

Seasonal dynamics of microcystins in Yanghe Reservoir, Northern China, and their responses to environmental factors

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Abstract

Although laboratory studies on *Microcystis* toxins are extensive, they often fail to fully explain the high variability observed in natural cyanobacterial blooms. This study investigated the seasonal dynamics of microcystins (MCs) and their environmental drivers in Yanghe Reservoir, a critical drinking water source in Northern China. From January to December 2023, water samples from five sites were analyzed for MC-LR, MC-RR, and MC-YR variants, alongside comprehensive physicochemical and biological parameters. The results demonstrated that total MC concentrations ranged from 0.24 to 22.61 µg/L, peaking during the summer months. Spatially, elevated toxin levels were predominantly observed in the western estuary. Dissolved MCs exhibited positive correlations with phosphorus and chlorophyll-a, but correlated negatively with nitrogen forms. Notably, the dissolved peaks of MC-LR and MC-YR preceded their intracellular maxima, a phenomenon that deviates from traditional laboratory-based models. With maximum dissolved concentrations (22.61 µg/L) significantly exceeding the World Health Organization (WHO) provisional guideline of 1.0 µg/L, this study highlights a severe public health risk. These findings underscore the urgent necessity for targeted nutrient control and enhanced, high-frequency monitoring strategies in this critical reservoir ecosystem.

1 INTRODUCTION

The global proliferation of cyanobacteria (blue-green algae) in freshwater ecosystems remains a pressing environmental and public health concern. These photosynthetic organisms frequently form dense, nuisance blooms in lakes, reservoirs, and rivers worldwide, a phenomenon exacerbated by anthropogenic eutrophication and climate change (Ballot et al. 2003, de Figueiredo et al. 2004, Fleming et al. 2002, Hotto et al. 2008, Makarewicz et al. 2009). Beyond ecological disruption, many cyanobacterial species produce a diverse array of potent secondary metabolites collectively known as cyanotoxins. Among these, microcystins (MCs) are the most frequently detected and extensively studied class due to their severe toxicity, chemical stability, and potential for bioaccumulation (Azevedo et al. 2002, Garcia et al. 2010, Wang et al. 2008, Wang et al. 2025, Zhang et al. 2009). To date, over 300 structural variants of microcystins have been identified, with microcystin-LR (MC-LR) recognized as one of the most toxic and globally prevalent (Hoerstmann et al. 2025, Kotak et al. 1995). Acute exposure to MCs, primarily through the ingestion of contaminated water or aquatic food sources, can induce severe hepatotoxicity, gastroenteritis, and, in extreme scenarios, fulminant liver failure and death (Carmichael 2001, Chen et al. 2016). Furthermore, chronic, low-dose exposure has been linked to the promotion of liver tumors and potential neurodegenerative consequences (Hu et al. 2016, Zegura et al. 2011). In recognition of these profound risks, the World Health Organization (WHO) established a provisional guideline value of 1.0 µg/L for MC-LR in drinking water (WHO, 2020).

In China, the management of cyanobacterial blooms and their associated toxins is a matter of paramount national concern. This is driven by the country's extensive reliance on surface waters for municipal supply, agriculture, and aquaculture, coupled with widespread eutrophication across numerous major watersheds (Ma et al. 2016, Qin et al. 2019, Wang et al. 2026, Zhang et al. 2018).

Yanghe Reservoir, located in Qinhuangdao City, Hebei Province, serves as a strategic water resource with a total storage capacity of 353 million m³. It fulfills multiple critical functions, including providing municipal drinking water for over one million residents, flood control, irrigation, and hydropower generation (Li et al. 2022). Since the 1980s, rapid socio-economic expansion within its catchment has resulted in increased nitrogen and phosphorus loading from agricultural runoff, domestic sewage, and other anthropogenic inputs. Consequently, the reservoir has experienced progressive eutrophication, characterized by recurrent, large-scale cyanobacterial blooms dominated by *Microcystis* spp. during the warm summer and autumn seasons (Hui et al. 2014). These blooms not only degrade water quality and ecological integrity but also pose a direct, acute threat to the safety of the drinking water supply, particularly because conventional water treatment processes (e.g., coagulation, sedimentation, filtration, and standard chlorination) are largely ineffective at eliminating dissolved microcystins (Jiao et al. 2026, Xue et al. 2023).

Previous limnological research on Yanghe Reservoir has predominantly focused on general physicochemical parameters, nutrient budgets, and phytoplankton community succession (Li et al. 2010). However, a systematic, annual-scale investigation specifically targeting the occurrence, spatial distribution, and environmental drivers of microcystin pollution is currently lacking. Such detailed knowledge is imperative for accurate ecological risk assessment and the formulation of effective mitigation strategies.

This study aims to address this critical knowledge gap through a comprehensive, high-frequency field investigation. Our primary objectives were to: (1) characterize the seasonal and spatial variations of three major MC variants (MC-LR, MC-RR, and MC-YR) in both dissolved and intracellular forms; (2) analyze concurrent dynamics of the phytoplankton community, emphasizing toxic cyanobacteria; (3) identify key environmental variables (physical, chemical, and biological) regulating MC production and persistence; and (4) evaluate potential human health risks by comparing measured toxin concentrations against established international safety guidelines. By integrating high-resolution toxin profiling with environmental monitoring, this study provides a holistic understanding of MC dynamics in a large, eutrophic drinking water reservoir and offers evidence-based insights for sustainable watershed management.

2 MATERIALS AND METHODS

2.1 Study Area and Sampling Sites

Yanghe Reservoir has a total storage capacity of 353 million m³ and an average depth of 5.7 m. Five sampling sites were selected to represent different hydrological and functional zones (Fig. 1). Site 1 (39.983237°N, 119.212804°E) and Site 2 (39.987182°N, 119.203878°E) are located at the agricultural diversion outlet. Sites 3 (40.00842°N, 119.168945°E) and Site 4 (39.998624°N, 119.255806°E) represent the inlet and outlet of the reservoir, respectively. Site 5 (40.000136°N, 119.214779°E) is situated in the pelagic zone (or center) of the reservoir.

2.2 Sample Collection and Field Measurements

A comprehensive sampling campaign was conducted monthly from January to December 2023. During periods of frequent bloom occurrence (April, May, July, August), sampling frequency was increased to twice per month to better capture rapid dynamics.

Water Samples: Integrated water column samples were collected using a 5-L Ruttner water sampler at each site. For nutrient and toxin analysis, composite samples were created by mixing equal volumes of water collected from the surface (0.5 m depth), middle (mid-depth), and bottom (0.5 m above sediment). For phytoplankton analysis, separate 1-L surface water samples were collected and immediately preserved with Lugol's iodine solution (1% final concentration).

Cyanobacterial Bloom Samples: Surface scum or high-biomass water was collected using a plankton net (200 μm mesh) when visible blooms were present. The concentrated material was transferred to clean polyethylene bottles.

In Situ Parameters: Water temperature (WT), pH, dissolved oxygen (DO), and electrical conductivity (EC) were measured on-site at each sampling point using a calibrated multi-parameter water quality sonde (YSI 6600 V2, USA). Water transparency was measured using a Secchi disk (SD).

2.3 Laboratory Analyses

2.3.1 Nutrient and Chlorophyll-a Analysis

All water samples for chemical analysis were kept on ice in the dark during transport and processed within 24 hours. Concentrations of total nitrogen (TN), total phosphorus (TP), nitrate-nitrogen (NO_3^- -N), ammonium-nitrogen (NH_4^+ -N), dissolved total nitrogen (DTN), dissolved total phosphorus (DTP), and phosphate (PO_4^{3-}) were determined according to standard methods (State Environmental Protection Administration of China, 2002). Chlorophyll-a (Chl-a) was extracted from water samples filtered onto Whatman GF/C filters using 90% hot ethanol and quantified spectrophotometrically (Lorenzen 1967).

2.3.2 Phytoplankton Analysis

For phytoplankton enumeration and identification, preserved water samples were settled in 50-mL Utermöhl chambers for at least 48 hours. Phytoplankton were examined, identified to genus or species level where possible, and counted using an inverted microscope (Olympus IX73) at 400x magnification. Cell counts were expressed as cells per liter (cells L^{-1}). The biovolume of dominant species was estimated using standard geometric shapes (Hillebrand et al. 1999).

2.3.3 Microcystin Extraction and Quantification

Intracellular Microcystins (IMCs): Lyophilized bloom material or filters containing phytoplankton were extracted three times with 10 mL of 75% (v/v) aqueous methanol containing 0.1% (v/v) trifluoroacetic acid (TFA) by sonication for 15 minutes followed by centrifugation. The combined supernatants were

evaporated to near dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 1 mL of 30% methanol and purified using solid-phase extraction (SPE) cartridges (Oasis HLB, 60 mg, Waters, USA). Microcystins were eluted with 3 mL of 90% methanol containing 0.1% TFA, dried under nitrogen, and reconstituted in 500 µL of HPLC-grade methanol for analysis.

Dissolved Microcystins (DMCs): For dissolved toxins, 500 mL of filtered (GF/C) water samples were acidified to pH 3 with formic acid and loaded onto preconditioned SPE cartridges (Oasis HLB). The elution and reconstitution steps were identical to those for IMCs.

HPLC-MS/MS Analysis: Quantification of MC-LR, MC-RR, and MC-YR was performed using an Agilent 1290 Infinity II HPLC system coupled to an Agilent 6470 Triple Quadrupole mass spectrometer. Separation was achieved on a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm) maintained at 40°C. The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient elution program was: 0–2 min, 25% B; 2–10 min, 25–70% B; 10–12 min, 70–100% B; 12–14 min, 100% B; 14–14.5 min, 100–25% B; followed by 5.5 min re-equilibration. The flow rate was 0.3 mL/min, and the injection volume was 5 µL. The mass spectrometer was operated in positive electrospray ionization (ESI+) mode with multiple reaction monitoring (MRM). The optimized MS parameters were: capillary voltage, 3500 V; nebulizer gas pressure, 45 psi; drying gas flow, 11 L/min; drying gas temperature, 300°C. The MRM transitions used for quantification were: MC-RR m/z 519.8 → 135.0 (collision energy, CE 70 V); MC-YR m/z 1045.5 → 135.0 (CE 85 V); and MC-LR m/z 995.5 → 135.0 (CE 80 V). Quantification was performed using external calibration curves prepared from certified reference standards (≥95% purity, Wako Pure Chemical Industries, Japan). The method detection limits (MDL) for MC-LR, -RR, and -YR in water were 0.01, 0.015, and 0.02 µg/L, respectively, and 0.05 µg/g dry weight for bloom samples. Recoveries for spiked samples ranged from 85% to 105%.

2.4 Data and Statistical Analysis

All data are presented as the mean ± standard deviation (SD) of duplicate measurements for each sample (n = 5 sites). Temporal trends were visualized using time-series plots. Differences in MC concentrations among seasons and sites were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The relationships between microcystin concentrations (for each variant and pool) and environmental variables were explored using Pearson correlation analysis. To identify the key environmental drivers of total MC concentration, multiple linear regression (stepwise method) was performed. All statistical analyses were conducted using R software (version 4.3.0, R Core Team, 2023), and significance was accepted at $p < 0.05$.

3 Results

3.1 Spatiotemporal Dynamics of Environmental Conditions

The physicochemical and biological characteristics of Yanghe Reservoir exhibited pronounced seasonal patterns throughout 2023 (Table 1). The surface water remained consistently alkaline, with pH values

ranging from 7.31 in winter to a peak of 9.41 in late summer, the latter driven by intense photosynthetic carbon fixation during cyanobacterial blooms. Water temperature (WT) followed a typical temperate seasonal trajectory, dropping to a minimum of 1.9°C in January and reaching a maximum of 29.0°C in July and August. Dissolved oxygen (DO) concentrations averaged 11.48 mg/L across all sites, displaying temporary daytime supersaturation (up to 18.64 mg/L) during bloom peaks, as well as localized bottom-water oxygen depletion during stratification events.

Nutrient dynamics reflected a characteristically eutrophic system with substantial temporal variability. Total nitrogen (TN) concentrations ranged from 0.27 to 5.69 mg/L, with noticeable concentration surges during spring runoff and high-intensity summer rainfall events. Total phosphorus (TP) concentrations ranged from 0.02 to 0.38 mg/L (mean: 0.08 mg/L), with organic phosphorus (OP) accounting for a major fraction (mean: 0.07 mg/L). The molar TN:TP ratio fluctuated dynamically between 7.6 (October) and 124.6 (April). Throughout the spring and early summer (January to July), the TN:TP molar ratio consistently exceeded the Redfield stoichiometric ratio (16:1), indicating potential phosphorus limitation for phytoplankton growth during the initial bloom development period.

Chlorophyll-a (Chl-a) concentrations, serving as a proxy for total phytoplankton biomass, exhibited extreme seasonal variation, ranging from 7.34 µg/L in winter to a spatial maximum of 346.63 µg/L recorded at the western estuary (Site 1 and Site 2) in August during a massive bloom event (reservoir-wide mean: 39.07 µg/L). Secchi depth (SD) inversely mirrored Chl-a trends, dropping to a minimum of 0.26 m during peak bloom conditions in late summer.

Table 1 Summary statistics (minimum, first quartile, median, mean, third quartile, and maximum) of the physical, chemical, and biological variables measured in Yanghe Reservoir.

variables	minimum	1 st quartile	median	mean	3 rd quartile	maximum
DO(mg/L)	6.15	9.87	12.14	11.48	13.09	18.64
pH	7.31	8.1	8.33	8.4	8.73	9.41
SD(m)	0.26	0.43	0.58	0.82	1.14	1.88
Temperature(°C)	1.9	8.9	15.8	16.18	24.2	29
SS(mg/L)	5	16.74	23.96	25.01	31.5	89.6
TN(mg/L)	0.27	1.19	1.5	1.71	1.84	5.69
DN(mg/L)	0.12	0.75	1.16	1.3	1.5	5.55
NH ₄ ⁺ -N(mg/L)	0	0.05	0.14	0.16	0.2	0.88
NO ₃ ⁻ -N(mg/L)	0.02	0.04	0.12	0.25	0.43	0.77
OP(mg/L)	0.01	0.03	0.06	0.07	0.09	0.22
DTP(mg/L)	0	0.01	0.01	0.02	0.03	0.15
TP(mg/L)	0.02	0.04	0.06	0.08	0.1	0.38
COD _{Mn} (mg/L)	1.66	2.95	3.69	4.13	4.89	21.75
CHL-a(μg/L)	7.34	17.62	31.97	39.07	44.76	346.63

3.2 Phytoplankton Community Composition and Succession

Phytoplankton community structure underwent a clear seasonal succession (Fig. 2). From January to March, the community was dominated by diatoms (Bacillariophyta) and chrysophytes, which are typical of cooler, nutrient-rich waters. A sharp transition occurred in April, as water temperatures rose above 15°C. From April through October, cyanobacteria overwhelmingly dominated the phytoplankton, constituting 93% to 100% of the total cell density. The cyanobacterial community itself was dominated by two species of *Microcystis*: *M. aeruginosa* and *M. flos-aquae*. The highest cyanobacterial cell density recorded was 9.68×10^6 cells L⁻¹ on August 15th at the central site 5, coinciding with the annual maximum in Chl-a concentration (95.97 μg/L). Following this peak, a gradual decline in cyanobacterial biomass was observed through autumn, with a resurgence of diatoms and other algae in late autumn and winter.

3.3 Temporal and Spatial Patterns of Microcystins

3.3.1 Dissolved Microcystins (DMCs)

All three targeted microcystin variants (MC-LR, MC-RR, and MC-YR) were quantitatively detected in the dissolved phase, though they exhibited distinct temporal trajectories and spatial patterns across Yanghe

Reservoir (Fig. 3). Toxin occurrence initiated in mid-to-late spring, with MC-LR being the first variant detected above the method detection limit in late April (reservoir-wide mean: 0.33 µg/L). Dissolved MC-RR and MC-YR became detectable approximately two weeks later, in mid-May.

The annual dynamics of total DMCs displayed a pronounced multi-peak profile, comprising a minor elevation in June (mean: 1.97 µg/L), a substantial surge in August (mean: 8.26 µg/L), and an annual maximum in October (mean: 19.84 µg/L) (Fig. 3). Notably, the peak timing differed significantly among the three variants:

MC-LR and MC-YR reached their primary dissolved maxima in August and September (reservoir-wide mean peaks of 4.21 ± 1.05 µg/L and 1.18 ± 0.32 µg/L, respectively) before gradually declining. Conversely, MC-RR demonstrated a delayed accumulation pattern, surging dramatically in October to reach a maximum concentration of 18.23 µg/L (reservoir-wide mean: 18.06 ± 3.12 µg/L), where it accounted for over 90% of the total dissolved toxin pool.

Spatially, DMC concentrations exhibited a clear gradient. Sites 1 and Site 2 in the western agricultural estuary consistently recorded 20%–40% higher DMC concentrations than the pelagic site (Site 5) and the dam outlet site 4 during the early and mid-bloom stages (June–September). However, during the late-autumn MC-RR surge in October and November, elevated dissolved toxin levels expanded across the entire reservoir, with peak total DMC concentrations exceeding 24.0 µg/L at Site 4 (Fig. 3). Toxin concentrations dropped rapidly in December, falling below 0.70 µg/L at all monitoring stations as water temperatures declined below 5°C.

3.3.2 Intracellular Microcystins (IMCs)

The cellular toxin quotas and variant compositions of intracellular microcystins (IMCs, expressed as pg/cell) exhibited distinct seasonal dynamics that shared key similarities with, yet diverged significantly from, the dissolved toxin pool (Fig. 4). Intracellular MC-LR was first detected in late April across all monitoring stations at low levels (reservoir-wide mean: 0.02 pg/cell), co-occurring with the initial rise of *Microcystis* cell density. The seasonal progression of total IMC quotas revealed a shift in dominant variants and cellular toxicity over time:

April to September (MC-LR Dominance): During the early-to-mid bloom development phase, MC-LR was the predominant intracellular variant, following an abundance hierarchy of MC-LR > MC-RR > MC-YR. The mean cellular MC-LR quota reached a localized peak in September (0.03 ± 0.01 pg/cell).

October Peak (MC-RR Surge): A striking transition occurred in October, coinciding with the senescence phase of the primary bloom. Total intracellular toxin quotas surged to an annual maximum across all sites, driven overwhelmingly by a sharp increase in intracellular MC-RR. The mean cellular quota of MC-RR reached 0.11 ± 0.03 pg/cell in October (accounting for >80% of total IMCs), with the highest individual site quota recorded at Site 4 (0.18 pg/cell; Fig. 4).

Late-Autumn and Winter Persistence: Following the October peak, intracellular MC-RR and MC-LR concentrations decreased rapidly in November (mean total IMCs: 0.05 pg/cell) and dropped below method detection limits by December. Interestingly, trace levels of intracellular MC-YR (0.01 pg/cell) persisted into January of the following year, long after MC-LR and MC-RR became undetectable.

Spatially, intracellular toxin quotas displayed notable heterogeneity. During the October peak, Site 4 (dam outlet) and Site 5 (pelagic zone) exhibited markedly higher cellular MC-RR quotas (0.18 pg/cell and 0.12 pg/cell, respectively) compared to the western estuary sites (Sites 1 and Site 2; ~0.065–0.08 pg/cell), reflecting spatial differences in cyanobacterial cell physiological status and biomass accumulation prior to overwintering.

3.3.3 Relationship between DMCs and IMCs

A comparative analysis revealed a complex relationship between the dissolved (DMC) and intracellular (IMC) pools. For MC-RR, the annual cycles of DMC-RR and IMC-RR were remarkably synchronous, with strong positive correlation ($r = 0.88$, $p < 0.001$). In contrast, for MC-LR and MC-YR, the dissolved peaks in September preceded the intracellular peaks. This asynchrony suggests that the release of these variants into the water column is not a simple, immediate consequence of cell lysis following biomass peaks, but may involve active excretion or be strongly modulated by environmental factors like rainfall and flushing.

3.4 Correlations between Microcystins and Environmental Factors

3.4.1 Dissolved Microcystins (DMCs)

Correlation analysis revealed distinct environmental associations for each DMC variant (Fig. 5). DMC-LR exhibited significant positive correlations with water temperature (WT, $r = 0.76$, $p < 0.01$), total phosphorus (TP, $r = 0.71$, $p < 0.01$), organic phosphorus (OP, $r = 0.69$, $p < 0.01$), and chlorophyll-a (Chl-a, $r = 0.82$, $p < 0.001$). It showed significant negative correlations with total nitrogen (TN, $r = -0.58$, $p < 0.05$), nitrate ($\text{NO}_3\text{-N}$, $r = -0.62$, $p < 0.05$), and Secchi depth (SD, $r = -0.73$, $p < 0.01$). DMC-YR showed a similar but weaker pattern of correlations. DMC-RR demonstrated the strongest positive correlation with phosphorus forms (TP: $r = 0.79$, $p < 0.001$; OP: $r = 0.79$, $p < 0.001$) and a strong positive correlation with pH ($r = 0.72$, $p < 0.01$). It was negatively correlated with dissolved oxygen (DO, $r = -0.66$, $p < 0.05$) and the TN:TP ratio ($r = -0.70$, $p < 0.01$).

3.4.2 Intracellular Microcystins (IMCs)

The correlations for intracellular toxins were generally weaker than for dissolved toxins (Fig. 6). IMC-RR showed the strongest pattern, with positive correlations with TP ($r = 0.75$, $p < 0.001$) and pH ($r = 0.70$, $p < 0.01$), and a negative correlation with DO ($r = -0.61$, $p < 0.05$). IMC-LR and IMC-YR showed few strong correlations with the measured nutrients, but IMC-YR was modestly correlated with DO.

4 Discussion

4.1. Drivers of Microcystin Seasonality and Spatial Distribution

The pronounced seasonal pattern of microcystins in Yanghe Reservoir, with peak concentrations in summer and autumn, aligns with observations from eutrophic lakes worldwide (Graham et al. 2010). This pattern is primarily driven by water temperature, which regulates the growth rates of *Microcystis* spp. The optimal growth temperature for many *Microcystis* strains is between 25°C and 30°C (Davis et al. 2009), which corresponds perfectly with the period of highest MC concentrations in our study (July–October). The significant positive correlation between DMC-LR and water temperature underscores the thermal control on both biomass accumulation and potentially toxin production (Xu et al. 2021, Zhang et al. 2017).

The spatial gradient, with higher MC levels in the western estuary (Sites 1 and Site 2), is likely a consequence of differential nutrient loading. This area receives inflow from agricultural drains, which are typically rich in phosphorus from fertilizers. The strong positive correlations between both DMC and IMC concentrations (especially MC-RR) and various phosphorus fractions (TP, OP) provide compelling evidence that phosphorus availability is a key driver of microcystin production in Yanghe Reservoir. This aligns with the observed TN:TP ratios often exceeding the Redfield ratio, indicating a system where phosphorus is the primary limiting nutrient for phytoplankton growth. Under P-limitation, increased P availability can lead to disproportionate increases in cyanobacterial biomass and their toxin production (Couto et al. 2015, Vézic et al. 2002, Zhang et al. 2023).

The negative correlations between dissolved MCs (particularly MC-LR) and various nitrogen forms were unexpected but not unprecedented (Kieley et al. 2023). Some studies suggest that under high nitrogen conditions, *Microcystis* may allocate more resources to growth rather than toxin synthesis (Gobler et al. 2016). Alternatively, the negative correlation could reflect the seasonal drawdown of inorganic nitrogen by the massive summer blooms, creating an inverse relationship between bloom intensity/N-depletion and MC accumulation (Major et al. 2018).

4.2. Congener-Specific Dynamics and the Dissolved-Intracellular Paradox

A key finding of this study is the divergent behavior of MC congeners. The consistent lag of MC-RR peaks (both dissolved and intracellular) relative to MC-LR and MC-YR has been previously documented in laboratory settings (Wei et al. 2006). This lag may originate from variations in biosynthetic pathways or cellular regulation among the variants. MC-RR, which lacks the leucine residue present in MC-LR, might be synthesized or excreted under distinct physiological conditions, potentially appearing later in the bloom cycle as cells encounter environmental stress.

The temporal asynchrony between dissolved and intracellular peaks for MC-LR and MC-YR challenges the conventional conceptual model derived from laboratory batch cultures, where dissolved toxin peaks invariably trail intracellular peaks due to progressive cell lysis (Gurbuz et al. 2009, Watanabe et al. 1992). Conversely, our field data reveal an inverse pattern. We propose two interconnected mechanisms to explain this phenomenon:

Hydrological Forcing: The primary dissolved peaks observed in September coincided with, or immediately followed, significant precipitation and surface runoff events (Newell et al. 2024). This sudden hydrological influx may have diluted the intracellular toxin pool within surface scums while simultaneously advecting dissolved toxins from littoral degradation zones or resuspended sediments into the pelagic sampling sites.

Population Asynchrony and Active Release: Within natural cyanobacterial blooms, individual cells occupy diverse growth phases and physiological states. While a subset of the population undergoes lysis, other cells actively proliferate and synthesize toxins. Emerging evidence indicates that *Microcystis* can actively release portions of its synthesized toxins independent of cell lysis, potentially functioning as an allelopathic mechanism or a form of intercellular signaling (Zilliges et al. 2011). Consequently, the peak in dissolved MC concentration may reflect the optimal product of the abundance of actively secreting cells and their respective release rates, rather than strictly mirroring the average intracellular toxin quota.

4.3. Environmental Correlates and Implications for Toxin Production

The strong link between MCs and phosphorus, and the contrasting relationship with nitrogen, provides critical information for management. Efforts to control MC risk in Yanghe Reservoir should prioritize the reduction of phosphorus loading from the watershed, particularly from non-point agricultural sources in the western catchment. The negative correlation of MC-RR with DO is ecologically intuitive; as blooms senesce and massive biomass decomposes, DO is consumed, and simultaneously, cell lysis releases stored MC-RR into the water.

The positive correlation of MC-RR with pH is notable. High pH during intense photosynthesis can favor the dominance of cyanobacteria like *Microcystis*, which possess efficient carbon-concentrating mechanisms (Zhang et al. 2024). This creates a positive feedback loop where blooms drive up pH, further favoring cyanobacteria and potentially influencing the congener profile of toxins produced.

4.4. Human Health Risk Assessment and Management Implications

The persistent detection of dissolved microcystins at concentrations far exceeding the WHO guideline value of 1.0 µg/L during the warm season represents a serious and ongoing threat to the safety of the drinking water supply for Qinhuangdao City. The maximum recorded concentration of 22.61 µg/L (total DMCs) in October highlights the potential for extreme exposure events. Conventional water treatment, as employed at the Yanghe waterworks, provides limited removal of dissolved toxins (Melaram et al. 2022). Therefore, reliance on treatment as the sole barrier is inadequate. A multi-barrier risk management approach is urgently required:

Source Control: Implement watershed management plans to reduce external nutrient (especially phosphorus) loading through improved agricultural practices, wastewater treatment upgrades, and riparian buffer restoration.

Enhanced Monitoring: Establish a real-time or near-real-time monitoring program for cyanobacterial biomass (using probes or satellite imagery) and MCs (using ELISA or LC-MS/MS) to provide early warning for water treatment plants.

Treatment Optimization: Pilot and implement advanced treatment technologies at the waterworks capable of removing dissolved MCs, such as activated carbon adsorption (powdered or granular), advanced oxidation processes (e.g., ozonation), or membrane filtration.

In-situ Management: Explore the feasibility of targeted, ecosystem-based in-lake measures, such as selective hypolimnetic aeration to prevent nutrient release from sediments, or artificial mixing to disrupt cyanobacterial buoyancy, with careful consideration of potential ecological side-effects.

5 CONCLUSION

This year-long, comprehensive study demonstrates that Yanghe Reservoir is subject to severe and recurrent toxic cyanobacterial blooms dominated by *Microcystis aeruginosa* and *M. flos-aquae*. These blooms result in the sustained production and release of microcystins, with MC-LR and MC-RR being the dominant variants. The dynamics of these toxins are governed by a complex interplay of environmental factors: seasonal warming initiates bloom development, while phosphorus availability appears to be a critical driver of both biomass and toxin production. The uncoupling of dissolved and intracellular toxin peaks for some congeners reveals limitations in applying laboratory culture models to natural systems and underscores the importance of hydrological events and population heterogeneity in regulating field-scale toxin dynamics.

Most critically, the concentration of dissolved microcystins frequently and substantially exceeded international health guidelines for drinking water, indicating a clear and present risk to public health. The reservoir's role as a vital drinking water source necessitates immediate and sustained action. The findings of this study strongly advocate for a shift from reactive to proactive management, centered on nutrient (P) reduction at the watershed scale, complemented by enhanced monitoring and upgrades to water treatment infrastructure. Future research should focus on elucidating the precise physiological and genetic mechanisms behind congener-specific production and release in response to environmental cues, which will further refine predictive models and management strategies.

Declarations

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Author contributions

Tao Han: Conceptualization, Methodology, Writing - original draft, Data curation.

Jinbang Cai: Investigation, Writing - review & editing.

Wei Zhuang: Validation, Writing - review & editing.

Zhen Yang: Writing - review & editing.

Guie Zhu: Investigation, Formal analysis.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval and consent to participate

Not applicable

Competing Interest

The authors declare that they have no competing interests.

Consent to publish

Not applicable.

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Figures

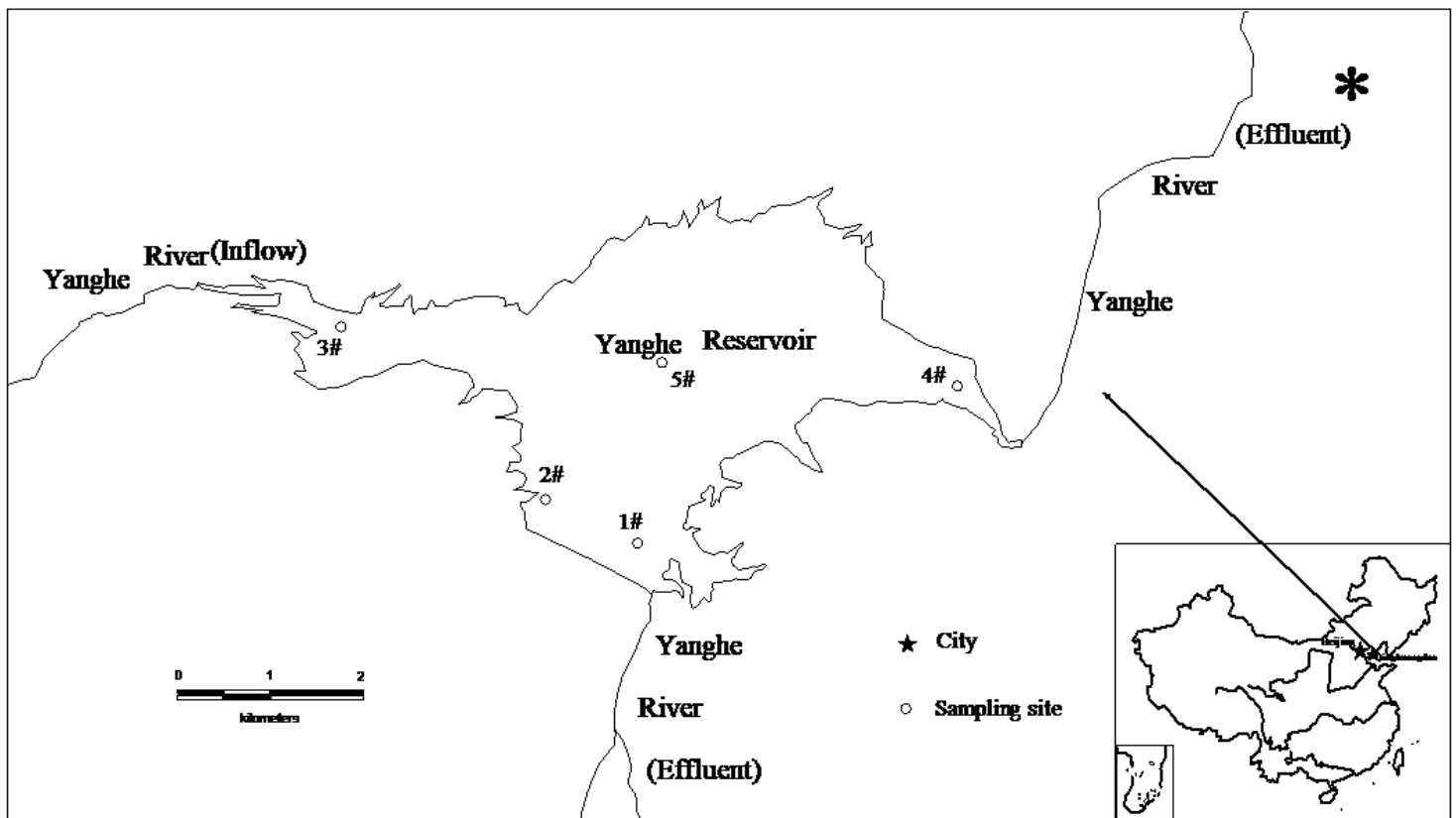


Figure 1

Five sampling sites of Yanghe Reservoir

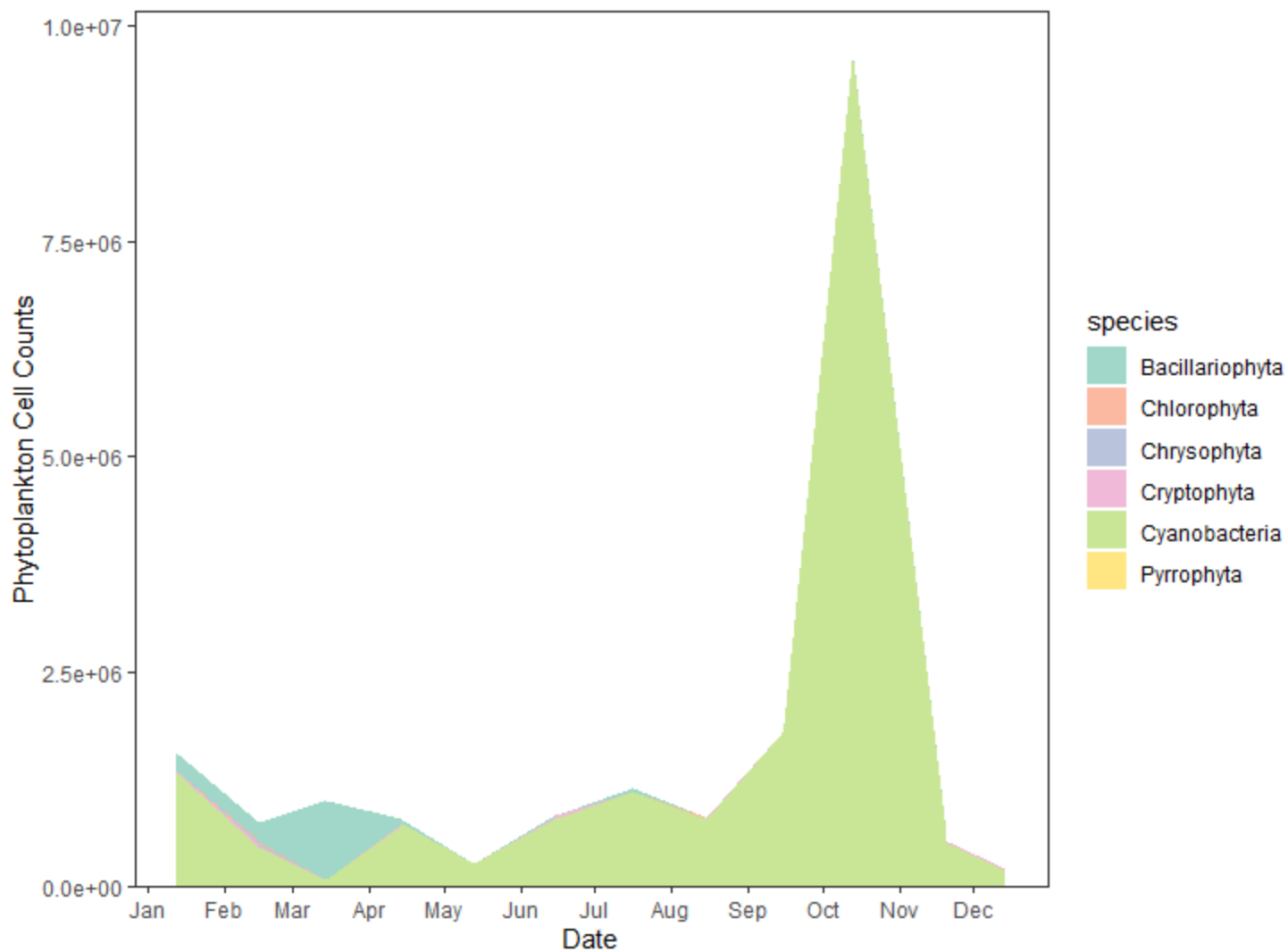


Figure 2

Phytoplankton Cell Counts

Monthly Variation of Dissolved MCs (2023)

MC Type MC-LR MC-YR MC-RR

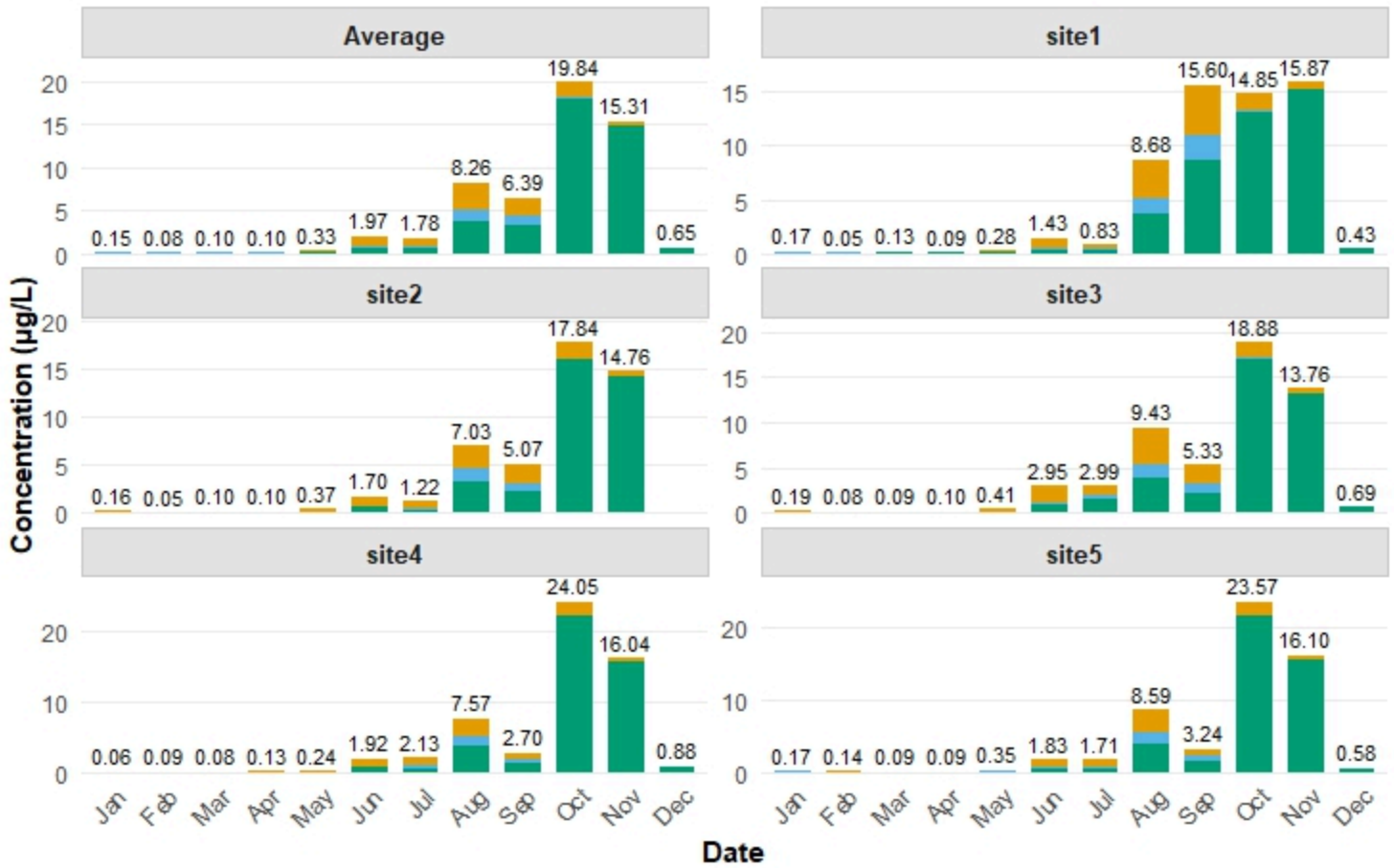


Figure 3

Seasonal changes in dissolved MC concentrations in the water column of Yanghe Reservoir

Monthly Variation of Intracellular MCS (2023)

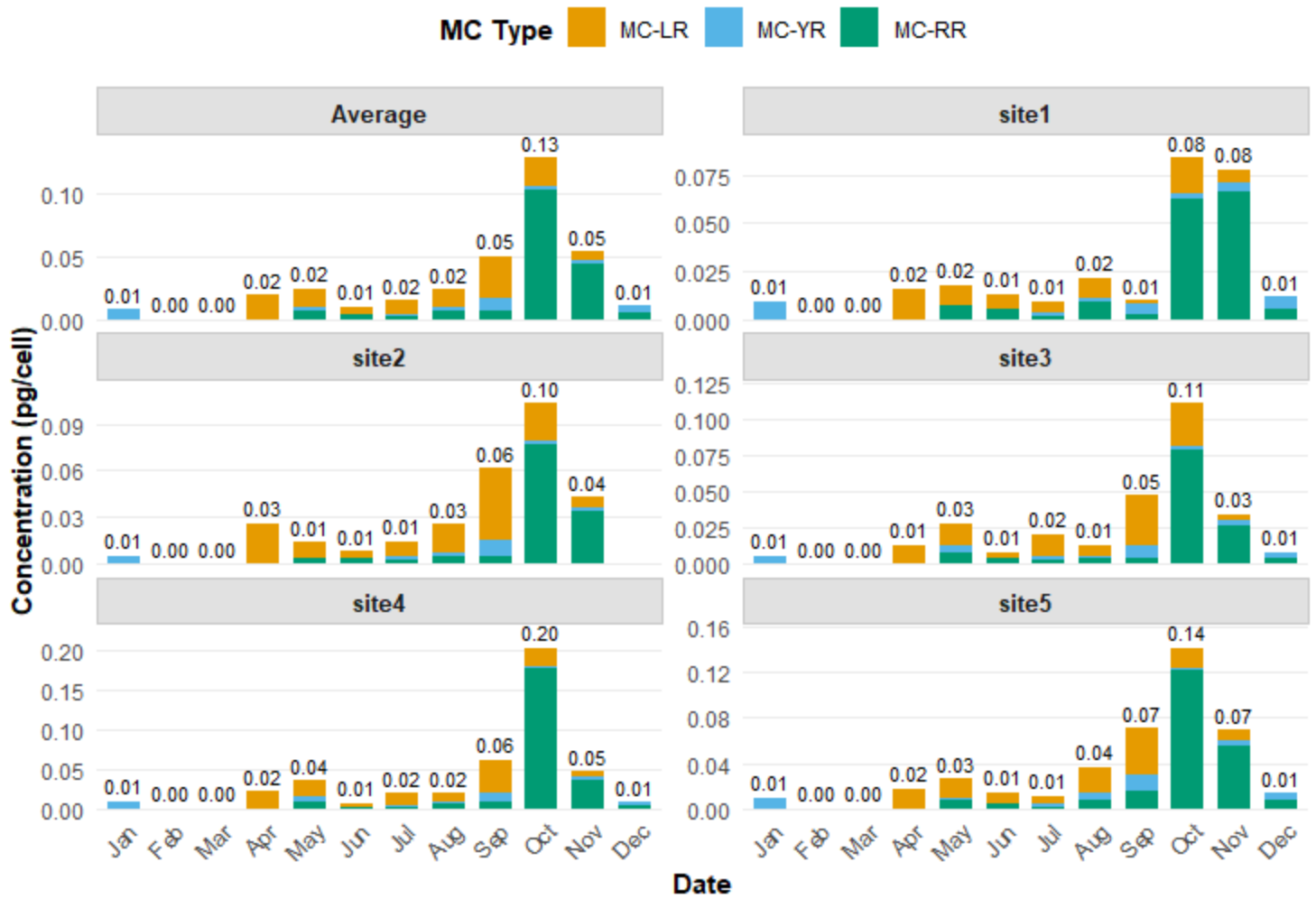


Figure 4

Seasonal changes in intracellular MC concentrations in the water column of Yanghe Reservoir

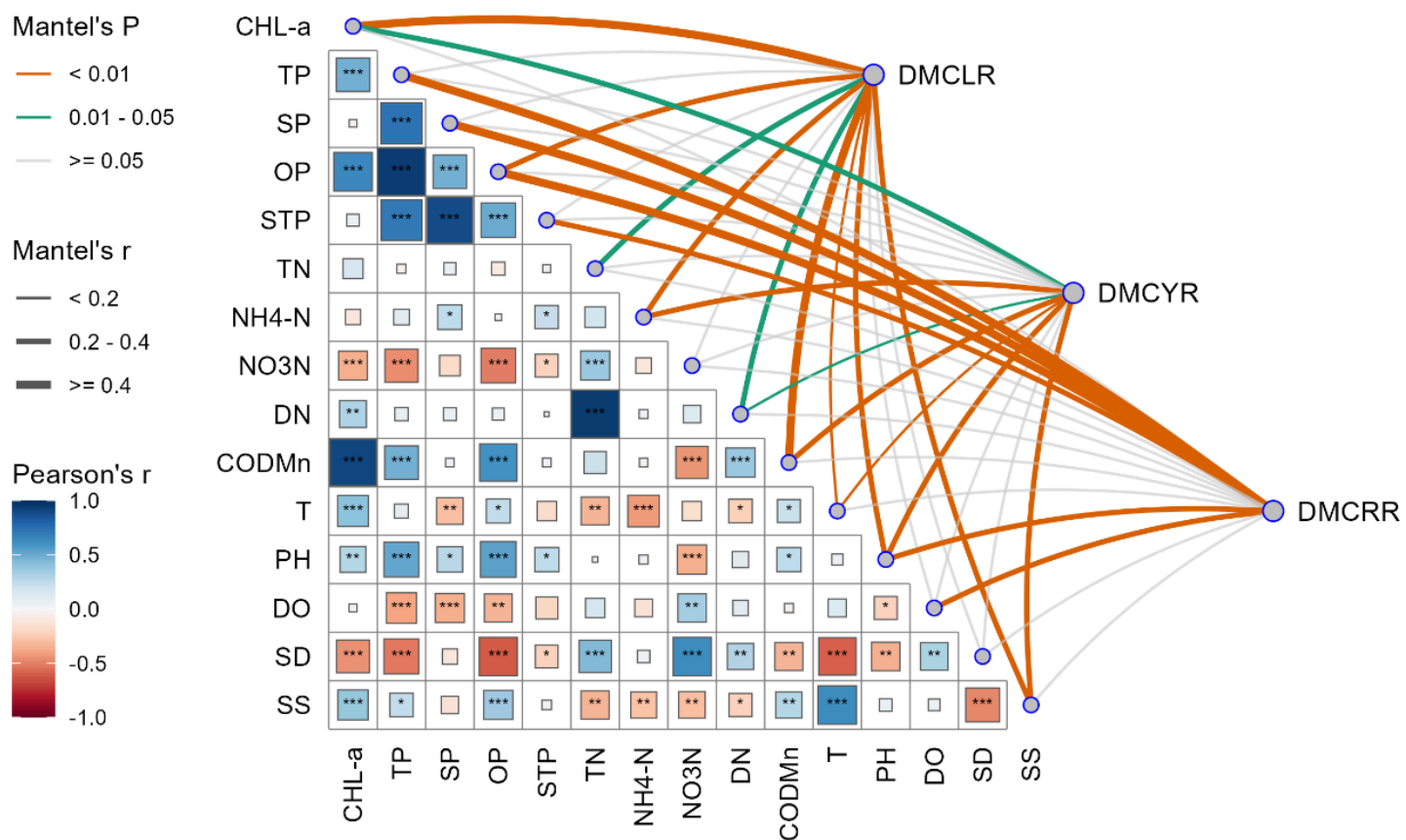


Figure 5

Correlation map between three dissolved microcystin (DMC) variants and environmental factors

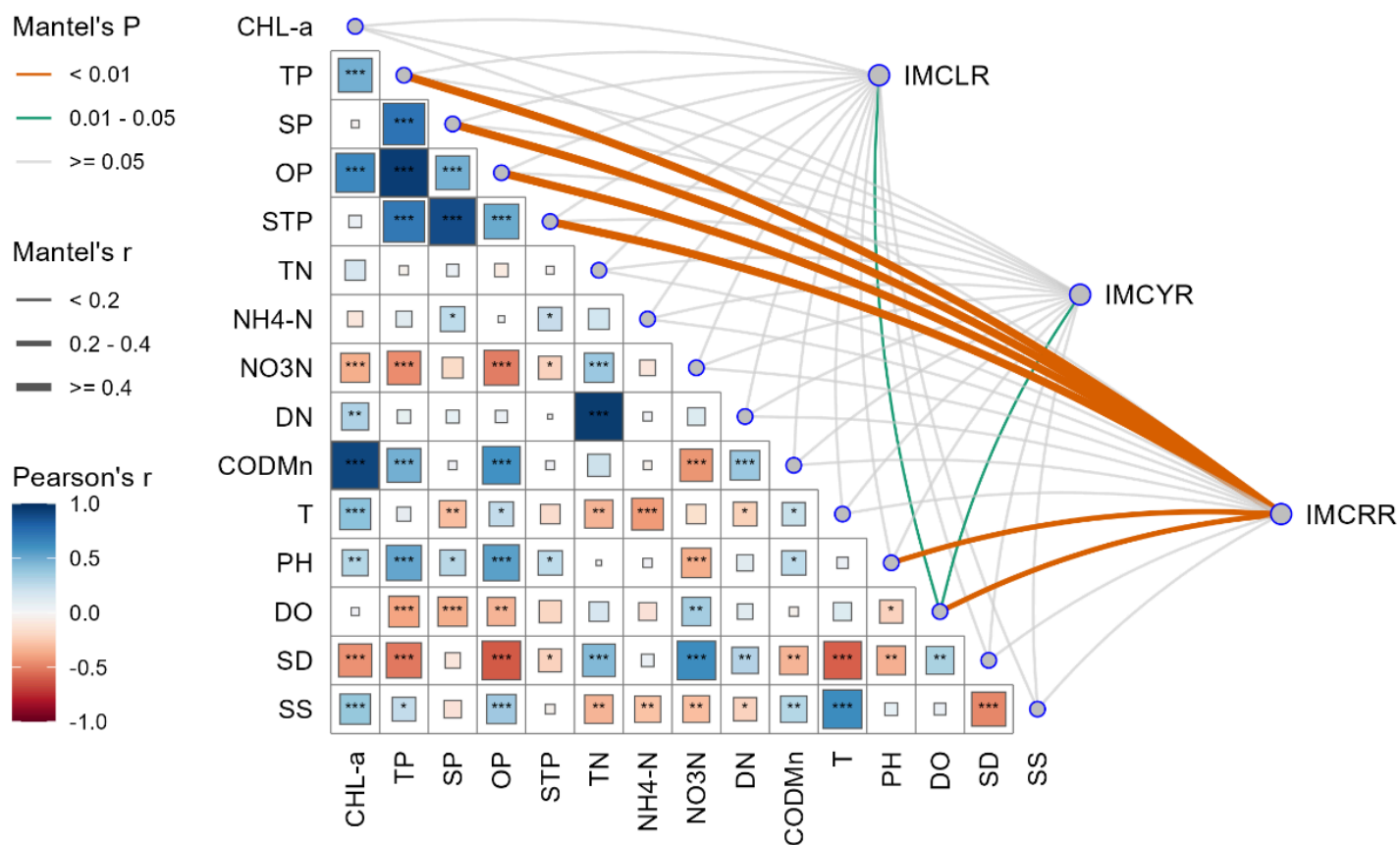


Figure 6

Correlation map between three Intracellular Microcystins (IMCs) variants and environmental factors

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