Science of the Total Environment 757 (2021) 143815

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Distribution of microcystins in environmental multimedia and their bioaccumulation characteristics in marine benthic organisms in the Geum River Estuary, South Korea

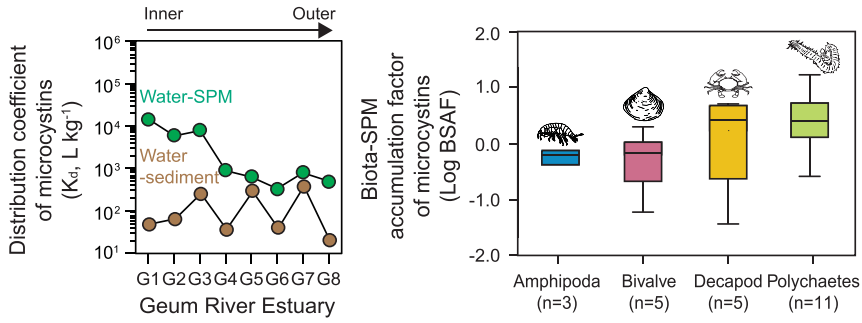
Mungi Kim [a,1], Dokyun Kim [b,1], Jaeseong Kim [c], Seongjin Hong [a,*], Kyung-Hoon Shin [b,*]

[a] Department of Ocean Environmental Sciences, Chungnam National University, Daejeon, Republic of Korea
[b] Department of Marine Sciences and Convergence Engineering, Hanyang University, Ansan, Republic of Korea
[c] Water & Eco-Bio Co., Ltd., Jungboo Building, Miryong-dong, Kunsan, Republic of Korea

HIGHLIGHTS

- Concentrations of MCs in water were strongly related to cyanobacterial density and biomass.
- MC-LR was the most abundant MC variant in environmental multimedia and organisms.
- Water-SPM distribution coefficients of MCs decreased from inner to outer estuary areas.
- The half-life of dissolved MCs in the estuary was longer than that of particulate MCs.
- Species-specific bioaccumulation of MCs occurred in tidal flat organisms.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 August 2020
Received in revised form 16 October 2020
Accepted 27 October 2020
Available online 19 November 2020

Guest Editor: Wenyou Hu

Keywords:
Microcystins
Distribution coefficient
Bioaccumulation
Estuary
HPLC-MS/MS

ABSTRACT

Spatio-temporal distributions and bioaccumulation characteristics of freshwater cyanobacterial toxins, such as microcystins (MCs) in the Geum River Estuary, South Korea, were investigated during summer. Environmental multimedia samples (water, suspended particulate matter (SPM), and sediments) and tidal flat organisms (polychaetes, decapods, amphipods, and bivalves) were collected from regions inside and outside of the estuary dam for MCs analysis. Phytoplankton communities in the Geum River (freshwater) and estuarine area (brackish water) were also analyzed in order to understand the relationship with MCs concentrations. Seasonal variation in the structure of phytoplankton communities was detected in the Geum River, with a relatively high density of Cyanophyta in summer. MC concentrations were strongly correlated to water temperature, chlorophyll *a*, and cyanobacterial density. MC-LR was the most abundant MC variants in environmental samples. Dissolved MCs remained for longer periods and were more widely distributed in the coastal environments compared to particulate MCs. The distribution coefficients between water and SPM ($K_{d\text{-}SPM}$) and between water and sediments ($K_{d\text{-}sediment}$) of MCs showed that the phase shift of MCs in the environmental samples occurred in the estuary. $K_{d\text{-}SPM}$ declined from the inside to outside regions of the estuary dam, and was mainly attributed to differences in the half-lives of MCs in dissolved (4.7 d for MC-LR) and particulate phases (0.44–0.52 d for MC-LR). Species-specific bioaccumulation of MCs occurred in tidal flat organisms, with relatively high bioaccumulation factors of MCs being detected in polychaetes and decapods compared to amphipods and bivalves. Overall, this study advances our understanding on the distribution, transport, fate, and bioaccumulation of MCs in estuarine and coastal environments.

* Corresponding authors.
E-mail addresses: hongseongjin@cnu.ac.kr (S. Hong), shinkh@hanyang.ac.kr (K.-H. Shin).
[1] Contributed equally to this work.

https://doi.org/10.1016/j.scitotenv.2020.143815
0048-9697/

1. Introduction

In recent decades, the intensity, frequency, and duration of harmful freshwater cyanobacteria blooms (CyanoHABs) have increased worldwide, due to the increased inflow of nutrients associated with urbanization and industrialization (Heisler et al., 2008; Paerl and Paul, 2012). An increase in water temperature and hydrodynamic patterns, due to global climate change, is also hypothesized to be related to CyanoHABs (Carey et al., 2012; O'Neil et al., 2012; Paerl and Huisman, 2009; Visser et al., 2016). CyanoHABs threaten freshwater ecosystems through the production of harmful metabolites in the form of peptides (Berry et al., 2008), with potential negative impacts on drinking water, irrigation, fishing, and recreational uses (Carmichael, 2012; Osborne et al., 2007). Microcystins (MCs) are representative cyanotoxins belonging to the hepatotoxin cyclic peptide group, and are produced by freshwater cyanobacteria, including *Anabaena*, *Aphanizomenon*, *Microcystis*, *Planktothrix*, *Nostoc*, and *Oscillatoria* (Chorus et al., 2000; Paerl and Otten, 2013). Over 240 MC variants have been identified to date (Spoof and Catherine, 2016). MCs are hepatotoxins that potentially promote the formation of liver tumors in mammals (Watanabe et al., 1996), leading to adverse effects, such as carcinogenicity, reproductive toxicity, neurotoxicity, immunity toxicity, and endocrine disruption (Chen et al., 2016; Valério et al., 2016).

Under field conditions, MCs remain in the form of intracellular toxins during the growth phase and steady-state phase of blooms (Jones and Orr, 1994). These toxins are released to the water column by cellular senescence and cell lysis, and exist as dissolved MCs (Sivonen and Jones, 1999). MCs are chemically stable in the environment, because heptapeptide has a cyclical structure (Krishnamurthy et al., 1989), and can be maintained even under high temperatures (Metcalf and Codd, 2000) and/or low pH (Harada, 1996) conditions. MCs produced in closed freshwater environments, such as lakes and reservoirs, enter coastal environments through estuaries (Kim et al., 2019). Dissolved MCs and/or cyanobacteria cells introduced to the marine environments partially accumulate in sediments (Kankaanpaa et al., 2009). In addition, MCs accumulate in a variety group of organisms, including fish, crustaceans, bivalve, insects, mammals, birds, and mollusks (Garcia et al., 2010; Gerard et al., 2009; Papadimitriou et al., 2010; Poste et al., 2011; Wilson et al., 2008). MCs may also be transferred to organisms in the upper trophic level through the food chain (Smith and Haney, 2006; Xie et al., 2005), even though some studies reported that biomagnification does not occur (Kim et al., 2019).

MCs have been detected in coastal organisms inhabiting the James River Estuary (Wood et al., 2014), San Francisco Estuary (Lehman et al., 2009), Geum River Estuary (Kim et al., 2019), Adriatic Sea (Rita et al., 2014), Isahaya Bay (Metcalf and Codd, 2000; Umehara et al., 2017), and Monterey Bay (Miller et al., 2010). In general, *Microcystis aeruginosa* is inhibited when salinity is below 10 psu (Preece et al., 2017); yet, some *Microcystis* spp. are able to survive in brackish (17.5 psu) and seawater (35 psu) conditions (Miller et al., 2010; Tonk et al., 2007). Although MCs that enter the marine environments are potentially persistent, most studies have been conducted in freshwater environments (Kim et al., 2019). Consequently, our current understanding of the environmental multimedia distribution (water, suspended particulate matter (SPM), and sediments) and fate of MCs in estuarine and coastal environments remains limited.

The Geum River Estuary has a partially mixed estuary characteristic, because the inflow of freshwater was completely blocked after the construction of an estuary dam in 1994 (Lee et al., 1999). Following dam construction, primary production increased significantly compared to before (Jeong et al., 2014). When the water level inside the estuary dam increases, the watergate is opened to discharge freshwater, with most freshwater being discharged in summer (> 80%), due to heavy rainfall. Approximately 6.4×10^9 tons of freshwater flows into the Yellow Sea through the estuary dam every year (Jeong et al., 2014; Kim et al., 2019). Phytoplankton in the Geum River includes Bacillariophyceae, Cyanobacteria, Chlorophyceae, Chrysophyceae, Dinoflagellatae, Cryptophyceae, and Euglena (Han et al., 2016). CyanoHABs have been frequently detected during the summer in recent years (Noh et al., 2014).

Here, we aimed to understand the environmental multimedia distribution and bioaccumulation of MCs in the Geum River Estuary during the period of freshwater discharge in summer. The specific objectives were to: (i) determine the abundance and composition of phytoplankton communities in inside (freshwater) versus outside (brackish water) of estuary dam; (ii) investigate the spatial and temporal distribution of MCs in water, SPM, and sediments, (iii) evaluate the distribution coefficients of MCs across environmental samples in the estuary; and (iv) determine the bioaccumulation of MCs in various tidal flat organisms. The results of the present study will provide a better understanding on the multimedia distribution, fate, and bioaccumulation characteristics of MCs in estuarine and coastal environments.

2. Materials and methods

2.1. Sampling and sample preparation

From May to October 2018, after freshwater discharge, surface water was collected inside (D1) and outside (D2) the Geum River Estuary dam, to analyze the phytoplankton community and MCs. On June 26–30, 2018, when there was a large quantity of freshwater discharge due to heavy rainfall, environmental multimedia samples (including water, SPM, and sediments) were collected from Sites G1 to G8, while tidal flat organisms were collected from Sites B1 and B2 (Fig. 1 and Fig. S1 of Supplementary Materials). Surface water (0.5–1 m depth) was collected using a Van Dorn sampler. For SPM, a 0.5–1 L of water sample was filtered using a pre-combusted glass fiber filter (GF/F, Whatman, Maidstone, England). The SPM was used for the analysis of chlorophyll-a (Chl *a*) and particulate MCs. The samples were immediately frozen at −80 °C until analysis. Surface sediments were collected at 0–0.5 cm depth using a Van Veen grab sampler, placed in a glass jar, and frozen at −20 °C until analysis.

During the sampling periods, tidal flat organisms were collected using 1-mm mesh sieve or collected directly from the tidal flat by hand, and included polychaetes (*Neanthes japonica*, *Neanthes* spp., *Nephtys* sp., *Glycera* spp., and *Lumbrineis* sp.), decapods (*Hemigrapsus* spp.), amphipod (*Mandibulophoxus* spp.), and bivalves (*Glauconome* sp.). To remove the gut contents, the organisms were depurated with filtered seawater for 4 h. The organisms were pooled, with five individuals of the same species. For decapods and bivalves, the liver and mid gland were dissected. Polychaetes and amphipods could not be dissected; thus, the whole body was used for MCs analysis. All biological samples were stored at −80 °C until analysis.

2.2. Measurement of water quality

From May to October, water quality parameters (such as temperature, salinity, pH, conductivity, and dissolved oxygen) were measured in situ using YSI Model Pro 30 (YSI Inc., Baton Rouge, LA). Surface water samples were used to analyze dissolved inorganic nutrients, including NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, and SiO_2. The samples were filtered through GF/F filters (Whatman), and were stored at −20 °C until analysis. Dissolved inorganic nutrients were analyzed using a nutrient autoanalyzer system (Quattro; Seal Analytical Gmbh, Norderstedt, Germany). On June 26–30, 2018, physical environmental factors (such as temperature, salinity, and pH) were measured in situ using the Hydrolab DS5X Multiparameter sonde (OTT Hydromet, Loveland, CO).

2.3. Phytoplankton taxa

Five hundred milliliters of seawater were collected, immediately preserved with acidic Lugol's solution (final concentration of 1%), and

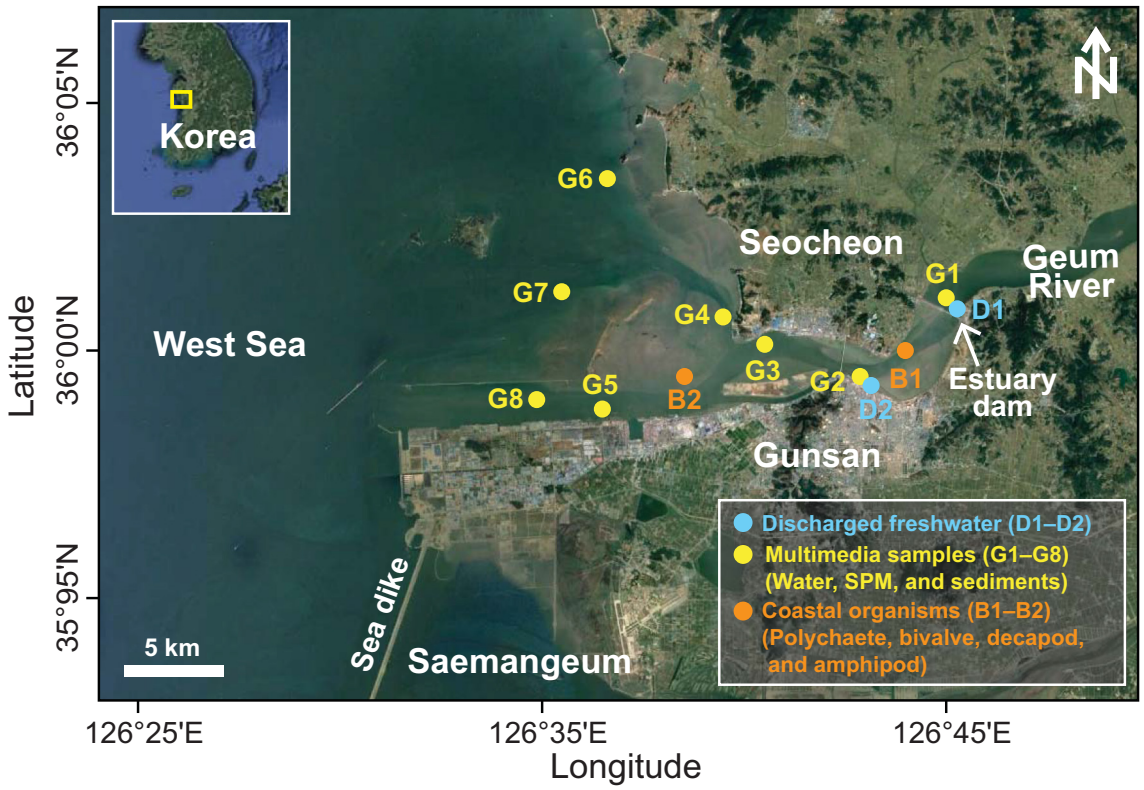


Fig. 1. Map showing the sampling sites in the Geum River Estuary. Discharged freshwater was collected at Sites D1 and D2 from May to October 2018. Environmental multimedia samples and benthic organisms were collected at Sites G1 to G8 and Sites B1 and B2 in June 2018, respectively.

transported to the laboratory to analyze the phytoplankton community. The samples preserved with Lugol's solution were concentrated to determine the abundance of phytoplankton based on a previously described method (Welch, 1948). After mixing the concentrated samples (> 50 times), each phytoplankton species in 1 mL Sedgwick-Rafter counting chamber was identified and counted using an optical microscope.

2.4. Extraction of MCs in the environmental samples

The method developed by Kim et al. (2019) to analyze MCs was followed. In brief, lyophilized filter, sediments, and organisms were placed in a conical tube containing butanol:methanol:water (1:4:15, v:v:v) solution. Then, 100 ng enkephalin (Sigma-Aldrich, Saint Louis, MO) was added to evaluate extraction efficiency. The samples were extracted for 24 h, sonicated for 5 min, centrifuged at 3000 rpm for 15 min, and the supernatants were transferred. This process was repeated three times. The extract (30 mL) was injected to an Oasis HLB cartridge (500 mg, 6 cc, Waters, Milford, MA) that was activated with 10 mL methanol and 10 mL distilled water. After sample loading, the cartridge was washed with 10 mL distilled water and 10 mL 20% methanol. It was then dried for 30 min using a vacuum pump. The samples were eluted with 10 mL methanol, and were concentrated to 1 mL under N_2 gas flow with a 40 °C heating mantle. Finally, 100 μL of 100 ng L^{-1} monolinuron (Sigma-Aldrich) was added as an internal standard.

2.5. HPLC-MS/MS analysis

The eluents were analyzed using a 1290 infinity II series HPLC (Agilent Technologies, Santa Clara, CA) combined with a QTRAP 6500 series electrospray ionization tandem mass spectrometer (AB Sciex, Framingham, MA). The separation of target compounds was conducted with a UPLC column (Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm, Agilent Technologies). The mobile phase was: (A) 0.1% formic acid in water and (B) 100% methanol. The injection volume was 10 μL, and the flow rate was 0.3 mL min^{-1}. All compounds (MC-LR, −RR, -YR, −LA, -LF, -LW, and -LY) were measured under the positive mode and multiple reaction monitoring (MRM). Detailed information on MRM and gradient conditions is presented in Tables S1 and S2.

2.6. Quality control

Calibration standards of 1, 10, 50, 100, 300, 500, and 1000 ng L^{-1} for seven MC variants were used, including MC-LR, −RR, -YR, −LA, -LF, -LW, and -LY ($R^2 > 0.997$ for all MCs). To check accuracy and repeatability, instrumental blanks and calibration standards of mid concentration were analyzed for every 10 injections of samples, as a running standard. Limit of detections (LODs) for seven MC variants were calculated as: 3.143 × SD (standard deviation, $n = 7$) for a one-sided 98% confidence interval. Limit of quantifications (LOQs) were calculated as: 10 × SD. The LODs for MC-LR, −RR, -YR, −LA, -LF, -LW, and -LY were 3.1, 1.1, 3.2, 5.5, 4.3, 5.3, and 6.1 ng L^{-1}, respectively, while those of LOQs were 10, 3.2, 10, 17, 14, 17, and 19 ng L^{-1}, respectively. The results of standard spike test ($n = 3$) indicated that the recovery of all MCs was satisfactory; the average recovery rates of MC-LR, −RR, -YR, −LA, -LF, -LW, and -LY were 96%, 101%, 92%, 87%, 95%, 101%, and 105%, respectively.

2.7. Statistical analysis

To determine the environmental factors affecting the composition of the phytoplankton community, Spearman's correlation analysis and principal component analysis (PCA) were conducted by applying R software (Version 3.4.2). Shapiro-Wilk's normality test was performed for normalization. To clarify any significant difference of concentrations between the inner and outer dam, and among organisms, the Mann-Whitney and Kruskal-Wallis tests were conducted, respectively. The significant level was set to 0.05 for all statistical analyses.

3. Results and discussion

3.1. Water quality parameters and phytoplankton community

Water quality parameters in the inside (D1) and outside (D2) regions of the estuary dam from May to October 2018 are shown in Tables S3 and S4. Water temperature rose to 37 °C in the inside region of the estuary dam (Site D1) in August, and exceeded 30 °C until the end of August. Water temperature in the Geum River Estuary (Site D2) was below that of D1, but also exceeded 30 °C in August. The inside of the estuary dam was completely separated from the seawater, and had almost zero salinity. In comparison, salinity in the outside region showed large fluctuations associated with the amount of freshwater that was discharged through the estuary dam. In particular, salinity in the water of D2 was almost zero in July, which was a season with large quantities of freshwater discharge (Shin, 2013; Yih et al., 2005). In August, when water temperature was high, pH was high at Site D1, which was attributed to a cyanobacteria bloom (Lopez-Archilla et al., 2004). This phenomenon was supported by high concentrations of Chl *a* and MCs detected at the same time.

The density of phytoplankton at Site D1 ranged from 171 to 139,828 cells mL^{-1} from May to October during the sampling period (Fig. 2a and Table S5). The greatest density of phytoplankton was recorded on June 15. At Site D1, a relatively higher density of phytoplankton was recorded in summer (June–August; mean: 52,845 cells mL^{-1}) compared to spring and fall (May, September, and October; mean: 5937 cells mL^{-1}). This seasonal trend was similar to that reported by previous studies (Bukaveckas et al., 2018; Singh et al., 2015; Wood et al., 2014). Bacillariophyta (diatoms) dominated in Site D1 in May and October (74.2 ± 11.7%), followed by Chlorophyta (green algae, 10.4 ± 1.6%), Cyanophyta (cyanobacteria, 10.4 ± 12.3%), and Cryptophyta (4.1 ± 4.1%). In comparison, in June, August, and September (not July), Cyanophyta accounted for more than 90% of total phytoplankton density, while MC producing cyanobacteria (such as *Microcystis* sp.) accounted for more than 95% cyanobacteria. In particular, a high density of cyanobacteria was recorded at Site D1 on June 15 and August 8. The recorded values exceeded the domestic cyanobacteria guideline (100,000 cells mL^{-1}) (Fig. 2a). This phenomenon frequently occurs in the Geum River during summer (Park et al., 2017). Thus, the risk of MCs to the aquatic ecosystem is of great concern, due to the cyanobacterial blooms that occur during this period (Joung et al., 2011; Park et al., 2017). In addition, on June 15 at Site D2, a high density of freshwater cyanobacteria was detected, which was attributed to freshwater being discharged through the estuary dam (Fig. S2). Consequently, MCs are expected to have an adverse impact on coastal organisms.

Statistical analysis was conducted to identify the major environmental factors regulating phytoplankton communities at Site D1 (Fig. S3). Spearman's rank correlation showed that the density of Cyanophyta was positively correlated with water temperature ($R^2 = 0.84$, $p < 0.05$) and TP concentrations ($R^2 = 0.40$, $p < 0.05$). Previous studies reported that water temperature, nutrient concentrations, and the ratios of nitrogen and phosphorus impact the growth of *Microcystis* (Paerl and Paul, 2012; Rinta-Kanto et al., 2009; Xie et al., 2003). The current study showed that the density of Cyanophyta was weakly correlated with nutrients; thus, water temperature seems to be a major factor regulating Cyanophyta blooms in the Geum River.

The regression between the relative compositions of cyanobacteria to total phytoplankton abundance and water temperature indicated that cyanobacteria density increased rapidly with increasing water temperature (Fig. 2b). In particular, at 26 °C water temperature or higher, cyanobacteria accounted for more than 95% of the total phytoplankton community. This might be because *Microcystis* has a competitive advantage through being able to grow at a relatively higher temperature than other species (Elliott et al., 2006; JÖHnk et al., 2008). A previous study reported similar results, with the number of cyanobacteria increasing

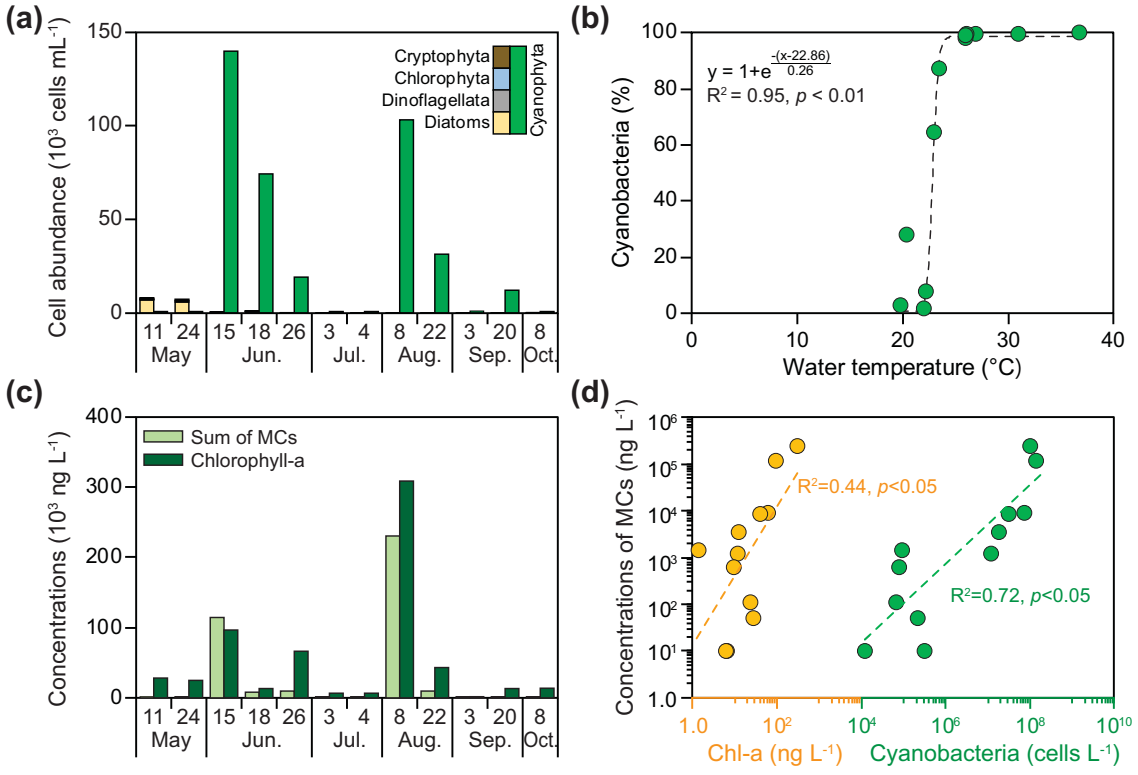


Fig. 2. (a) Cell abundance of each phytoplankton group at Site D1, (b) portion of cyanobacteria out of all phytoplankton based on water temperature, (c) concentration of MCs and Chl *a* at Site D1, and (d) linear relationships between Chl *a* and abundance of cyanobacteria, in comparison to concentrations of MCs.

with greater water temperature or residence time (Cha et al., 2017). In the Geum River, the water temperature at Site D1 ranged from 26 to 36.8 °C during the summer, which was similar to the optimum water temperature for the growth of cyanobacteria (Bui et al., 2018).

3.2. Concentrations of MCs and Chl a in discharged freshwater

Concentrations of particulate and dissolved MCs in discharged freshwater from May to October ranged from 0.05 to 235 μg L^{-1} and 0.02 to 3.8 μg L^{-1}, respectively (Figs. 2c and S4). MC concentrations in discharged water tended to be greater in June and August, during which the distribution trend was very similar to the density of cyanobacteria. There were more particulate MCs compared to dissolved MCs. Thus, cyanobacteria cells were discharged directly to the estuary with freshwater. The composition of particulate MCs in discharged water showed that the MC-RR (62%) contributed more than MC-LR (31%) and MC-YR (6.1%). MC-LA, -LF, -LW, and -LY were not detected in most samples (Fig. S4a). For the composition of dissolved MCs, MC-LR concentrations (55%) were relatively greater compared to MC-RR (30%) and MC-YR (9.9%) concentrations (Fig. S4b). These results were consistent with previous studies, in which particulate MCs composition in freshwater environments was ordered as (highest to lowest): MC-RR, -LR, and -YR (Kim et al., 2019; Singh et al., 2015). The lower contributions of MC-RR in dissolved MCs compared to those of particulate MCs seemed to be attributed to MC-RR having one more arginine group compared to other MC variants. The unusual structure of MC-RR increases its tendency to form cation-bridges, and improves its adsorption affinity for cation-exchange sites in organic or clay materials (Wu et al., 2011). Thus, dissolved MC-RR could be adsorbed into organic materials and suspended particles in the water column.

Chl *a* concentrations in discharged water ranged from 1.3 to 308 μg L^{-1}, and showed similar trends to the distributions of cyanobacterial density and MC concentrations (Fig. 2c). The highest Chl *a* concentration was recorded on August 8, with the highest MC concentrations also being detected at this time. Particulate MC concentrations were significantly correlated with Chl *a* concentration ($R^2 = 0.44$, $p < 0.05$) and cyanobacterial density ($R^2 = 0.72$, $p < 0.05$) (Fig. 2d). This phenomenon might be attributed to MC-producing cyanobacteria, such as *Microcystis*, which accounted for more than 95% of total phytoplankton density in the Geum River during summer. Thus, Chl *a* concentration and the density of cyanobacteria could be useful indicators of MC concentrations in water during summer (Bukaveckas et al., 2018; Yuan et al., 2014). Based on these regressions, the MCs per cyanobacteria cell quota and MCs per Chl *a* were calculated as 1.12 pg MCs $cell^{-1}$ and 0.76 μg MCs Chl a^{-1}, respectively. These values exceeded that of a previous study conducted in the Geum River (Kim et al., 2019) and other regions elsewhere (Chorus et al., 2000; Shi et al., 2015). Thus, cyanobacteria in the Geum River watershed seemed to have a relatively high MC quota compared to previous studies, raising concerns on its potential adverse effects to the aquatic ecosystem. Based on the amount of freshwater discharged through the estuary dam and concentrations of MCs in water, a total of 2.2 tons of MCs were discharged to the Geum River Estuary from May to October 2018. This value was comparable to the quantity of MCs discharged to the Geum River Estuary in 2017 (4.4 tons of MCs discharged) (Kim et al., 2019).

The concentration of MCs in water showed the strongest positive correlation with Cyanophyta and water temperature, and also showed a significant positive correlation with DO, pH, and Chl *a* (Fig. S3). It is considered that as the water temperature in summer increases, suitable conditions for Cyanophyta are made, thereby increasing the phytoplankton biomass (Chl *a*), and at the same time increasing the production of MCs. The increase in DO and pH in water could be attributed to blooms of Cyanophyta.

3.3. Spatial and temporal distributions of MCs in environmental multimedia samples

During the period of freshwater discharge, the distribution and fate of cyanobacteria and MCs in the coastal environment were investigated by daily monitoring (5 days, June 26–30, 2018). Particulate MCs were detected at all sites (G1–G8) (Fig. 3a and Tables S6 and S7). Particulate MC concentrations inside and outside the estuary dam ranged from 0.04 to 7.8 μg L^{-1} (mean: 2.05 μg L^{-1}) and from 0.01 to 2.68 μg L^{-1} (mean: 0.41 μg L^{-1}), respectively. The greatest concentration of particulate MCs was recorded at Site G1 (inside the estuary dam) on the first day of the sampling campaign (June 26). The concentration rapidly decreased for 4 days, which was attributed to the dilution effect of heavy rainfall. In parallel, comparatively lower concentrations of particulate MCs were detected in the estuarine area (i.e., Sites G2 − G8), compared to G1 (except for June 27). Particulate MC concentrations decreased during the sampling period.

Spatially, particulate MC concentrations tended to decline from the inner to outer regions. Particulate MC concentrations might have declined because cyanobacteria cells were lysed in seawater through osmotic pressure (Umehara et al., 2015), with cell lysis increasing at higher salinities (Tonk et al., 2007). Consequently, MCs could be released to the water column as the dissolved phase. There was a negative correlation between the concentrations of particulate MCs and salinity ($R^2 = 0.19$, $p < 0.05$). Particulate MCs also showed a positive relationship with Chl *a*, which was similar to that shown for freshwater ($R^2 = 0.45$, $p < 0.05$). The composition of particulate MCs differed slightly among sites. Relative compositions were ordered, on average as: MC-RR (49.5%) > MC-LR (42.6%) > MC-YR (6.2%) > MC-LY (3.2%) > MC-LW (0.6%). MC-LY and MC-LW had relatively greater contributions in the estuarine areas compared to freshwater areas.

For dissolved MCs, concentrations in the inside (G1) and outside (G2 − G8) regions were 0.51–1.9 μg L^{-1} (mean: 1.2 μg L^{-1}) and 0.6–3.3 μg L^{-1} (mean: 1.5 μg L^{-1}), respectively (Fig. 3b and Table S8). There was no significant difference in dissolved MC concentrations between the inside and outside regions of the estuary dam. Compared to particulate MC concentrations, dissolved MC concentrations were relatively higher, except on June 26.

Dissolved MC concentrations (mean: 1.5 μg L^{-1}) were relatively higher on the outside of the estuary dam during the sampling period. This phenomenon was attributed to cyanobacteria cells entering the marine environment being destroyed. Dissolved MCs in seawater were relatively stable compared to particulate MCs, and remained in the water column for a few days as in previous studies (Lahti et al., 1997; Zastepa et al., 2014). The composition of dissolved MCs was ordered, on average, as: MC-LR (87%) > MC-RR (4.9%) > MC-YR (1.9%) > MC-LW (2.3%) > MC-LA (1.8%) > MC-LY (1%). The proportion of MC-LR in dissolved MCs was greater compared to that in particulate MCs. MC-LA that was not detected in SPM was detected as the dissolved phase in the water column. MC-LA exhibits toxicity similar to MC-LR, which has the highest toxicity of MC variants (Chorus et al., 2000). Thus, compositional changes of MC variants that occurred in the estuarine area might increase the potential risk of MCs in the marine environment.

For sedimentary MCs, concentrations in the inside and outside regions of the estuary dam ranged from 0.06 to 0.5 μg g^{-1} (mean: 0.28 μg g^{-1}) and from 0.02 to 0.68 μg g^{-1} (mean: 0.19 μg g^{-1}), respectively (Fig. 3c and Table S9). On the first and second days (June 26–27) of sampling, MC concentrations in sediments tended to decrease with increasing distance from G1; however, after the third day (June 28), concentrations appeared to increase at Sites G1, G2, and G3. The distribution of sedimentary MCs fluctuated more compared to dissolved MCs (Chen et al., 2008). Previous studies reported that the heterogeneity of MCs in sediments causes differences in concentrations across sampling sites (Xue et al., 2020). In particular, MCs have high adsorption capacity in clay-silt sized sediments (Maghsoudi et al., 2015; Munusamy et al.,

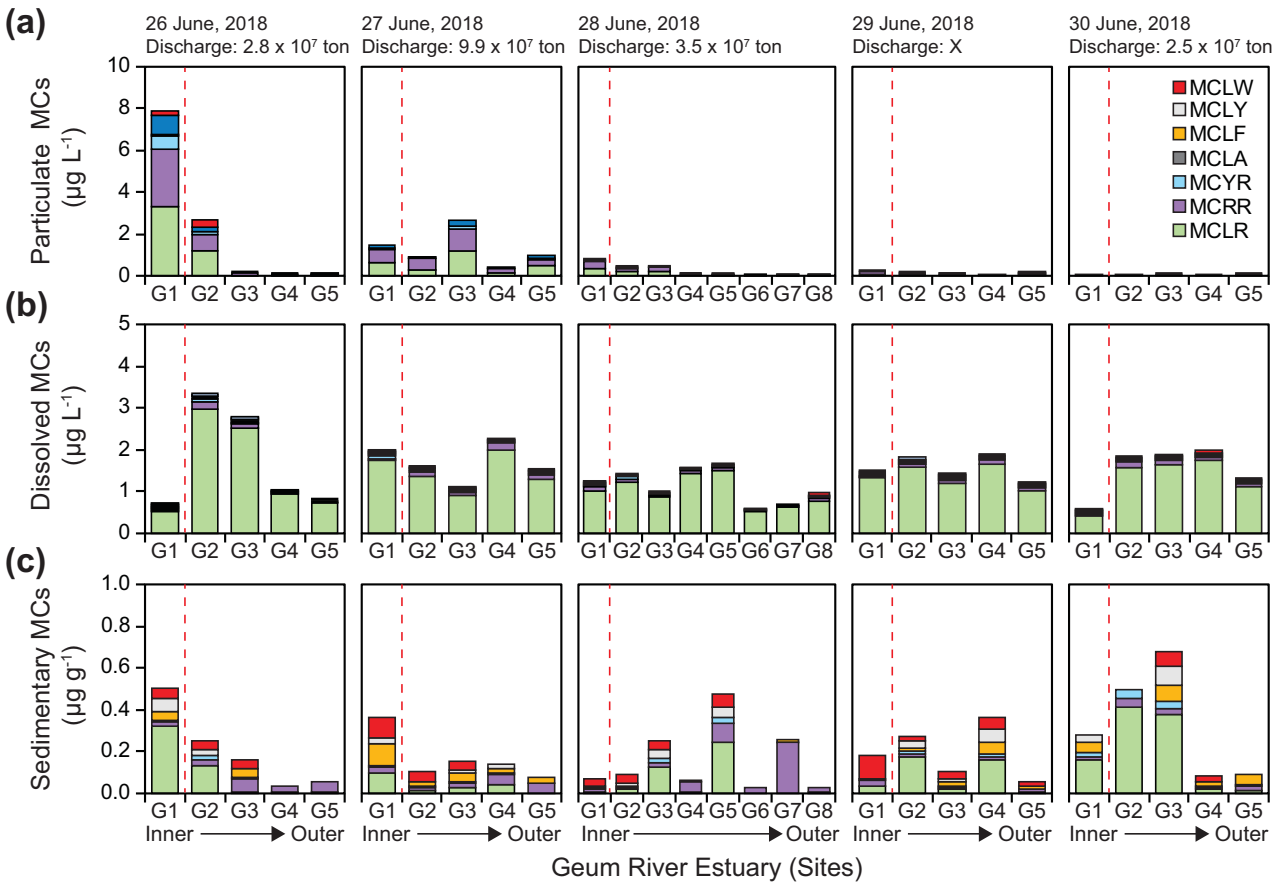


Fig. 3. Concentrations of MCs in environmental multimedia samples. (a) Particulated MCs, (b) dissolved MCs, and (c) sedimentary MCs. Red lines represent the estuary dam.

2012). The composition of sediment MC variants was ordered as: MC-LR (29%) > MC-RR (26.6%) > MC-LW (19.4%) > MC-LF (13.8%) > MC-LY (7.2%) > MC-YR (3.3%). The proportion of MC-LW and MC-LF was relatively higher compared to other environmental variables, such as dissolved MCs and particulate MCs. This phenomenon was attributed to the hydrophobic characteristics of MC-LW and MC-LF (Vesterkvist and Meriluoto, 2003). Previous studies reported that MCs strongly adsorb to sediments, due to the hydrophobic interaction between MCs and organic material (Liu et al., 2008). Consequently, dissolved and particulate MCs settle on sediment in estuarine areas, which then adsorb the MCs, resulting in their remaining for a few days. During the sampling period, there was no significant relationship between the concentrations of MCs in the three media, such as water, SPM, and sediments ($p < 0.05$). This means that MCs introduced into the Geum River Estuary show a media-specific distribution pattern in the coastal environment.

3.4. Distribution coefficients of MCs between environmental media

To understand the distribution behavior of MCs among environmental multimedia in the estuary, the field-based distribution coefficients of MCs between water and SPM ($K_{d\text{-}SPM}$) and between water and sediments ($K_{d\text{-}sediment}$) were calculated. $K_{d\text{-}SPM}$ had the highest value at Site G1, and declined towards the regions outside the estuary dam. $K_{d\text{-}SPM}$ also decreased during the sampling period (Fig. 4a). This phenomenon might be attributed to the rapid decreasing concentrations of particulate MCs and increasing dissolved MCs in brackish water. During the sampling period, particulate MCs remained at an average of 0.75 μg L^{-1} at Site G3, with an average salinity of 11 psu; however, concentrations at Sites G4 — G8 (mean: 0.14 μg L^{-1}) were comparatively low, with an average salinity of 21 psu. Relatively high concentrations of dissolved MCs was maintained at Sites G4 — G8 compared to particulate MCs; consequently, $K_{d\text{-}SPM}$ appeared to decline from the inner to outer regions of the Geum River Estuary.

The half-life of particulate MCs at Site G1 was calculated as 0.44, 0.42, 0.41, and 0.41 d for MC-LR, MC-RR, MC-YR, and MC-LY, respectively (Table 1). In addition, the half-life of particulate MCs at Site G2 was 0.52, 1.16, and 0.72 d for MC-LR, MC-RR, and MC-YR, respectively. The half-life of dissolved MCs at Site G1 could not be calculated, due to high variability of MC variants, except for MC-LY. The half-life of dissolved MC-LR and -YR at Site G2 was 4.7 and 1.9 d, respectively. Dissolved MCs had a relatively longer half-life in the environment compared to particulate MCs. This finding was consistent with the results of previous studies (Table 1). $K_{d\text{-}sediment}$ varied among sites and sampling dates, and did not show any clear spatial or temporal trend (Fig. 4b). In addition to sedimentary MCs having greater variability compared to dissolved MCs, they were also affected by other environmental factors, such as the production of MCs by benthic cyanobacteria (Ihle et al., 2005) and adsorption of MCs in sedimentary organic matter (Maghsoudi et al., 2015).

3.5. Bioaccumulation characteristics of MCs in tidal flat organisms

MCs were detected in all tidal flat organisms collected from Sites B1 and B2, with ranges of 0.68 to 1.9 μg g^{-1} (mean: 1.2 ± 0.34 μg g^{-1}), 0.38 to 1.2 μg g^{-1} (mean: 0.81 ± 0.42 μg g^{-1}), 0.38 to 13 μg g^{-1} (mean: 4.9 ± 3.5 μg g^{-1}), and 0.4 to 7.0 μg g^{-1} (mean: 3.8 ± 2.6 μg g^{-1}) for bivalves, amphipods, polychaetes, and decapods, respectively (Fig. 5). Relatively higher concentrations of MCs were recorded in polychaetes and decapods compared to bivalves and amphipods ($p < 0.05$). Out of all MC variants, tidal flat organisms had the greatest concentrations of MC-LR.

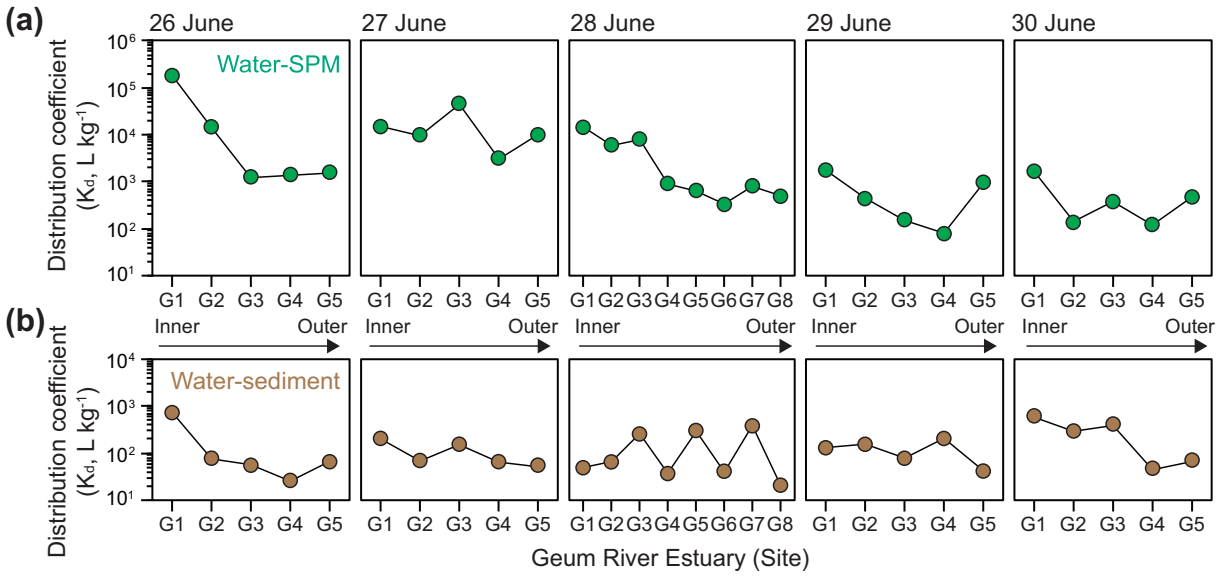


Fig. 4. (a) Water-SPM distribution coefficients and (b) water-sediment distribution coefficients of MCs from the inner to outer estuary dam.

MC-RR was lowest in organisms, but was highest in environmental media, such as water, SPM, and sediments. This result might be attributed to the chemical properties of MC-RR. MC-RR has relatively high polarity and hydrophilicity out of all MC variants (Diez-Quijada et al., 2019; Fastner et al., 1998). Hydrophilic MCs are more easily excreted compared to relatively hydrophobic MC variants (Gupta et al., 2003; Kim et al., 2019). Polychaetes had relatively higher proportions of MC-YR compared to other organisms. Decapods had the highest proportion of MC-LW compared to other organisms. This result was consistent with a previous study showing that the MC-YR contributed more to freshwater oligochaetes compared to other MC variants (Xue et al., 2016). Polychaetes appear to be able to selectively degrade MCs that are consumed with relatively little energy. Previous studies reported that MC-LW has higher cell permeability compared to other relatively hydrophilic MCs (Vesterkvist and Meriluoto, 2003). Furthermore, hydrophobic MCs might not require a bile-acid transporter to penetrate the cell lipid membrane of animals or bacteria (Sivonen and Jones, 1999). Consequently, the bioaccumulation patterns of MCs in intertidal organisms are species-specific, due to the metabolic capacity of organisms and differences in the chemical properties of MC variants.

The field-based biota-SPM accumulation factor ($BSAF_{SPM}$) and biota-sediment accumulation factor ($BSAF_{sediment}$) were calculated to determine the bioaccumulation characteristics of MCs between organisms (Fig. 6). For calculation of BSAF, concentrations of MCs in the liver of organisms (dry mass basis) and concentrations of MCs in sediments and SPM (dry mass basis) were used. $BSAF_{SPM}$ of MCs ranged from −0.56 to 1.22 (mean: 0.35), −1.6 to 0.71 (mean: 0.097), −1.34 to 0.37 (mean: −0.26), and − 0.56 to −0.058 (mean: −0.27) in polychaetes,

Table 1
Half-life of microcystins under field and/or laboratory conditions reported in previous studies and this study.

Samples	Country	Study area	Condition (Field/laboratory)	MC variants	Half-life (days)	References
Dissolved MCs	Australia	Lake Burragorang	Laboratory	MC-LR	2.2–22	Ho et al. (2012)
	Canada	Lake Ottawa	Field	MC-LA	2.8 and 16	Zastepa et al. (2014)
			Laboratory	MC-LA	11–251	
	China	Lake Taihu	Field	MC-LR	0.85–13	Chen et al. (2008)
				MC-RR	0.9–11.5	
			Laboratory	MC-Dha^{7}LR	0.96–16	
				MC-LR	0.75–6.5	
				MC-RR	0.61–5.9	
				MC-Dha^{7}LR	0.92–12.7	
	Finland	Lake Tuusula	Field	MC-LR	10	Lahti et al. (1997)
	Japan	Lake Kasumigaura	Laboratory	MC-LR	5–11	Li et al. (2011)
	Scotland	River Carron	Laboratory	MC-LR	6.5	Manage et al. (2016)
	South Korea	Geum River Estuary	Field	MC-LR	4.7	This study
				MC-YR	1.9	
Particulate MCs	Canada	Lake Ottawa	Field	MC-LA	1.5 and 6.5	Zastepa et al. (2014)
			Laboratory	MC-LA	5–55	
	Finland	Lake Tuusula	Field	MC-LR	4.7	Lahti et al. (1997)
	South Korea	Geum River Estuary	Field	MC-LR	0.44–0.52	This study
				MC-RR	0.52–1.2	
				MC-YR	0.41–0.72	
				MC-LY	0.41	
Sedimentary MCs	China	Lake Dianchi	Laboratory	MC-LR	2.5–14.7	Chen et al. (2010)
		Lake Taihu	Field	MC-LR	0.87–1.2	Chen et al. (2008)
				MC-RR	0.94–1.15	
				MC-Dha^{7}LR	0.83–1.2	

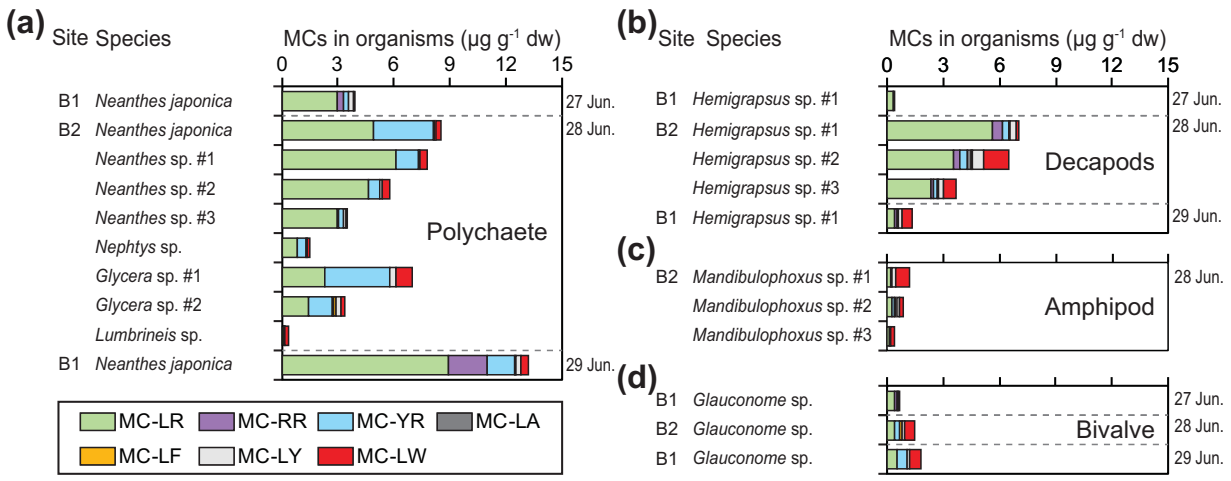


Fig. 5. Concentrations of MCs in marine benthic organisms, including (a) polychaetes, (b) decapods, (c) amphipods, and (d) bivalves collected from Sites B1 and B2.

decapods, bivalves, and amphipods, respectively. Similar to the MC concentrations detected in organisms, $BSAF_{SPM}$ of polychaetes and decapods were relatively higher compared to those in bivalves and amphipods. In addition, $BSAF_{sediment}$ was similar to $BSAF_{SPM}$. $BSAF_{sediment}$ of MCs in polychaetes (mean: 1.5) and decapods (mean: 1.3) were relatively greater compared to that of bivalves (mean: 0.93) and amphipods (mean: 0.84).

Various biotic and abiotic factors impact the toxicokinetics and bioaccumulation of MCs in aquatic organisms, including exposure pathways, feeding habits, food sources, duration of exposure, and concentration (Ibelings and Chorus, 2007). Polychaetes, decapods, and amphipods mainly consume sedimentary organic matter (Kim et al., 2019); consequently, they can ingest aggregates containing MCs (Umehara et al., 2017). In comparison, bivalves are filter-feeding organisms, which tend to ingest suspended particles directly, including cyanobacteria cells in the water column (van Egmond and Jonker, 2004). Overall, differences to $BSAF_{SPM}$ and $BSAF_{sediment}$ of MCs in the tidal flat organisms of the Geum River Estuary were regulated by feeding habits and metabolic capacity, in addition to differences in the chemical properties of MC variants and exposure duration.

4. Conclusions

In this study, we investigated the composition of the phytoplankton community in the Geum River, the distributions and fate of MCs in environmental multimedia samples, and the bioaccumulation of MCs in intertidal organisms. The results showed that phytoplankton density was greater in summer compared to other seasons, and that MCs producing cyanobacteria were dominant in the Geum River. Large quantities of MCs might enter the marine environment via freshwater discharge from the estuary dam during summer. MCs showed specific distribution patterns in the environmental multimedia samples. MC-LR was a major MC variant in most environmental media, and the elevated proportion of MC-RR was found in particulate MCs. Species-specific bioaccumulation of MCs was detected in tidal flat organisms, with MC-YR and MC-LW, specifically accumulating in polychaetes and decapods, respectively. Overall, the current study advanced our understanding on the distributions, fate, and bioaccumulation of MCs in various environmental variables of the estuarine environment. Further studies are needed to understand the phase shift of MCs in marine environmental multimedia, species-specific metabolism, and their bioaccumulation in coastal organisms.

CRediT authorship contribution statement

Mungi Kim: Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft. **Dokyun Kim:** Conceptualization, Investigation, Formal analysis. **Jaeseong Kim:** Investigation, Formal analysis, Data curation. **Seongjin Hong:** Conceptualization, Writing - original draft, Writing - review & editing, Project

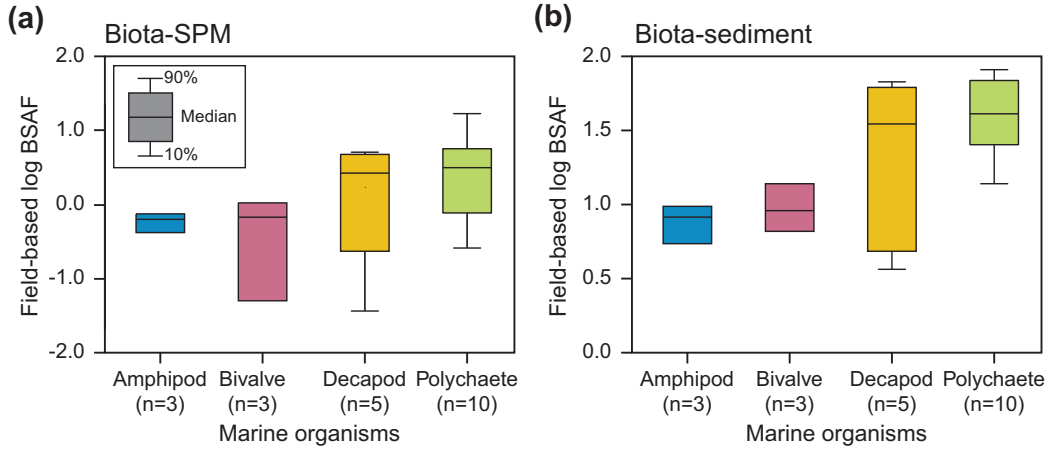


Fig. 6. (a) Field-based biota-SPM accumulation factor and (b) biota-sediments accumulation factor of MCs relative to marine benthic organisms.

administration, Funding acquisition, Supervision. **Kyung-Hoon Shin:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision.

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.

Acknowledgments

This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIP) (2016R1E1A1A01943004 and 2020R1A4A2002823). This research was also supported by a grant (20163MFDS641) from Ministry of Food and Drug Safety in 2020.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143815.

References

Berry, J.P., Gantar, M., Perez, M.H., Berry, G., Noriega, F.G., 2008. Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. Mar. Drugs 6, 117–146.

Bui, T., Dao, T.S., Vo, T.G., Lurling, M., 2018. Warming affects growth rates and microcystin production in tropical bloom-forming Microcystis strains. Toxins (Basel) 10, 123.

Bukaveckas, P.A., Franklin, R., Tassone, S., Trache, B., Egerton, T., 2018. Cyanobacteria and cyanotoxins at the river-estuarine transition. Harmful Algae 76, 11–21.

Carey, C.C., Ibelings, B.W., Hoffmann, E.P., Hamilton, D.P., Brookes, J.D., 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Res. 46, 1394–1407.

Carmichael, W.W., 2012. Health effects of toxin-producing cyanobacteria: "the CyanoHABs". Hum. Ecol. Risk. Assess. 7, 1393–1407.

Cha, Y., Cho, K.H., Lee, H., Kang, T., Kim, J.H., 2017. The relative importance of water temperature and residence time in predicting cyanobacteria abundance in regulated rivers. Water Res. 124, 11–19.

Chen, W., Song, L., Peng, L., Wan, N., Zhang, X., Gan, N., 2008. Reduction in microcystin concentrations in large and shallow lakes: water and sediment-interface contributions. Water Res. 42, 763–773.

Chen, X., Yang, X., Yang, L., Xiao, B., Wu, X., Wang, J., Wan, H., 2010. An effective pathway for the removal of microcystin LR via anoxic biodegradation in lake sediments. Water Res. 44, 1884–1892.

Chen, L., Chen, J., Zhang, X., Xie, P., 2016. A review of reproductive toxicity of microcystins. J. Hazard. Mater. 301, 381–399.

Chorus, I., Falconer, I.R., Salas, H.J., Bartram, J., 2000. Health risks caused by freshwater cyanobacteria in recreational waters. J. Toxicol. Env. Heal. B 3, 323–347.

Diez-Quijada, L., Puerto, M., Gutierrez-Praena, D., Llana-Ruiz-Cabello, M., Jos, A., Camean, A.M., 2019. Microcystin-RR: occurrence, content in water and food and toxicological studies. A review. Environ. Res. 168, 467–489.

Elliott, J.A., Jones, I.D., Thackeray, S.J., 2006. Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate Lake. Hydrobiologia 559, 401–411.

Fastner, J., Flieger, I., Neumann, U., 1998. Optimised extraction of microcystins from field samples—a comparison of different solvents and procedures. Water Res. 32, 3177–3181.

Garcia, A.C., Bargu, S., Dash, P., Rabalais, N.N., Sutor, M., Morrison, W., Walker, N.D., 2010. Evaluating the potential risk of microcystins to blue crab (Callinectes sapidus) fisheries and human health in a eutrophic estuary. Harmful Algae 9, 134–143.

Gerard, C., Poullain, V., Lance, E., Acou, A., Brient, L., Carpentier, A., 2009. Influence of toxic cyanobacteria on community structure and microcystin accumulation of freshwater molluscs. Environ. Pollut. 157, 609–617.

Gupta, N., Pant, S.C., Vijayaraghavan, R., Rao, P.V.L., 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188, 285–296.

Han, S.R., Cho, K., Yoon, J.-H., Lee, J.-J., Yoo, S., Choi, I.-C., Joo, H.-J., Cheon, S.-U., Lim, B.-J., 2016. Phytoplankton community structure of midstream of Geum River on 2014 and 2015. Korean J. Environ. Biol. 49, 375–384.

Harada, K., 1996. Chemistry and detection of microcystins. In: Watanabe, M.F., Harada, K.-I., Carmichael, W.W., Fujiki, H. (Eds.), toxic Microcystis. CRC press, Boca Raton, FL., 103-148.

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Gobler, C., Dortch, Q., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., Suddleson, M., 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8, 3–13.

Ho, L., Tang, T., Hoefel, D., Vigneswaran, B., 2012. Determination of rate constants and half-lives for the simultaneous biodegradation of several cyanobacterial metabolites in Australian source waters. Water Res. 46, 5735–5746.

Ibelings, B.W., Chorus, I., 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. Environ. Pollut. 150, 177–192.

Ihle, T., Jähnichen, S., Benndorf, J., 2005. Wax and wane of Microcystis (Cyanophyceae) and microcystins in Lake sediments: a case study in Quitzdorf reservoir (Germany) 1. J. Phycol. 41, 479–488.

Jeong, Y.H., Yang, J.S., Park, K., 2014. Changes in water quality after the construction of an estuary dam in the Geum River estuary dam system, Korea. J. Coastal Res. 298, 1278–1286.

JÖHnk, K.D., Huisman, J.E.F., Sharples, J., Sommeijer, B.E.N., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Chang. Biol. 14, 495–512.

Jones, G.J., Orr, P.T., 1994. Release and degradation of microcystin following algicide treatment of a Microcystis aeruginosa bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. Water Res. 28, 871–876.

Joung, S.-H., Oh, H.-M., Ko, S.-R., Ahn, C.-Y., 2011. Correlations between environmental factors and toxic and non-toxic Microcystis dynamics during bloom in Daechung reservoir, Korea. Harmful Algae 10, 188–193.

Kankaanpaa, H.T., Sjovall, O., Huttunen, M., Olin, M., Karlsson, K., Hyvarinen, K., Sneitz, L., Harkonen, J., Sipia, V.O., Meriluoto, J.A., 2009. Production and sedimentation of peptide toxins nodularin-R and microcystin-LR in the northern Baltic Sea. Environ. Pollut. 157, 1301–1309.

Kim, D., Hong, S., Choi, H., Choi, B., Kim, J., Khim, J.S., Park, H., Shin, K.H., 2019. Multimedia distributions, bioaccumulation, and trophic transfer of microcystins in the Geum River estuary, Korea: application of compound-specific isotope analysis of amino acids. Environ. Int. 133, 105194.

Krishnamurthy, T., Szafraniec, L., Hunt, D.F., Shabanowitz, J., Yates, J.R., Hauer, C.R., Carmichael, W.W., Skulberg, O., Codd, G.A., Missler, S., 1989. Structural characterization of toxic cyclic peptides from blue-green algae by tandem mass spectrometry. P. Natl. Acad. Sci. USA 86, 770–774.

Lahti, K., Rapala, J., Färdig, M., Niemelä, M., Sivonen, K., 1997. Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in lake water. Water Res. 31, 1005–1012.

Lee, S.-H., Kwon, H.-K., Choi, H.-Y., Yang, J.-S., Choi, J.-Y., 1999. Physical environment changes in the Keum River estuary by the dyke gate operation: II. Salinity structure and estuary type. Korean J. Environ. Biol. 4, 255–265.

Lehman, P.W., Teh, S.J., Boyer, G.L., Nobriga, M.L., Bass, E., Hogle, C., 2009. Initial impacts of Microcystis aeruginosa blooms on the aquatic food web in the San Francisco estuary. Hydrobiologia 637, 229–248.

Li, J., Shimizu, K., Sakharkar, M.K., Utsumi, M., Zhang, Z., Sugiura, N., 2011. Comparative study for the effects of variable nutrient conditions on the biodegradation of microcystin-LR and concurrent dynamics in microcystin-degrading gene abundance. Bioresour. Technol. 102, 9509–9517.

Liu, G., Qian, Y., Dai, S., Feng, N., 2008. Adsorption of microcystin LR and LW on suspended particulate matter (SPM) at different pH. Water Air Soil Pollut. 192, 67–76.

Lopez-Archilla, A.I., Moreira, D., Lopez-Garcia, P., Guerrero, C., 2004. Phytoplankton diversity and cyanobacterial dominance in a hypereutrophic shallow lake with biologically produced alkaline pH. Extremophiles 8, 109–115.

Maghsoudi, E., Prevost, M., Vo Duy, S., Sauve, S., Dorner, S., 2015. Adsorption characteristics of multiple microcystins and cylindrospermopsin on sediment: implications for toxin monitoring and drinking water treatment. Toxicon 103, 48–54.

Manage, P.M., Edwards, C., Lawton, L.A., 2016. Biodegradation of microcystin-LR by natural bacterial populations. Interdisciplinary studies on environmental chemistry — environmental research in Asia, Eds., Y. Obayashi, T. Isobe, A. Subramanian, S. Suzuki and S. Tanabe, Tokyo, 277-285.

Metcalf, J.S., Codd, G.A., 2000. Microwave oven and boiling waterbath extraction of hepatotoxins from cyanobacterial cells. FEMS Microbiol. Lett. 184, 241–246.

Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler, M., Miller, W.A., Toy-Choutka, S., Dominik, C., Hardin, D., Langlois, G., Murray, M., Ward, K., Jessup, D.A., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. PLoS One 5, 1–11.

Munusamy, T., Hu, Y.L., Lee, J.F., 2012. Adsorption and photodegradation of microcystin-LR onto sediments collected from reservoirs and rivers in Taiwan: a laboratory study to investigate the fate, transfer, and degradation of microcystin-LR. Environ. Sci. Pollut. R. 19, 2390–2399.

Noh, S., Park, H., Choi, H., Lee, J., 2014. Effect of climate change for cyanobacteria growth pattern in Chudong station of Lake Daechung. J. Korean Soc. Water Environ. 30, 377–385.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.

Osborne, N.J., Shaw, G.R., Webb, P.M., 2007. Health effects of recreational exposure to Moreton Bay, Australia waters during a Lyngbya majuscula bloom. Environ. Int. 33, 309–314.

Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1, 27–37.

Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65, 995–1010.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46, 1349–1363.

Papadimitriou, T., Kagalou, I., Bacopoulos, V., Leonardos, I.D., 2010. Accumulation of microcystins in water and fish tissues: an estimation of risks associated with microcystins in most of the Greek Lakes. Environ. Toxicol. 25, 418–427.
Park, Y., Pyo, J., Kwon, Y.S., Cha, Y., Lee, H., Kang, T., Cho, K.H., 2017. Evaluating physico-chemical influences on cyanobacterial blooms using hyperspectral images in inland water, Korea. Water Res. 126, 319–328.
Poste, A.E., Hecky, R.E., Guildford, S.J., 2011. Evaluating microcystin exposure risk through fish consumption. Environ. Sci. Technol. 45, 5806–5811.
Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in estuarine and marine waters: environmental implications and human health risk. Harmful Algae 61, 31–45.
Rinta-Kanto, J.M., Konopko, E.A., DeBruyn, J.M., Bourbonniere, R.A., Boyer, G.L., Wilhelm, S.W., 2009. Lake Erie Microcystis: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. Harmful Algae 8, 665–673.
Rita, D.P., Valeria, V., Silvia, B.M., Pasquale, G., Milena, B., 2014. Microcystin contamination in sea mussel farms from the Italian southern Adriatic coast following Cyanobacterial blooms in an artificial reservoir. J. Ecosyst. 2014, 1–11.
Shi, K., Zhang, Y., Xu, H., Zhu, G., Qin, B., Huang, C., Liu, X., Zhou, Y., Lv, H., 2015. Long-term satellite observations of microcystin concentrations in Lake Taihu during Cyanobacterial bloom periods. Environ. Sci. Technol. 49, 6448–6456.
Shin, Y.K., 2013. A ecological study of phytoplankton community in the Geum river estuary. Korean J. Limnol. 46, 524–540.
Singh, S., Rai, P.K., Chau, R., Ravi, A.K., Neilan, B.A., Asthana, R.K., 2015. Temporal variations in microcystin-producing cells and microcystin concentrations in two fresh water ponds. Water Res. 69, 131–142.
Sivonen, K., Jones, G., 1999. Cyanobacterial toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. 1. E&FN Spon, London, UK., pp. 43–112.
Smith, J.L., Haney, J.F., 2006. Foodweb transfer, accumulation, and depuration of microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (Lepomis gibbosus). Toxicon 48, 580–589.
Spoof, L., Catherine, A., 2016. Appendix 3. Tables of microcystins and nodularins. In: Meriluoto, J., Spoof, L., Codd, J. (Eds.), handbook of cyanobacterial monitoring andcyanotoxin analysis. Wiley, Hoboken. 526-537.
Tonk, L., Bosch, K., Visser, P.M., Huisman, J., 2007. Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. Aquat. Microb. Ecol. 46, 117–123.
Umehara, A., Komorita, T., Tai, A., Takahashi, T., Orita, R., Tsutsumi, H., 2015. Short-term dynamics of cyanobacterial toxins (microcystins) following a discharge from a coastal reservoir in Isahaya Bay, Japan. Mar. Pollut. Bull. 92, 73–79.
Umehara, A., Takahashi, T., Komorita, T., Orita, R., Choi, J.W., Takenaka, R., Mabuchi, R., Park, H.D., Tsutsumi, H., 2017. Widespread dispersal and bio-accumulation of toxic microcystins in benthic marine ecosystems. Chemosphere 167, 492–500.
Valério, E., Vasconcelos, V., Campos, A., 2016. New insights on the mode of action of microcystins in animal cells-a review. Mini-Rev. Med. Chem. 16, 1032–1041.
van Egmond, H.P., Jonker, M., 2004. Worldwide regulations for mycotoxins in food and feed in 2003. FAO food and nutrition paper 81.
Vesterkvist, P.S., Meriluoto, J.A., 2003. Interaction between microcystins of different hydrophobicities and lipid monolayers. Toxicon 41, 349–355.
Visser, P.M., Verspagen, J.M.H., Sandrini, G., Stal, L.J., Matthijs, H.C.P., Davis, T.W., Paerl, H.W., Huisman, J., 2016. How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145–159.
Watanabe, M.F., Harada, K.-I., Carmichael, W.W., Fujiki, H., 1996. Toxic Microcystis. CRC, Boca Raton. CRC press. 103-148.
Welch, P.S., 1948. Limnological Methods. 381. The Blaikston Co., Philadelphia.
Wilson, A.E., Gossiaux, D.C., Höök, T.O., Berry, J.P., Landrum, P.F., Dyble, J., Guildford, S.J., 2008. Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (Perca flavescens). Can. J. Fish. Aquat. Sci. 65, 1487–1497.
Wood, J.D., Franklin, R.B., Garman, G., McIninch, S., Porter, A.J., Bukaveckas, P.A., 2014. Exposure to the cyanotoxin microcystin arising from interspecific differences in feeding habits among fish and shellfish in the James River Estuary, Virginia. Environ. Sci. Technol. 48, 5194–5202.
Wu, X., Xiao, B., Li, R., Wang, C., Huang, J., Wang, Z., 2011. Mechanisms and factors affecting sorption of microcystins onto natural sediments. Environ. Sci. Technol. 45, 2641–2647.
Xie, L., Xie, P., Li, S., Tang, H., Liu, H., 2003. The low TN:TP ratio, a cause or a result of Microcystis blooms? Water Res. 37, 2073–2080.
Xie, L., Xie, P., Guo, L., Li, L., Miyabara, Y., Park, H.D., 2005. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. Environ. Toxicol. 20, 293–300.
Xue, Q., Steinman, A.D., Su, X., Zhao, Y., Xie, L., 2016. Temporal dynamics of microcystins in Limnodrilus hoffmeisteri, a dominant oligochaete of hypereutrophic Lake Taihu, China. Environ. Pollut. 213, 585–593.
Xue, Q., Steinman, A.D., Xie, L., Yao, L., Su, X., Cao, Q., Zhao, Y., Cai, Y., 2020. Seasonal variation and potential risk assessment of microcystins in the sediments of Lake Taihu, China. Environ. Pollut. 259, 113884.
Yih, W.-H., Myung, G.-O., Yoo, Y.-D., Kim, Y.-G., Jeong, H.-J., 2005. Semiweekly variation of spring phytoplankton community in relation to the freshwater discharges from Keum River estuarine weir, Korea. The Sea 10, 154–163.
Yuan, L.L., Pollard, A.I., Pather, S., Oliver, J.L., D'Anglada, L., 2014. Managing microcystin: identifying national-scale thresholds for total nitrogen and chlorophylla. Freshw. Biol. 59, 1970–1981.
Zastepa, A., Pick, F.R., Blais, J.M., 2014. Fate and persistence of particulate and dissolved microcystin-LA from Microcystis blooms. Hum. Ecol. Risk. Assess. 20, 1670–1686.

<Supplementary Materials>

Distribution of microcystins in environmental multimedia and their bioaccumulation characteristics in marine benthic organisms in the Geum River Estuary, South Korea

Mungi Kim[1], Dokyun Kim[1], Jaeseong Kim, Seongjin Hong[*], Kyung-Hoon Shin[*]

Supplementary Tables

Supplementary Figures

---

[1] M.K. and D.K. contributed equally to this work.

[*]**Corresponding authors.**
E-mail addresses: hongseongjin@cnu.ac.kr (S. Hong); shinkh@hanyang.ac.kr (K.H. Shin).

Supplementary Tables

Table S1. Instrumental conditions for the analysis of microcystins using HPLC-MS/MS.

Instrument	HPLC: Agilent Infinity 1290 II, MS/MS: SCIEX Qtrap 6500
Column	Poroshell 120 EC-C18. 4.6 × 50 mm, 2.7 μm
Column temperature	30 ℃
Mobile phase	A: Methanol, B: 0.1% formic acid in water
Mobile phase gradient	70% A (0-1 min) → 70–0% A (1-7 min) → 0% A (7-11 min) → 0-70% A (11-12 min) 30% B (0-1 min) → 30-100% B (1-7 min) → 100% B (7-11 min) → 100-30% B(11-12 min)
Injection volume	10 μL
Flow rate	0.3 mL min^{-1}
Ion source	ESI+ (electrospray ionization)
Ion spray voltage	5500 V
Source temperature	600 ℃
Curtain gas	N_2 (35 psi)
Ion source gas 1	N_2 (50 psi)
Ion source gas 2	N_2 (50 psi)

Table S2. Conditions of tandem mass spectrometry parameters for the analysis of microcystins.

Compounds	Molecular weight	MRM transition Parent ion → Daughter ion (m/z)	DP (volts)	CE (volts)	CXP (volts)	Dwell (msec)
MC-LR	995.2	995.4 → 103.1 (ESI+)	41	129	14	50
MR-RR	1038.2	519.9 → 103.0 (ESI+)	146	37	6	50
MC-YR	1045.2	1045.4 → 103.0 (ESI+)	16	127	14	50
MC-LA	910.0	910.4 → 135.0 (ESI+)	201	89	14	150
MC-LF	986.2	986.4 → 135.1 (ESI+)	261	93	16	150
MC-LY	1002.2	1002.5 → 134.9 (ESI+)	246	93	18	150
MC-LW	1025.2	1025.5 → 135.2 (ESI+)	291	97	8	150
Monolinuron[a]	214.7	215.0 → 126.2 (ESI+)	111	25	6	150
Enkephalin[b]	555.6	556.2 → 120.1 (ESI+)	141	71	8	50

[a] Internal standard.
[b] Surrogate standard.

Table S3. Water quality parameters and concentrations of microcystins in discharged water collected from the Geum River (Site D1) from May to October, 2018.

Parameters	11 May	24 May	15 Jun.	18 Jun.	26 Jun.	3 Jul.	4 Jul.	8 Aug.	22 Aug.	3 Sep.	20 Sep.	8 Oct.
Temperature (°C)	20	22	27	26	26	22	24	37	31	23	27	20
Salinity (psu)	0.28	0.14	0.16	0.17	0.17	0.07	0.06	0.24	0.16	0.08	0.13	0.16
pH	9.2	9.2	9.5	8.8	9.4	8.1	7.8	10.8	9.8	7.5	8.4	8.4
DO (mg L^{-1})[a]	9.3	10.1	10.4	6.9	7.1	3.8	5.8	12	6.9	4.5	8.6	8.4
SS (mg L^{-1})[b]	14	20.4	48	44	18	57	21	64	37	6.4	5.4	4.8
NO_2-N (mg L^{-1})	0.047	0.055	0.13	0.11	0.089	0.046	0.041	0.001	0.002	0.034	0.024	0.033
NO_3-N (mg L^{-1})	1.9	1.5	1.8	1.4	0.82	1.6	1.8	ND[g]	ND	2.1	1.6	2.6
NH_4-N (mg L^{-1})	0.063	0.091	0.025	0.041	0.071	0.33	0.24	0.021	0.017	0.16	0.024	0.097
TDN (mg L^{-1})[c]	2.7	2.9	4.2	4.3	1.8	2.5	2.9	6.9	0.99	2.9	2.4	3.2
PO_4-P (mg L^{-1})	0.012	0.011	0.011	0.012	0.018	0.036	0.062	0.018	0.094	0.086	0.029	0.19
TP (mg L^{-1})[d]	0.079	0.076	0.49	0.37	0.12	0.17	0.13	0.94	0.16	0.13	0.064	0.24
SiO_2-Si (mg L^{-1})	0.35	0.11	0.43	0.49	0.49	0.27	0.32	0.26	0.37	0.41	0.27	0.36
Chl.a (μg L^{-1})	28	24	96	13	65	6.9	6.3	308	42	1.3	12	9.6
PMCs (μg L^{-1})[e]	0.051	0.11	116	3.6	9.1	ND	ND	236	8.4	1.4	1.2	0.62
DMCs (μg L^{-1})[f]	0.063	0.021	0.66	3.8	1.1	0.11	0.17	0.94	0.38	0.072	0.27	0.039

[a] Dissolved oxygen.
[b] Suspended solids.
[c] Total dissolved nitrogen.
[d] Total phosphorus.
[e] Particulate microcystins.
[f] Dissolved microcystins.
[g] Not detected.

Table S4. Water quality parameters in seawater collected from the Geum River Estuary (Site D2) from May to October, 2018.

Parameters	11 May	24 May	15 Jun	18 Jun	26 Jun	3 Jul	4 Jul	8 Aug	22 Aug	3 Sep	20 Sep	8 Oct
Temperature (°C)	18.6	20.6	21.5	24.5	24.5	24	23.0	32.1	30.3	24.6	24.5	20.3
Salinity (psu)	16.9	14.0	11.5	7.9	18.1	0.3	0.3	28.3	17.2	0.6	18.1	2.0
pH	8.1	7.8	7.7	8.0	7.9	7.5	7.5	8.1	8.3	7.4	7.2	8.2
DO (mg L^{-1})[a]	7.8	6.3	5.4	6.1	4.8	4.0	4.7	3.7	4.6	3.6	5.4	6.6
SS (mg L^{-1})[b]	27.2	30.4	78.5	104	33.6	180	64	42	41	20.8	32	21.6
NO_2-N (mg L^{-1})	0.039	0.051	0.041	0.097	0.054	0.087	0.078	0.019	0.023	0.042	0.045	0.031
NO_3-N (mg L^{-1})	0.71	0.76	0.72	0.93	0.32	1.6	1.7	0.043	0.034	2.2	0.71	1.7
NH_4-N (mg L^{-1})	0.43	0.49	0.37	0.16	0.36	0.49	0.41	0.19	0.35	0.17	0.42	0.17
TDN (mg L^{-1})[c]	2.02	2.1	1.6	2.9	1.5	2.9	3.4	0.68	0.71	3.4	2.4	2.9
PO_4-P (mg L^{-1})	0.037	0.038	0.087	0.031	0.062	0.075	0.066	0.063	0.11	0.089	0.073	0.073
TP (mg L^{-1})[d]	0.095	0.108	0.15	0.26	0.11	0.31	0.24	0.12	0.19	0.14	0.13	0.15
SiO_2-Si (mg L^{-1})	0.109	0.406	0.39	0.38	0.25	0.28	0.36	0.22	0.31	0.43	0.23	0.29
Chl.a (μg L^{-1})	8.8	8.9	21	15.1	5.3	9.3	7.1	15	16	4.9	14	17

[a] Dissolved oxygen.
[b] Suspended solids.
[c] Total dissolved nitrogen.
[d] Total phosphorus.

Table S5. Abundance of phytoplankton in the water column of the Geum River (Site D1, inside of dam) and estuarine area (Site D2, outside of dam) during May to October, 2018.

Site	Date (dd-month)	Diatom (cells mL^{-1})	Dinoflagellate (cells mL^{-1})	Euglenophyceae (cells mL^{-1})	Chlorophyceae (cells mL^{-1})	Cryptophyceae (cells mL^{-1})	Cyanophyceae (cells mL^{-1})
D1	11-May	7735	5.7	-	792	211	228
	24-May	6463	-[a]	-	800	808	69
	15-Jun.	104	-	-	153	2.3	139569
	18-Jun.	277	8.3	-	59	20.1	74526
	26-Jun.	85	11	-	153	2.3	18596
	03-Jul.	157	-	-	-	-	13
	04-Jul.	47	1.3	-	-	-	330
	08-Aug.	6	-	0.43	-	-	103974
	22-Aug.	57	3.4	-	23	-	31125
	03-Sep.	49.5	0.75	-	0	2.3	96
	20-Sep.	47	-	-	11	48	12055
	08-Oct.	170	2.2	0.86	36	0.43	80
D2	11-May	351	22	16	86	1985	54
	24-May	70	409	32	84	551	-
	15-Jun.	77	51	-	63	48	9650
	18-Jun.	335	2	-	872	-	15
	26-Jun.	58	5	-	-	14	393
	03-Jul.	105	-	-	-	2	-
	04-Jul.	12	-	-	-	-	5
	08-Aug.	41	-	-	-	-	123
	22-Aug.	512	-	-	-	-	516
	03-Sep.	38	-	-	-	-	34
	20-Sep.	662	-	-	-	-	18
	08-Oct.	91	-	-	12	0	29

[a] - Not detected.

Table S6. Cell abundance of phytoplankton in seawater collected from the Geum River Estuary (Site D2) from May to October, 2018.

Date	26 June				27 June				28 June				29 June				30 June			
Parameter	T[a]	S[b]	pH	EC[c]	T	S	pH	EC	T	S	pH	EC	T	S	pH	EC	T	S	pH	EC
GR1	25	0.2	8.5	0.5	29	0.2	8.5	0.4	25	0.2	7.8	0.3	27	0.2	7.7	0.1	21	0.0	7.9	0.0
GR2	25	9.5	8.2	16	27	0.6	7.9	1.1	25	1.6	7.7	2.9	26	9.9	7.8	17	24	18	7.4	29
GR3	24	19	8.0	32	26	2.5	8.2	4.5	25	0.8	7.6	1.5	25	15	7.8	25	24	19	7.5	30
GR4	23	28	7.9	46	25	16	8.0	21	24	20	7.8	33	25	21	7.9	36	24	19	7.5	37
GR5	23	30	7.9	44	26	12	8.0	26	24	18	7.9	29	27	23	7.9	33	24	23	7.3	30
GR6	-[d]	-	-	-	-	-	-	-	24	28	8.1	44	-	-	-	-	-	-	-	-
GR7	-	-	-	-	-	-	-	-	23	29	8.0	45	-	-	-	-	-	-	-	-
GR8	-	-	-	-	-	-	-	-	24	16	7.9	25	-	-	-	-	-	-	-	-

[a] Temperature (℃).
[b] Salinity (psu).
[c] Electronical conductivity (μS/cm).
[d] not surveyed.

Table S7. Concentrations of particulate MCs in seawater from the Geum River Estuary.

Date	Site	MC-LR (μg L^{-1})	MC-RR (μg L^{-1})	MC-YR (μg L^{-1})	MC-LA (μg L^{-1})	MC-LF (μg L^{-1})	MC-LY (μg L^{-1})	MC-LW (μg L^{-1})	∑MCs (μg L^{-1})
26 June	GR1	3.34	2.71	0.59	<LOD[a]	0.07	0.87	0.23	7.81
	GR2	1.19	0.77	0.15	<LOD	<LOD	0.19	0.37	2.68
	GR3	0.05	0.12	0.01	<LOD	<LOD	<LOD	<LOD	0.18
	GR4	0.02	0.05	0.005	<LOD	<LOD	<LOD	<LOD	0.08
	GR5	0.03	0.04	0.01	<LOD	<LOD	<LOD	<LOD	0.08
27 June	GR1	0.61	0.62	0.10	<LOD	<LOD	0.14	<LOD	1.48
	GR2	0.27	0.60	0.06	<LOD	<LOD	<LOD	<LOD	0.92
	GR3	1.19	1.03	0.17	<LOD	<LOD	0.28	<LOD	2.7
	GR4	0.10	0.24	0.02	<LOD	<LOD	<LOD	<LOD	0.36
	GR5	0.51	0.26	0.09	<LOD	<LOD	0.11	<LOD	0.97
28 June	GR1	0.32	0.35	0.04	<LOD	<LOD	0.07	<LOD	0.78
	GR2	0.18	0.16	0.03	<LOD	<LOD	0.07	<LOD	0.44
	GR3	0.22	0.22	0.03	<LOD	<LOD	<LOD	<LOD	0.46
	GR4	0.04	0.05	0.01	<LOD	<LOD	<LOD	<LOD	0.10
	GR5	0.03	0.03	0.01	<LOD	<LOD	<LOD	<LOD	0.07
	GR6	0.00	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
	GR7	0.02	0.02	0.003	<LOD	<LOD	<LOD	<LOD	0.04
	GR8	0.01	0.02	0.003	<LOD	<LOD	<LOD	<LOD	0.03
29 June	GR1	0.03	0.09	0.004	<LOD	<LOD	<LOD	<LOD	0.12
	GR2	0.02	0.02	0.004	<LOD	<LOD	<LOD	<LOD	0.05
	GR3	0.01	0.01	0.003	<LOD	<LOD	<LOD	<LOD	0.01
	GR4	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
	GR5	0.04	0.03	0.01	<LOD	<LOD	<LOD	<LOD	0.08
30 June	GR1	0.01	0.04	<LOD	<LOD	<LOD	<LOD	<LOD	0.04
	GR2	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	0.02
	GR3	0.02	0.02	0.005	<LOD	<LOD	<LOD	<LOD	0.05
	GR4	0.01	0.01	<LOD	<LOD	<LOD	<LOD	<LOD	0.01
	GR5	0.02	0.01	0.003	<LOD	<LOD	<LOD	<LOD	0.04

[a] <LOD: Below limit of detection.

Table S8. Concentrations of dissolved MCs in seawater from the Geum River Estuary.

Date	Site	MC-LR (μg L^{-1})	MC-RR (μg L^{-1})	MC-YR (μg L^{-1})	MC-LA (μg L^{-1})	MC-LF (μg L^{-1})	MC-LY (μg L^{-1})	MC-LW (μg L^{-1})	∑MCs (μg L^{-1})
26 June	GR1	0.52	0.04	0.01	0.04	0.01	0.04	0.02	0.70
	GR2	3.0	0.18	0.08	0.03	<LOD[a]	0.02	0.05	3.3
	GR3	2.5	0.10	0.06	0.01	<LOD	0.01	0.08	2.8
	GR4	0.93	0.03	0.02	<LOD	<LOD	<LOD	0.04	1.02
	GR5	0.72	0.03	0.02	<LOD	<LOD	<LOD	0.01	0.78
27 June	GR1	1.7	0.06	0.04	0.02	0.01	0.02	0.04	1.9
	GR2	1.3	0.13	0.03	0.02	<LOD	0.02	0.02	1.6
	GR3	0.91	0.06	0.02	0.03	<LOD	0.02	0.03	1.1
	GR4	2.0	0.16	0.04	<LOD	<LOD	0.03	0.04	2.3
	GR5	1.2	0.10	0.03	0.03	<LOD	0.01	0.04	1.5
28 June	GR1	1.03	0.08	0.04	0.04	<LOD	0.01	0.04	1.3
	GR2	1.2	0.07	0.04	0.06	<LOD	<LOD	0.04	1.4
	GR3	0.86	0.05	0.03	0.05	<LOD	<LOD	0.02	0.99
	GR4	1.4	0.08	0.03	0.01	<LOD	0.01	0.03	1.6
	GR5	1.5	0.09	0.03	0.03	<LOD	<LOD	0.01	1.6
	GR6	0.51	0.03	0.01	<LOD	<LOD	<LOD	0.03	0.57
	GR7	0.63	0.04	0.01	<LOD	<LOD	<LOD	<LOD	0.67
	GR8	0.77	0.07	0.02	0.02	<LOD	0.01	0.04	0.92
29 June	GR1	1.3	0.01	0.03	0.05	0.02	0.01	0.02	1.5
	GR2	1.6	0.09	0.03	0.03	<LOD	0.02	0.06	1.8
	GR3	1.2	0.08	0.02	0.02	0.01	0.01	0.04	1.4
	GR4	1.7	0.08	0.02	0.01	<LOD	0.01	0.03	1.8
	GR5	1.02	0.07	0.02	0.01	<LOD	0.02	0.02	1.6
30 June	GR1	0.43	0.01	0.01	0.02	<LOD	0.01	0.02	0.5
	GR2	1.6	0.12	0.02	0.01	<LOD	0.03	0.02	1.8
	GR3	1.7	0.10	0.02	0.01	<LOD	0.01	0.03	1.8
	GR4	1.8	0.08	0.02	0.02	<LOD	0.02	0.07	2.0
	GR5	1.1	0.05	0.02	0.01	<LOD	0.01	0.02	1.3

[a] <LOD: Below limit of detection.

Table S9. Concentrations of MCs in sediments from the Geum River Estuary.

Date	Site	MC-LR (ng g^{-1})	MC-RR (ng g^{-1})	MC-YR (ng g^{-1})	MC-LA (ng g^{-1})	MC-LF (ng g^{-1})	MC-LY (ng g^{-1})	MC-LW (ng g^{-1})	∑MCs (ng g^{-1})
26 June	GR1	324	20	3.2	<LOD[a]	42	62	48	451
	GR2	135	26	18	<LOD	LOD	29	41	208
	GR3	5.8	63	1.1	<LOD	40	<LOD	43	110
	GR4	1.6	25	LOD	<LOD	<LOD	<LOD	<LOD	26
	GR5	0.9	49	LOD	<LOD	<LOD	<LOD	<LOD	50
27 June	GR1	98	26	13	<LOD	100	33	99	270
	GR2	14	17	1.7	<LOD	24	<LOD	46	57
	GR3	31	21	6.4	<LOD	38	18	41	115
	GR4	46	48	5.5	<LOD	21	19	<LOD	139
	GR5	LOD	52	LOD	<LOD	23	<LOD	<LOD	75
28 June	GR1	6.8	14	1.6	<LOD	<LOD	6.3	32	29
	GR2	18	10	2.5	<LOD	<LOD	16	45	47
	GR3	122	24	20	<LOD	<LOD	41	45	206
	GR4	3.7	53	0.72	<LOD	<LOD	<LOD	<LOD	57
	GR5	244	88	32	<LOD	<LOD	51	62	414
	GR6	LOD	23	LOD	<LOD	<LOD	<LOD	<LOD	22
	GR7	LOD	244	LOD	<LOD	14	<LOD	<LOD	258
	GR8	0.8	18	LOD	<LOD	<LOD	<LOD	<LOD	19
29 June	GR1	32	34	5.2	<LOD	<LOD	<LOD	114	71
	GR2	173	19	14	<LOD	15	30	26	250
	GR3	19	11	2.4	<LOD	23	14	37	69
	GR4	159	14	18	<LOD	56	59	58	306
	GR5	1.1	13	LOD	<LOD	16	LOD	17	30
30 June	GR1	158	17	19	<LOD	53	36	<LOD	283
	GR2	413	46	38	<LOD	<LOD	<LOD	<LOD	497
	GR3	382	24	338	<LOD	73	96	69	612
	GR4	20	10	1.6	<LOD	24	<LOD	29	55
	GR5	15	19	2.1	<LOD	45	<LOD	<LOD	81

[a] <LOD: Below limit of detection.

Table S10. Concentrations of MCs in intertidal organisms from the Geum River Estuary.

Species	Site	Date	MC-LR (μg g^{-1})	MC-RR (μg g^{-1})	MC-YR (μg g^{-1})	MC-LA (μg g^{-1})	MC-LF (μg g^{-1})	MC-LY (μg g^{-1})	MC-LW (μg g^{-1})	∑MCs (μg g^{-1})
Polychaete										
Neanthes japonica	B1	27 June	2.9	0.3	0.27	0.023	0.038	0.22	0.044	3.9
Neanthes japonica	B2	28 June	4.9	0.027	3.2	0.027	0.022	0.11	0.21	8.5
Neanthes sp. #1			6.1	0.044	1.2	<LOD	<LOD	0.031	0.39	7.8
Neanthes sp. #2			4.6	0.035	0.55	<LOD	<LOD	0.19	0.38	5.8
Neanthes sp. #3			2.9	0.094	0.25	<LOD	0.018	0.14	0.059	3.5
Nephtys sp.			0.85	<LOD[a]	0.45	<LOD	<LOD	0.074	0.13	1.5
Glycera sp. #1			2.3	<LOD	3.4	<LOD	0.068	0.37	0.83	7.02
Glycera sp. #2			1.5	<LOD	1.2	0.059	0.149	0.22	0.23	3.4
Lumbrineris sp.			0.03	<LOD	0.057	0.013	0.014	0.052	0.21	0.38
Neanthes japonica	B1	29 June	8.9	2.08	1.4	0.017	0.075	0.27	0.38	13
Decapod										
Hemigrapsus sp.	B1	27 June	0.31	<LOD	<LOD	<LOD	<LOD	<LOD	0.11	0.42
Hemigrapsus sp. #1	B2	28 June	5.6	0.54	0.34	0.05	0.039	0.36	0.12	7.04
Hemigrapsus sp. #2			3.5	0.36	0.38	0.21	0.081	0.62	1.3	6.5
Hemigrapsus sp. #3			2.3	0.15	0.15	<LOD	0.11	0.24	0.68	3.7
Hemigrapsus sp.	B1	29 June	0.36	0.18	0.056	<LOD	<LOD	0.22	0.51	1.3
Amphipod										
Mandibulophoxus sp. #1	B2	28 June	0.18	<LOD	0.042	<LOD	0.061	0.15	0.77	1.2
Mandibulophoxus sp. #2			0.26	<LOD	0.11	0.069	0.11	0.14	0.18	0.86
Mandibulophoxus sp. #3			0.12	<LOD	0.044	<LOD	<LOD	0.021	0.19	0.38
Bivalve										
Glauconome sp. #1	B1	27 June	0.43	0.084	0.041	<LOD	<LOD	0.043	0.085	0.68
Glauconome sp. #2	B2	28 June	0.42	<LOD	0.22	0.042	0.087	0.13	0.54	1.4
Glauconome sp. #3	B1	29 June	0.56	<LOD	0.52	<LOD	<LOD	0.13	0.6	1.8

[a] <LOD: Below limit of detection.

Supplementary Figures

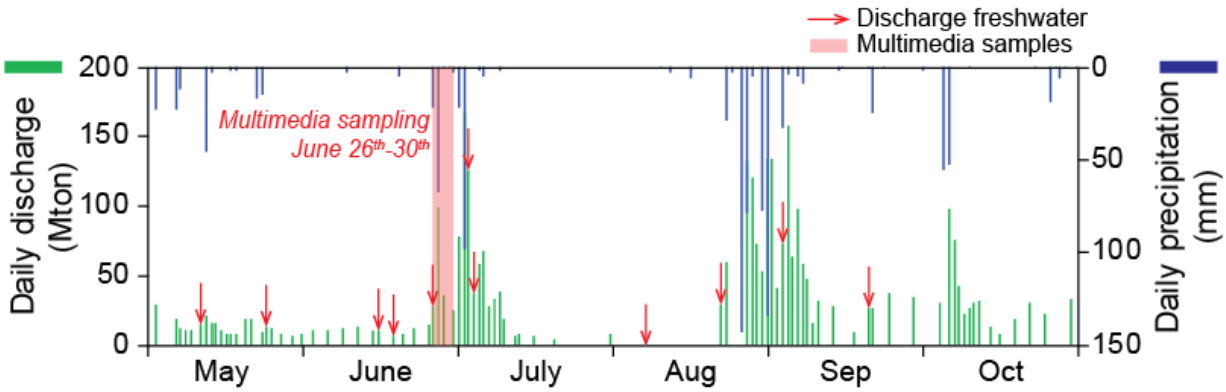


Fig. S1. Daily discharge (Mton) and precipitation (mm) in the Geum River Estuary. Surface water samples for identifying phytoplankton communities were collected on certain days (red arrow). Environmental multimedia samples were collected on certain days (red box).

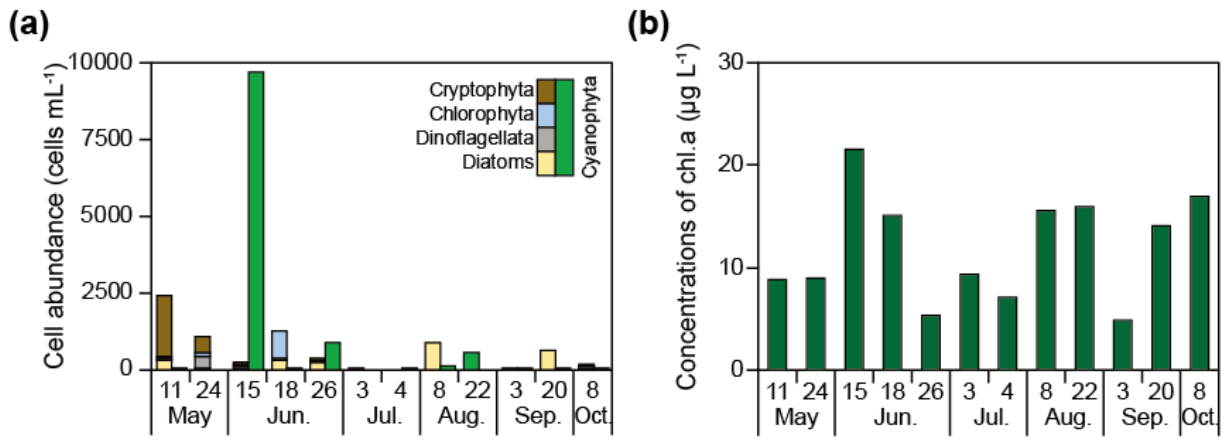


Fig. S2. (a) Abundance of phytoplankton and (b) concentrations of Chl *a* in seawater at Site D2 (outside of estuary dam) in the Geum River Estuary.

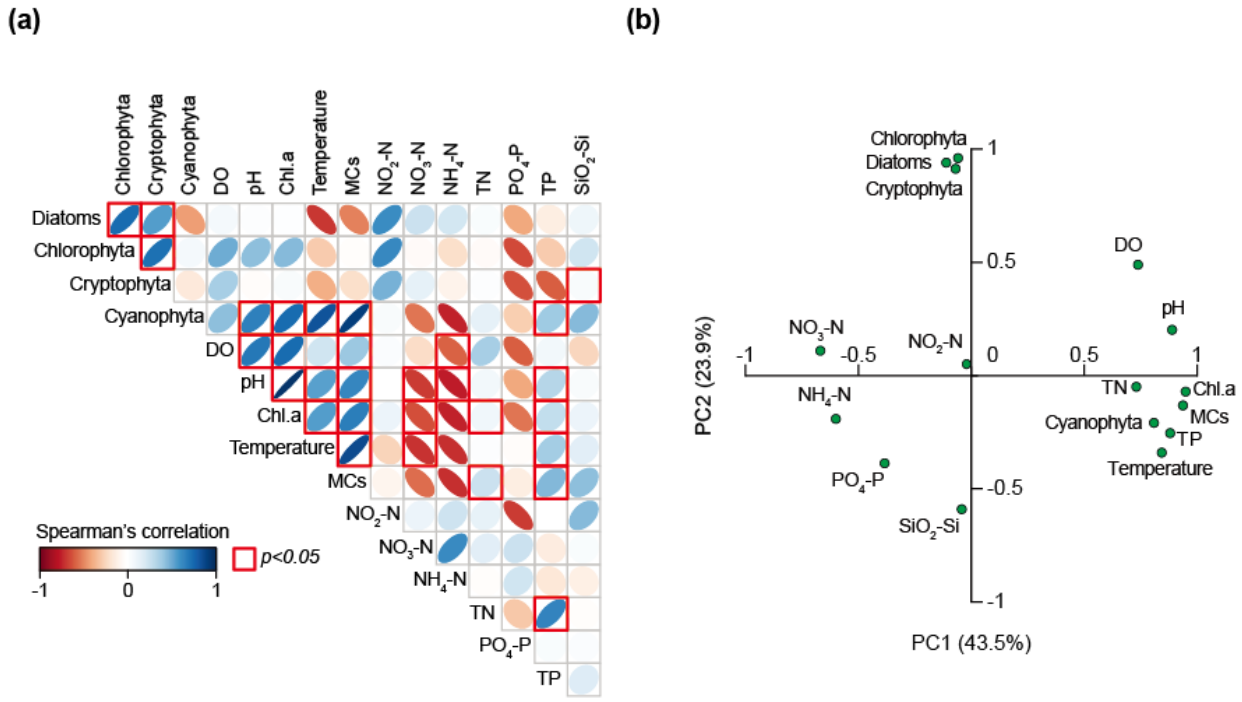


Fig. S3. (a) Spearman's rank correlation between environmental variables and phytoplankton communities in the Geum River and (b) results of the principal component analysis.

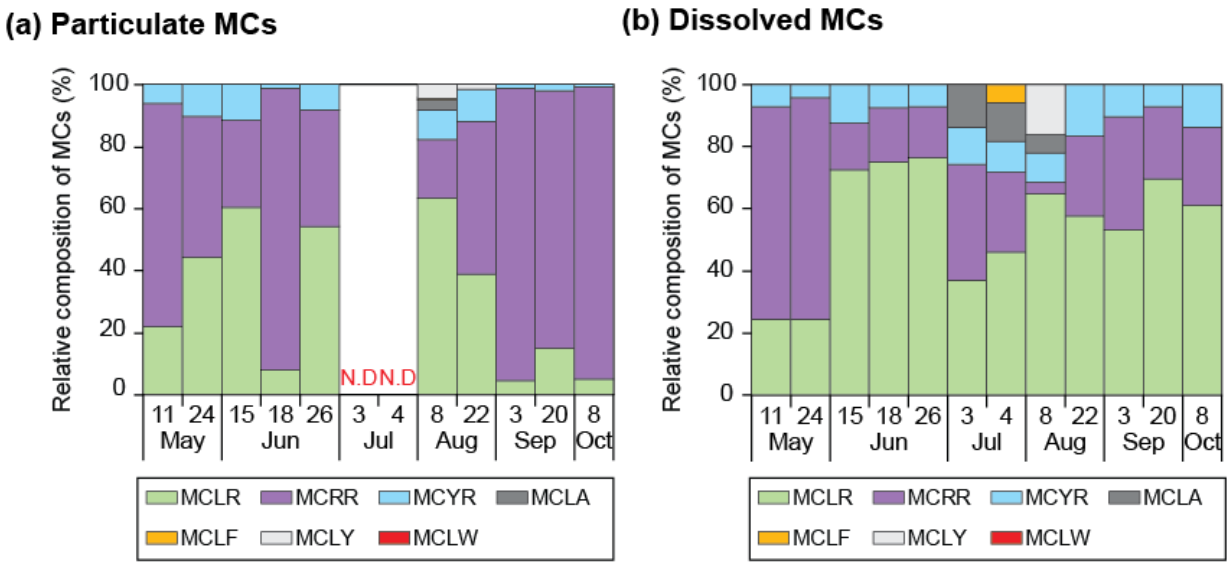


Fig. S4. Relative composition of (a) particulate MCs and (b) dissolved MCs inside the estuary dam of the Geum River.