the local city municipal council has undertaken several short-term strategies at the study site, including enforcement of public health precautionary measures at the freshwater lake to restrict recreational water activities such as fishing and swimming. These immediate actions address the impact of the studies in environmental stewardship and evidence-based water management policies to mitigate cyanotoxin-associated public health risks.

Conclusions

This study provided insights into cyanotoxin profiling at a recreational freshwater source and found that MC-RR was the most prevalent MC isotype in the water body. The CCK-8 cell viability assay revealed substantial cytotoxicity caused by MC-enriched water samples, indicating that MCs cause significant dermal toxicity. However, membrane permeabilization caused by the cyanotoxins may not be the primary mechanism for the observed dermal toxicity. Network toxicology revealed the probability of dermal toxicity mechanisms caused by cyanotoxins. The PPI networks displayed three distinguished clusters confined to vascular and inflammatory signaling pathways, epigenetic regulation, and immune responses. These overlapping target proteins were significantly enriched in Notch signaling pathways, MECP2-related pathways, cell cycle regulation, STAT3 signaling, and chromatin organization, which are vital in skin development and homeostasis. The findings offered a different perspective of potential yet neglected risks of cyanotoxin exposure through the dermal route in recreational freshwater. Our results also demonstrated differential cytotoxicity and membrane permeabilization effects from the different cyanotoxins. Our findings highlight the importance of evidence-based water management policies for mitigating cyanotoxin-associated health risks.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and available upon request from corresponding author.

Author contributions

Vivian Jia Weng Chan (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing—original draft, Writing—review & editing), Lee Wei Chang (Funding acquisition, Project administration, Visualization, Writing—review & editing), Faddrine Holt Jang (Writing—review & editing), Mohd Syamil Razak (Data curation, Formal analysis), and Michelle Khai Khun Yap (Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing)

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Conflicts of interest

The authors disclose that there are no competing interests.

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