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Multimedia distributions and the fate of microcystins from freshwater discharge in the Geum River Estuary, South Korea: Applicability of POCIS for monitoring of microalgal biotoxins☆

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ABSTRACT

Here, we investigated the characteristics of the environmental multimedia distribution of microcystins (MCs) introduced from freshwater discharge through the estuary dam of the Geum River. In addition, the applicability of a passive sampling device (polar organic chemical integrative sampler, POCIS) for monitoring MCs was evaluated. Surface water, suspended solids (SS), sediments, and oysters were collected from the inner and outer estuary dam. Seven MC variants were analyzed using HPLC-MS/MS. POCIS was deployed at three sites over one week, and MCs were monitored for four weeks from August to September 2019. Before POCIS was deployed in the field, compounds-specific sampling rates of MCs were determined as functions of water temperature (10, 20, and 30 °C), flow rate (0, 0.38, and 0.76 m s^{-1}), and salinity (0, 15, and 30 psu) in the laboratory. The sampling rates of MCs in POCIS increased significantly with increasing water temperature and flow rate, whereas salinity did not significantly affect the sampling rates between freshwater and saltwater. The MCs in the Geum River Estuary mainly existed as particulate forms (mean: 78%), with relatively low proportions of dissolved forms (mean: 22%), indicating that MCs were mainly contained in cyanobacterial cells. There was no significant correlation among the concentrations of MCs in water, SS, sediments, and oysters. Time-weighted average concentrations of MCs from POCIS were not significantly correlated with the concentrations of MCs in water and oysters. The metabolites of MCs, including MC-LR-GSH, MC-LR-Cys, MC-RR-GSH, and MC-RR-Cys, were detected in oysters (no metabolites were detected in POCIS). Overall, POCIS can be useful for monitoring dissolved MCs in the aquatic ecosystem, particularly in calculating time-weighted average concentrations, but it seems to have limitations in evaluating the contamination status of total MCs, mainly in particulate form.

Credit author contribution statement

Mungi Kim: Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft. Seongjin Hong: Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition, Supervision. Jihyun Cha: Investigation, Formal analysis. Youngnam Kim: Investigation, Formal analysis. Chang-Eon Lee: Investigation, Formal analysis. Yoonyoung An: Investigation, Formal analysis. Kyung-Hoon Shin: Writing – review & editing, Project administration, Funding acquisition.

1. Introduction

Microcystins (MCs) are representative biotoxins produced by harmful freshwater cyanobacteria (such as *Microcystis*, *Dolichospermum*, *Planktothrix*, *Nostoc*, and *Oscillatoria*), and are widely distributed in freshwater environments globally (Chorus et al., 2000; Paerl and Otten, 2013). MCs belonging to the hepatotoxin cyclic peptide group threaten aquatic ecosystems (Carmichael, 2012; Osborne et al., 2007) and cause

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serious health problems in humans (Buratti et al., 2017; Svirčev et al., 2017). To date, over 250 MC variants have been detected (Spoof and Catherine, 2016), with representative MCs in aquatic ecosystems, including MC-LR, -RR, and -YR (Liu and Sun, 2015). MCs inhibit the activity of protein phosphatase 2A, altering the expression of microRNA, and induce DNA damage, cytoskeleton disruption, autophagy, and apoptosis (Chen et al., 2019; Liu et al., 2018; Yang et al., 2018). To manage the potential risk of MCs, the World Health Organization (WHO) has developed a tentative guideline allowing up to 1 μg MCs L^{-1} in human drinking water, with many countries using this guideline (WHO, 2020).

MCs have a cyclical heptapeptide structure that is chemically stable (Krishnamurthy et al., 1989), and they are resistant at high temperatures (e.g., 40 °C) and under extreme pH conditions (e.g., pH 1) (Harada et al., 1996). MCs exist in the water column in a form contained in cyanobacterial cells (particulate phase) (Jones and Orr, 1994), and are released into the water through cell destruction and/or exocytosis (dissolved phase) (Sivonen and Jones, 1999). MCs are introduced to estuarine environments through freshwater discharge, and occur in dissolved and particulate forms that might partially accumulate in sediments (Kankaanpaa et al., 2009). Previous studies showed that MCs in estuarine environments have a low correlation between environmental media samples, such as water, suspended solids (SS), and sediments, and exhibit heterogeneous distributions depending on the extent of toxic cyanobacteria present (Kim et al., 2019, 2021). Since toxic algae have patchy distributions, the concentrations of MCs vary greatly depending on sampling methods. In addition, MCs accumulate in tidal flat organisms, including amphipods, bivalves, decapods, and polychaetes (Kim et al., 2021; Umehara et al., 2015), and can be transferred to a higher trophic level through the food chain (Smith and Haney, 2006; Xie et al., 2005). A previous study indicated that MCs do not biomagnify through the marine food chain, due to biodilution and/or metabolic processes (Kim et al., 2019).

To date, the multimedia distributions and bioaccumulation of MCs in the marine environment are not clearly understood, with most monitoring studies being performed using grab sampling. Traditional grab sampling methods have been used to monitor pollutants in the aquatic environments, but lack temporal representativeness because they only reflect the water quality at the time of sampling (Allan et al., 2006). In addition, when a compound is present at low concentrations, it is often difficult to detect. Thus, to use grab sampling to track the dynamic behavior of pollutants in the aquatic environment, repeated sampling is required, which is expensive and labor-intensive (Li et al., 2016). Passive samplers, such as the polar organic chemical integrative sampler (POCIS), are considered viable alternatives to grab sampling. POCIS has been widely used to collect polar organic pollutants, including pesticides and pharmaceuticals (Bailly et al., 2013; Guibal et al., 2018; Li et al., 2010; Van Metre et al., 2017). POCIS measures the time-weighted average (TWA) concentrations of target compounds present in the aquatic environment, and provides sensitive data over long periods facilitating the large-scale monitoring of contaminants in the aquatic environment (Lohmann et al., 2017; Xiong et al., 2019).

The Geum River Estuary has been completely blocked from the inflow of seawater since the estuary dam was constructed in 1994, and has partially mixed estuary characteristics (Lee et al., 1999). During freshwater discharge in summer monsoon periods, cyanobacteria that produce MCs (such as *Microcystis* sp. and *Dolichospermum* sp.) flow into the estuary at a density of more than 10,000 cells mL^{-1} (Kim et al., 2018). More than 171 species of phytoplankton have been reported in the Geum River Estuary, with dominant species, including Bacillariophyceae, Dinoflagellata, Chlorophyceae, and Cyanophyceae (Kim et al., 2018, 2019). Previous studies confirmed the introduction of MCs into the estuary, which were transported far into marine environments along the surface low-salinity layer, bioaccumulating in marine organisms (Kim et al., 2021).

The present study investigated the environmental distribution and bioaccumulation characteristics of MCs in various media introduced from freshwater discharge through the estuary dam of the Geum River during summer. In addition, the applicability of POCIS in monitoring MCs in water and organisms was evaluated. The main objectives were to: (i) measure compound-specific sampling rates of MCs in POCIS under various environmental conditions, (ii) investigate the multimedia distributions of MCs in the inner and outer estuary dam during summer, (iii) apply POCIS to estimate TWA concentrations in water compared to grab samples, and (iv) compare the concentrations and compositions of detected MCs in POCIS with oysters collected from the Geum River Estuary.

2. Materials and methods

2.1. Preparation of POCIS

POCIS, metal holders, and canisters used in the present study were obtained from Environmental Sampling Technologies Inc. (St. Joseph, MO). POCIS contained 0.2 ± 0.01 g Oasis HLB sorbent (Waters, Milford, MA), and the sorbent is enclosed between two polyethersulfone (PES) membranes (0.1 μm pore size, 90 mm diameter). All membrane-sorbent-membranes were fixed using metal holder washers. The exchange surface area of POCIS used in the present study was about 47.5 cm^2 (both sides).

2.2. Laboratory calibration of POCIS

Laboratory calibration experiments were conducted to determine compound-specific sampling rates of MCs in POCIS (MC-LR, -RR, and -YR), depending on the effects of water temperature, flow rate, and salinity. To apply POCIS to the estuarine environment, major physical factors that might affect the sampling rates of MCs were considered. Three water temperature conditions (10, 20, and 30 °C) were set. The effects of flow rate (turbulent conditions) and salinity were evaluated at 0, 0.38, and 0.76 m s^{-1} and 0, 15, and 30 psu, respectively. The flow rate experiment was conducted on a horizontal mechanical shaker, controlled by the revolutions per minute (rpm), and was converted to flow rate (50 rpm = 0.38 m s^{-1}) (Sesa et al., 2020). These flow rates could be observed in the actual estuarine environments (0.1–1.3 m s^{-1}) (Korea Hydrographic and Oceanographic Agency (KHOA), 2021).

The MCs were spiked in 2 L deionized water or salinity-adjusted water using sea salt to obtain a final concentration of 2 μg L^{-1}. The beakers were stirred to reach equilibrium for 1 h. POCIS was immersed in deionized water overnight to avoid an increase in sampling at the beginning of the experiments (Alvarez, 1999). All beakers were covered with aluminum foil to prevent photolysis and minimize volatilization.

One POCIS per one beaker was deployed vertically and exposed for 7 days. All experiments were conducted in duplicate. To determine the loss of MCs by volatilization, adsorption, or decomposition during the experiments, a blank experiment was performed without adding POCIS to deionized water spiked with MCs (positive control). As a negative control, POCIS was added to deionized water without MCs to evaluate contamination during the experiment. The results indicated that there was no loss or contamination of MCs during the experiments.

Sampling rates were calculated according to a previously described method (MacLeod et al., 2007). To calculate the sampling rates, MC concentration was determined by aliquoting 10 mL water from each beaker every 24 h, and was calculated according to Eqs. (1) and (2) (Fig. S1).

$$C_w(t) = C_w(0)\exp[-(k_u + k_D)t] = C_w(0)\exp[-kt] \quad \text{(Eq. 1)}$$

$$\mathrm{Ln}[C_w(t) / C_w(0)] = -kt \quad \text{(Eq. 2)}$$

where k_u and k_D are the uptake rate constant and dissipation rate constant, respectively; $C_w(0)$ and $C_w(t)$ are the concentrations in water at

the start of the experiment and at time t, respectively. Sampling rates (R_s) were expressed by the following equation (Eq. (3)):

$$R_s = k_u V_T \quad \text{(Eq. 3)}$$

where k_u indicates the corrected uptake rate constant and is obtained by subtracting k_D from k. V_T and k are the volume of water in the beaker and the slope of the decrease of water concentration (Ln [$C_w(t)/C_w(0)$]) during the exposure time, respectively.

2.3. Sample collection and field deployment of POCIS

The sampling period was determined by the presence of cyanobacteria blooms in the Geum River and freshwater discharge to the estuary following heavy rainfall. From August to September 2019, POCISs were deployed at three sites [one inner region (G1) and two outer regions (G2 and G3) of the estuary dam] in the Geum River Estuary for one week (repeated four times over four weeks in total) (Fig. 1). The deployment period of POCIS was chosen as 7 days because the laboratory calibration resulted in linear adsorption over a period of more than 7 days. In a previous study, the adsorption of MCs was also shown to be linear for 7 days (Kohoutek et al., 2010). During the deployment periods, temperature loggers [HOBO 8 K Pendant® Temperature/Alarm (Waterproof) Data Logger, Onset Computer Corporation, Bourne, MA] were placed inside canisters to measure in situ water temperature. POCIS was immersed in distilled water and transported to the sampling sites. After sampling was completed, samples were transported to the laboratory in a cool box and stored at −20 °C until extraction. During POCIS deployments, there was no biofouling of the membrane surface.

The amount of freshwater discharge and daily precipitation data for Geum River Estuary in 2019 were obtained from Korea Rural Community Corporation (Korea Rural Community Corporation (KRC), 2019) and Korea Meteorological Administration (Korea Meteorological Administration (KMA), 2019), respectively. Water quality parameters (temperature, salinity, and pH) were measured in situ using a calibrated multi-probe (YSI-650 MDS, YSI Inc., Yellow Springs, OH) during sampling campaigns. Environmental multimedia samples (such as surface water, SS, and sediments) were collected from each site at the time of deployment and retrieval of POCIS. SS was used to analyze particulate MCs, and were collected by filtration of 500 mL of surface water using GF/F filters (Whatman, Dassel, Germany). Concentration of chlorophyll-a (Chl-a) in filter samples (GF/F) was measured according to the previously reported method (Su et al., 2010). The filtrate was used to analyze dissolved MCs. Sediment samples were collected at a depth of 0.5 cm. Oyster samples (*Crassostrea gigas*) were collected from sites G2 and G3, and were analyzed by pooling 20–30 individuals from each site. Filters, sediments, and oysters were lyophilized and stored at −20 °C until extraction.

2.4. Extraction of MCs in environmental multimedia samples and POCIS

MCs were extracted from environmental samples following a previously described method (Kim et al., 2019, 2021). In brief, weighed samples, including lyophilized filter, sediments (1–2 g), and oysters (0.5–1 g), were transferred to a 50-mL Teflon tube. Ten milliliters of butanol:methanol:water (1:4:15, v:v:v) was added for extraction. Enkephalin was used as a surrogate standard and was added (100 ng) before sample extraction to evaluate extraction efficiency (Xu et al., 2008). Samples were stirred overnight and sonicated for 3 min to enhance extraction efficiency. The samples were centrifuged at 3500 g for 10 min, and the supernatants were transferred to a conical tube. This process was repeated three times, and the supernatants were combined (total 30 mL). Before clean up using a solid-phase extraction (SPE) cartridge, the extract was diluted in deionized water to reduce organic solvent content (<10%). The diluted extract (SS, sediments, or oysters) and surface water samples (1 L) were loaded to an Oasis HLB cartridge (500 mg, 6 cc, Waters) that had been preconditioned using 10 mL of 100% methanol followed by 10 mL deionized water. After loading samples, the cartridges were washed with 10 mL of 20% methanol to remove interferences. MCs were eluted using 10 mL of 100% methanol, and were evaporated at 40 °C under nitrogen gas. Finally, the eluent was reconstituted with 1 mL methanol. Monolinuron was used as an internal standard to check instrumental sensitivity (Draper et al., 2013).

After laboratory calibration experiments and field deployment, retrieved POCIS instruments were disassembled, and the HLB sorbent was carefully transferred to a 6-mL empty SPE cartridge fitted with polyethylene frits. Samples were eluted in the same manner as described in this section.

2.5. HPLC-MS/MS analysis

HPLC-MS/MS analyses were carried out using Agilent 1290 infinity II LC system (Agilent Technologies, Santa Clara, CA) coupled with Sciex QTrap 6500 MS/MS system (AB Sciex, Framingham, MA). All of the target MCs (MC-LR, -RR, -YR, -LA, -LF, -LW, and -LY) were separated with a Poroshell 120 EC-C18 (4.6 × 50 mm, 2.7 μm, Agilent Technologies). The column temperature was set at 30 °C. Mobile phases

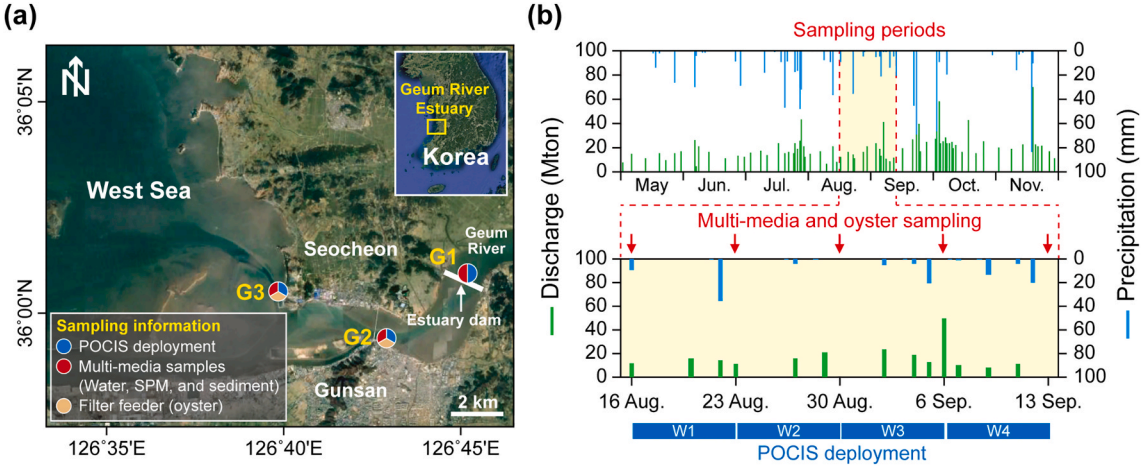

Fig. 1. **(a)** Map showing the sampling sites in the Geum River Estuary. Environmental multimedia samples and filter feeders (oysters) were collected at Sites G1 to G3 from August to September 2019. **(b)** Daily discharge of freshwater (Mton) and precipitation (mm) in the Geum River Estuary from May to November. Red dotted box indicates the sampling periods for this study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

consisted of (A) 0.05% formic acid in water and (B) 0.05% formic acid in acetonitrile. The injection volume was 10 μL, and the flow rate of the mobile phase was 0.3 mL min^{-1}. Target MCs, surrogate and internal standards (enkephalin and monolinuron) were detected in positive mode. Multiple reaction monitoring (MRM) was used for the qualitative and quantitative analysis of MCs. Detailed information on MRM transition for each compound and instrumental condition is provided in Tables S1 and S2.

2.6. Calculation of time-weighted average concentrations

When POCIS is exposed to the aquatic environment, polar organic compounds are absorbed into it. When compounds are absorbed linearly, the receiving phase of POCIS acts like an infinite sink. TWA concentrations of target compounds (C_w, ng L^{-1}) can be calculated from the amount of accumulated compounds in POCIS (Alvarez et al., 2004), according to Eq. (4):

$$C_w = \frac{C_s \times M_s}{R_s \times t} \quad \text{(Eq. 4)}$$

where C_s, M_s, R_s, and t are the concentration of MCs in POCIS (ng g^{-1}), sorbent weight (g), sampling rates (L day^{-1}), and exposure time (day), respectively.

2.7. QA/QC

An eight-level calibration curve (range: 1.0–1000 ng L^{-1}) was used for the quantitative analysis of MCs in the samples. Samples exceeding the ranges of the calibration curve were further diluted and quantified. Limit of detections (LODs) were calculated as being 3.143 times the standard deviation of responses of blank samples (or lowest concentration standard materials). The LODs of MCs ranged from 1.4 to 5.1 ng L^{-1} (details in Table S3). The limit of quantification (LOQs) was calculated as being 10 times the standard deviation of responses of blanks. The LOQs of MCs ranged from 4.2 to 15 ng L^{-1}. The spike test was performed by adding a mid concentration of standards (100 ng L^{-1}, n = 3) to deionized water. The recovery rates of MCs were satisfactory, ranging from 85 to 96% (Table S3). Surrogate recoveries ranged from 73 to 104%, with a mean of 88%.

Mass balance (MB) in the POCIS experiments was calculated according to the following equation (Eq. (5)):

$$\text{MB} \quad (\%) = 100 \times (M_t / M_0) \quad \text{(Eq. 5)}$$

where M_t is the sum of mass in POCIS and water at the end of the uptake experiment; M_0 is the mass in water at the start of the experiment. After 7 days of spiking MCs, MB of MC-LR, -RR, and -YR was 89%, 92%, and 87%, respectively, and no significant difference in MCs concentration was found in control (without POCIS). Thus, it was confirmed that most of the MCs absorbed to POCIS for 7 days were preserved. Field blank POCIS was used to assess contamination during deployment and transport. The field blank POCIS was exposed to the atmosphere at the time of deployment and recovery of POCIS. All target MCs were not detected in the field blank samples.

2.8. Statistics and data analysis

Statistical analyses were performed using R software (Ver. 3.6.3). Shapiro-Wilk's normality test was conducted to verify the normal distribution of the data. Since the variables in this study did not satisfy normality, Mann-Whitney test and Kruskal-Wallis test were carried out to evaluate differences in concentrations between the sampling sites and sampling rates of MC variants, respectively. The Mann-Whitney test was also used to determine the difference in concentrations among sampling sites (inside and outside of dam). The Kruskal-Wallis test was used to evaluate significant differences in sampling rates among the three groups (MC-LR, -RR, and -YR). Spearman's rank correlation and principal component analysis (PCA) were performed to investigate the correlation between water quality parameters and the concentrations of MCs in environmental media samples. When the concentrations of MCs were less than the LOD, LOD/2 was used for data analysis. The sample size (n) was 15 for particulate and dissolved MCs in grab water samples.

3. Results and discussion

3.1. POCIS sampling rates for MCs under various environmental conditions

Compound-specific sampling rates of MC-LR, -RR, and -YR in POCIS as functions of water temperature, flow rate, and salinity, which are representative physical factors, were measured in the laboratory. The sampling rates of MC-LR, -RR, and -YR in POCIS under various conditions were 0.069–0.39 L d^{-1}, 0.16–0.71 L d^{-1}, and 0.101–0.39 L d^{-1}, respectively (Table 1). The sampling rates of MCs in POCIS differed by up to 5.7 times depending on physical conditions. Thus, it is important to apply appropriate sampling rates to obtain more accurate TWA concentrations.

The sampling rates of MCs increased by 3.8–5.7 fold as water temperature increased (10, 20, and 30 °C; Table 1). The effect of water temperature on the sampling rates of MCs in POCIS has not been reported previously; however, similar results were obtained in studies conducted on other polar organic compounds, including pesticides, pharmaceuticals, and endocrine-disrupting substances (Li et al., 2010; Yabuki et al., 2016). The solubility of the compound and the water-ethanol partition coefficient might alter with increasing temperature, facilitating mass transfer from water to the adsorbent (Wezel, 1998). In this study, the sampling rates did not increase significantly for all target MCs with increasing temperatures from 20 to 30 °C. Li et al. (2010) found that some pharmaceuticals, such as sotalol and citalopram, showed no significant increase with increasing temperature from 15 to 25 °C. The effect of temperature on sampling rates appeared to be compound-specific, and MCs did not show a significant increase of sampling rates above a certain temperature. The sampling rates of MC-RR were relatively higher than those of other MC variants, while the sampling rates of MC-LR and -YR showed similar values. Similar results were also reported in the previous study (Jasa et al., 2019). This result was attributed to the chemical structure of MC-RR having two arginine groups, it being more hydrophilic compared to other MCs (Diez-Quijada et al., 2019).

The flow rate also had a significant effect on the sampling rates of MCs in POCIS (Table 1). As the flow rates increased (0, 0.38, and 0.76 m s^{-1}), the sampling rate of MCs in POCIS increased 2.7, 3.4, and 2.2 fold in MC-LR, -RR, and -YR, respectively. The sampling rates of MCs in previous studies were greater under turbulent conditions compared to static conditions (Kohoutek et al., 2010). This trend was also found for other organic compounds (Djomte et al., 2018; Guibal et al., 2020; Kaserzon et al., 2013). The mass transfer of compounds from water to the adsorbent is controlled by the thickness of the water boundary layer. Under high flow rate conditions, the turbulence of the water thins the water boundary layer; thus, the mass transfer of compounds occurs more easily compared to low flow rate conditions (Harman et al., 2012). The sampling rates of MCs in POCIS increased significantly as the flow rate increased from 0 to 0.38 m s^{-1}; however, there was no significant difference in the sampling rate when it increased from 0.38 to 0.76 m s^{-1}. In previous studies, the sampling rates of MCs significantly increased when changing from the static to turbulent conditions, but when the flow rate increased from 0.2 to 0.4 m s^{-1}, the sampling rates of MCs did not increase significantly (Jasa et al., 2019). Thus, the sampling rates of MCs do not increase further under high flow rate conditions (Harman et al., 2012; Jasa et al., 2019).

Under various salinity conditions (0, 15, and 30 psu), the sampling rates in POCIS did not show a significant difference for all MCs ($p >$

Table 1
Sampling rates (R_s, L/d) of microcystins in POCIS under various conditions reported in previous studies and this study.

Membrane (mesh size)	Sorbent (g)	Surface area (cm^2)	Water temperature (°C)	Static/Turbulent (Flow rates)	Salinity (psu)	Sampling rates (L day^{-1})			References
						MC-LR	MC-RR	MC-YR	
Polycarbonate (0.45 μm)	HLB (0.04 g)		22 ± 2	Static	0	0.022	0.017		Kohoutek et al. (2008)
Polycarbonate (0.45 μm)	HLB (0.04 g)	14.1	22 ± 2	Static	0	0.017	0.022		Kohoutek et al. (2010)
		14.1		Turbulent		0.087	0.09		
Polycarbonate (0.45 μm)	HLB (0.09 g)		21 ± 2	Static	0	0.018	0.028	0.006	Jasa et al. (2019)
		18.2		0.01 m s^{-1}		0.08	0.151	0.071	
				0.2 m s^{-1}		0.101	0.193	0.088	
				0.4 m s^{-1}		0.107	0.212	0.095	
Polyethersulfone (0.1 μm)	HLB (0.22 g)		20 ± 1	17 mL min^{-1}	0	0.043			Brophy et al. (2019)
Polyethersulfone (0.1 μm)	HLB (0.2 g)	47.5	10 ± 1	Turbulent (0.38 m s^{-1})	15	0.069	0.16	0.101	This study
		47.5	20 ± 1			0.36	0.63	0.36	
			30 ± 1			0.39	0.71	0.38	
			20 ± 1	Static	15	0.14	0.19	0.17	
				Turbulent (0.38 m s^{-1})		0.36	0.63	0.36	
				Turbulent (0.76 m s^{-1})		0.38[a]	0.64[a]	0.37[a]	
			20 ± 1	Turbulent (0.38 m s^{-1})	0	0.36	0.53	0.38	
					15	0.36[b]	0.63[b]	0.36[b]	
					30	0.36[c]	0.56[c]	0.39[c]	

[a] These sampling rates of MCs were used to calculate TWA concentrations in Site G1.
[b] These sampling rates of MCs were used to calculate TWA concentrations in Site G2.
[c] These sampling rates of MCs were used to calculate TWA concentrations in Site G3.

0.05). Previous studies reported that salinity affects sampling rates in a compound-specific manner (Bayen et al., 2014; Togola and Budzinski, 2007). Similar to water temperature and flow rate, the sampling rates of MC-RR were greater compared to MC-LR and -YR under all salinity conditions. Overall, the sampling rates of MCs mainly depend on water temperature and flow rate, with salinity having no significant effect.

The sampling rates of MCs in POCIS under the various conditions obtained from this study were generally higher than those previously reported (Table 1). Using the sampling rates of target compounds in POCIS reported in previous studies may have uncertainty in calculating the TWA concentrations. This issue is associated with several controlling factors, such as temperature (Yabuki et al., 2016), salinity (Togola and Budzinski, 2007), flow rate (Alvarez et al., 2007; Guibal et al., 2020), biofouling (Harman et al., 2009), calibration method, and the calculation methods of sampling rates (Morin et al., 2012). The sampling rates of MCs were slightly different because the major factors (such as membrane type, temperature, flow rate, salinity, sampler type, and amount of sorbent) differed compared to previous studies (Morin et al., 2012). The TWA concentration of MCs using POCIS is sometimes uncertain because it is very difficult to measure the sampling rates at all flow rates and temperature conditions. The sampling rates obtained in this study were applied to calculate the TWA concentrations of MCs from POCIS set in the Geum River Estuary in site- and compound-specific manners (Table 1 and Table S4).

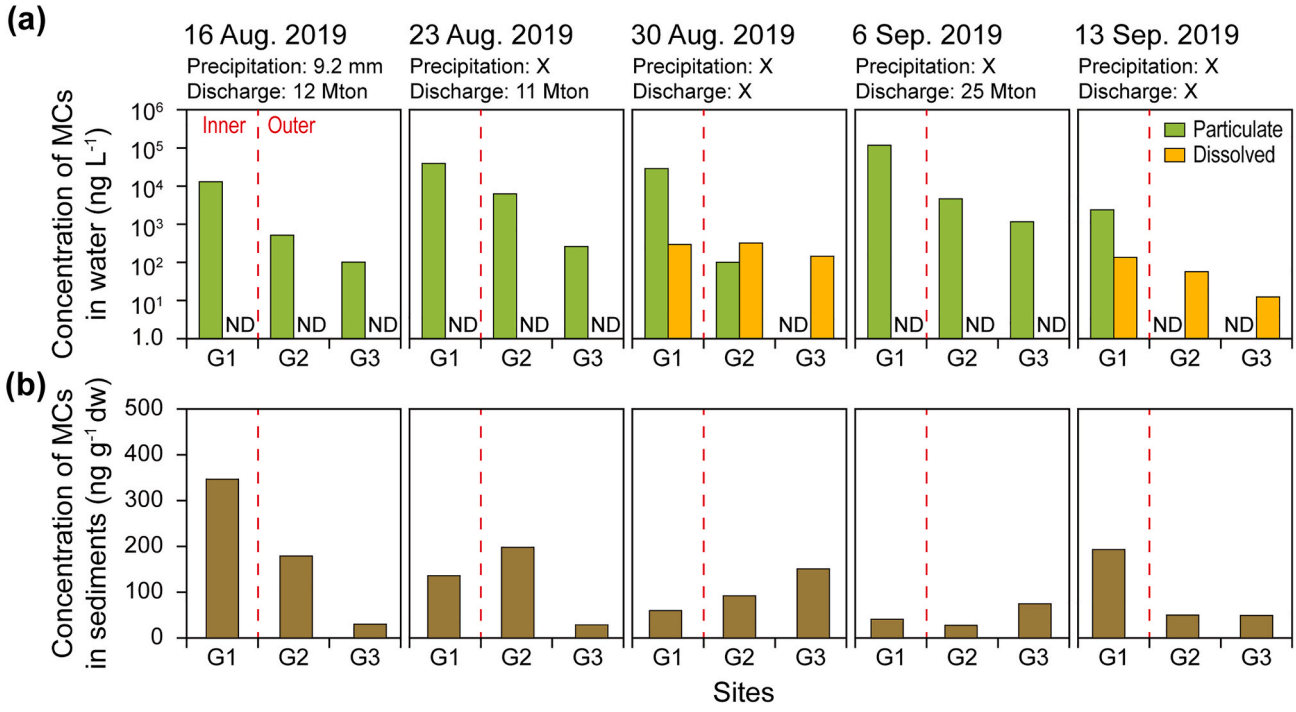

Fig. 2. **(a)** Concentrations of particulate and dissolved MCs and **(b)** concentrations of sedimentary MCs in the Geum River Estuary.

3.2. Distribution and composition of MCs in environmental multimedia samples

The concentrations of particulate MCs inside (Site G1) and outside (Sites G2 and G3) of the estuary dam were 2400–120,000 ng L^{-1} (mean: 40,700 ng L^{-1}) and below limit of detection (<LOD)–6020 ng L^{-1} (mean: 1200 ng L^{-1}), respectively (Fig. 2a, Fig. S2, and Table S5). During the study period, the concentrations of particulate MCs fluctuated over time, with the concentration being maximal at Site G1 on September 6 (120,000 ng L^{-1}), and then it decreased. The concentration of particulate MCs was significantly correlated with Chl-a concentration ($p < 0.05$, Fig. 3b), indicating that particulate MCs were directly affected by the density of the MC producing cyanobacteria. Previously, water temperature was identified as a major factor affecting the density of cyanobacteria that produce MCs, with cyanobacteria accounting for a large portion of the phytoplankton population (>95%) in the Geum River from June to August (Kim et al., 2021). The average water temperature was 29 °C at Site G1 from August 16 to September 6, which is the ideal temperature for cyanobacteria blooms (Bui et al., 2018). After September 6, the water temperature was 24 °C, which was relatively low; thus, the growth of cyanobacteria seemed to decline, with Chl-a concentrations also significantly decreasing over this period.

Spatially, the concentrations of particulate MCs tended to decrease from inside the estuary dam to outside areas. MC concentrations significantly differed between inner and outer regions ($p < 0.05$). MCs are intracellular toxins that mainly exist as particulate MCs in freshwater environments. However, in estuarine and coastal environments, cell lysis is promoted by salt and released into the water column (dissolved phase) (Umehara et al., 2015). Our results were consistent with those previously reported in the Geum River Estuary, whereby particulate MCs introduced to the estuarine environment through freshwater discharge appear to be rapidly degraded (Kim et al., 2019, 2021). The half-life of particulate MCs in the Geum River Estuary was reported as 0.44–0.52 days (Kim et al., 2021). The average proportion of particulate MCs during the sampling period was calculated (n = 15). The percentage of MC-RR was the greatest (65%) in particulate MCs, followed by MC-LR (25%), -LY (3.6%), and -LA (2.8%) (Fig. S2). This trend was similar to the compositions of MCs in *Microcystis* spp., with cyanobacteria containing a large portion of particulate MCs (Graham et al., 2010; Srivastava et al., 2012).

Dissolved MCs were detected at relatively lower concentrations compared to particulate MCs, with concentrations varying with the timing of sampling (Table S6). Dissolved MCs were detected only on August 30 and September 13 (Fig. 2a). This period was 1–2 days after freshwater discharge. The other sampling dates occurred during or immediately after freshwater discharge. The salinity at Site G2 (outside of the estuary dam) significantly decreased during the period of freshwater discharge (Table S4). In general, *Microcystis* spp. survives below 10 psu (Preece et al., 2017); thus, samples collected immediately after freshwater discharge were present as particulate MCs (microalgal cells). Samples collected 1–2 days after freshwater discharge had a salinity of 15 psu or more in the estuarine area. Some particulate MCs seemed to be decomposed and released into the water column as dissolved MCs. The half-life of dissolved MCs in the Geum River Estuary is relatively long compared to particulate MCs (Kim et al., 2021). There was no significant difference in concentration between the inside and outside of the dam,

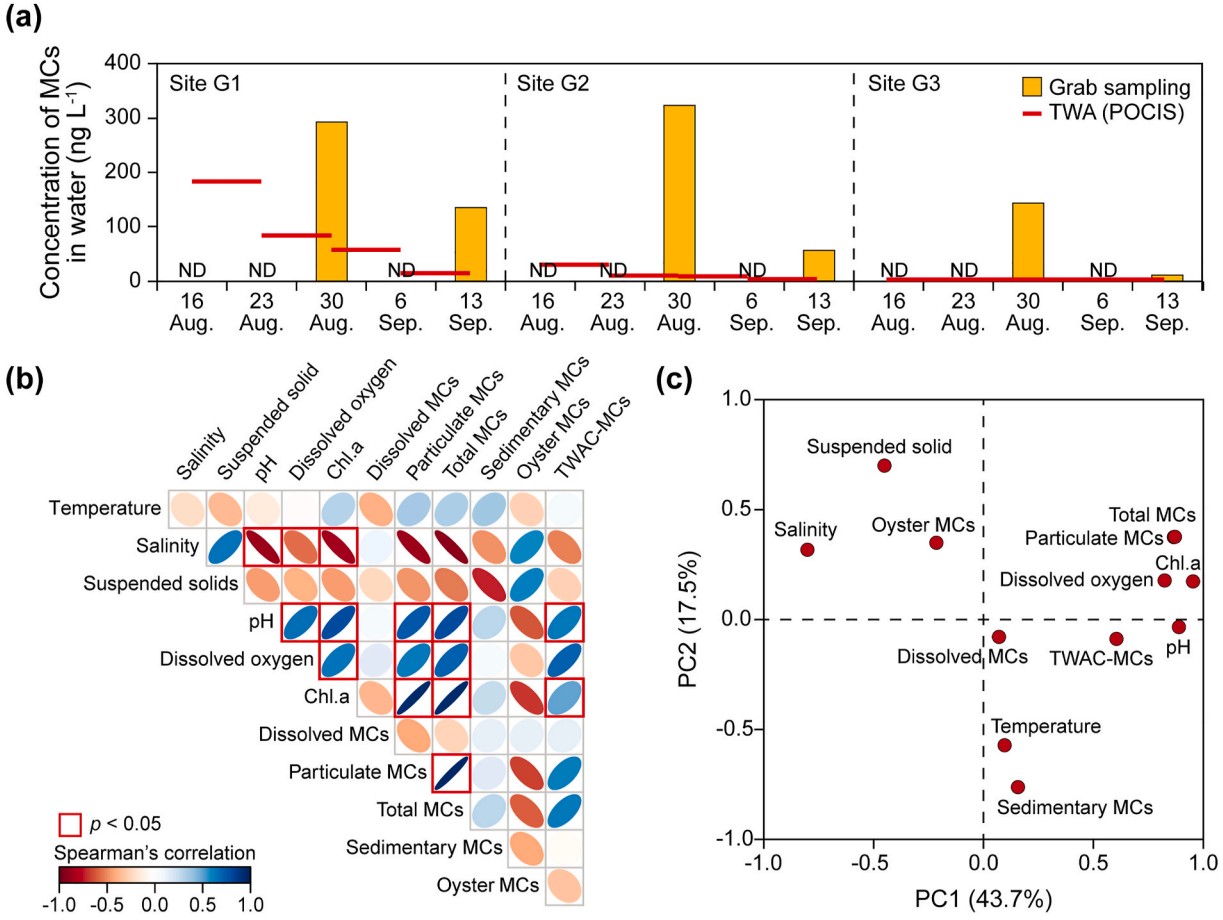

Fig. 3. **(a)** TWA concentrations of MCs from POCIS and dissolved MCs in grab water samples at Sites G1–G3 in the Geum River Estuary. **(b)** Spearman's rank correlation between water quality parameters and concentrations of MCs in multimedia samples and POCIS. **(c)** Results of the principal component analysis (PCA) (Total MCs: particulate + dissolved MCs; TWAC-MCs: time-weighted average concentrations of MCs).

with the concentrations fluctuating without any particular pattern. The average proportion of dissolved MCs during the sampling period was calculated (n = 15). MC-LR was the predominant dissolved MC (57%) in the Geum River Estuary, followed by MC-RR (27%), -YR (7.1%), and -LW (4.7%) (Fig. S2). The compositions of MCs between the particulate and dissolved phases differed slightly. Overall, the MCs in the Geum River Estuary were mainly present as particulate MCs (n = 15, mean: 78%), with relatively low concentrations of dissolved MCs (n = 15, mean: 22%) being found.

MC concentrations in the inner and outer estuary dam were 41–347 ng g^{-1} dw (mean: 155 ng g^{-1}) and 28–198 ng g^{-1} (mean: 88 ng g^{-1}), respectively (Fig. 2b and Table S7). Similar to water, MC concentration fluctuated during the sampling period. MC-LR was the dominant sedimentary MC (39%), followed by MC-LW (21%), MC-RR (14%), and MC-LF (12%) (Fig. S2). The composition of MCs in sediments is determined by the physicochemical properties of individual MCs, rather than reflecting the composition of sources of MCs (i.e., cyanobacteria) (Vesterkvist and Meriluoto, 2003). For example, contributions of MC-LW, -LY, and -LF in sediments were significantly higher compared to particulate and dissolved MCs. These MC variants are relatively hydrophobic. This trend was also found in a previous study (Kim et al., 2021). The hydrophobic interaction of MCs with organic materials causes significant adsorption to sediments (Liu et al., 2008). Overall, MCs derived from toxic cyanobacteria appeared to be directly precipitated and accumulated in sediments and/or transformed to the dissolved phase. In estuarine areas, the compositional change of MCs during this process seems to occur as a result of differences in properties between MC variants.

3.3. Comparison of MC concentrations between POCIS and grab samples

The TWA concentrations of MCs in water were calculated from POCIS deployed for seven days (four sampling campaigns from August to September) at three sites on the Geum River Estuary. The obtained concentrations were compared with those of dissolved MCs from the grab water samples (Fig. 3a). MCs were detected in all POCIS samples for four weeks (Fig. 3a and Table S8). The TWA concentrations of MCs at Sites G1, G2, and G3 ranged from 15 to 180 ng L^{-1} (mean: 85 ng L^{-1}), 4.1–28 ng L^{-1} (mean: 13 ng L^{-1}), and 2.1–4.4 ng L^{-1} (mean: 3.6 ng L^{-1}), respectively. In the case of grab water samples, dissolved MCs were not detected in many samples, whereas the TWA concentrations of MCs from POCIS were detectable. This result indicates that the concentrations of dissolved MCs were highly variable. The TWA concentrations of MCs and dissolved MCs were within the same order, but it was difficult to compare concentrations directly.

The average concentrations of dissolved MCs from grab samples collected on the placement and retrieval of POCIS were not significantly correlated with the TWA concentrations of MCs (Fig. S3). Particulate MCs exist in dissolved form in the water column through cell destruction and extracellular release, and can be absorbed into POCIS. The pore size of the membrane filter used in POCIS is 0.1 μm; consequently, suspended solids and microorganisms (bacteria) cannot enter POCIS. Thus, once absorbed into POCIS, MCs adsorbed to HLB sorbent could be preserved without decomposition (Wang et al., 2008). Alternatively, dissolved MCs in the water column might be degraded or biotransformed due to microbial activity (Chen et al., 2008; Lezcano et al., 2018; Santos et al., 2020). The composition of MCs absorbed in POCIS was dominated by MC-RR (49%) and -LR (43%), and showed a slight difference in composition from dissolved and particulate MCs. This phenomenon might be related to the difference in persistence in the water column for each compound and the difference in the sampling rate of MCs in POCIS. MC-RR seems to be detected relatively greater in POCIS due to the high sampling rate compared to other MC variants. In addition, dissolved MC-LR existed predominantly because of its high persistence in the water column compared to MC-RR. PCA analysis showed that particulate MCs, dissolved MCs, and TWAC-MCs from POCIS were distinguished due to differences in MCs composition (Fig. 3c). The POCIS accumulated dissolved MCs from the water during the deploying period, whereas grab water samples only captured MCs on the day of sampling. For this reason, the concentration of dissolved MCs in grab samples likely differs to the TWA concentrations in POCIS samples. Similar trends have been observed in previous studies (Brophy et al., 2019; Kohoutek et al., 2010). In contrast, Jasa et al. (2019) reported a significant relationship between TWA-MCs and MCs concentrations in grab water samples, and they also found a significant positive relationship between dissolved and particulate MCs.

Chl-a concentrations showed a significant relationship with concentrations of particulate MCs and total MCs ($p < 0.05$, Fig. 3b and Fig. S3), while no significant relationship with concentrations of dissolved MCs was found ($p > 0.05$, Fig. 3b). This result seemed to be due to particulate MCs could include MCs producing cyanobacteria. The tendency of total MCs concentration to show significant relationships with concentrations of particulate MCs and Chl-a was also confirmed by PCA results (Fig. 3c). A significant positive relationship was previously found between Chl-a and the concentrations of particulate MCs. The concentration of MCs per Chl-a in the Geum River Estuary conducted in 2017–2018 was 0.02–0.76 μg MCs Chl-a^{-1} (Kim et al., 2019, 2021). In the current study, MC concentrations per Chl-a ranged from 0.003 to 0.62 μg MCs Chl-a^{-1} (mean: 0.15 μg MCs Chl-a^{-1}), which was similar to previous results. Thus, MC concentrations in the Geum River Estuary during summer did not increase or decrease significantly, and appeared to remain at a similar level over 3 years.

The TWA concentrations of MCs showed a positive relationship with Chl-a ($p < 0.05$, Fig. S3). This result was attributed to large amounts of dissolved MCs being released into the water column when relatively high densities of toxic cyanobacteria were present, with MCs being subsequently absorbed into the POCIS. In the case of September 6, which showed a great concentration of particulate MCs, MCs were detected in the POCIS samples, but dissolved MCs were not detected in the grab water samples. This seems to be because POCIS reflects the average concentration during the sampling period. Overall, POCIS is useful for monitoring the average concentrations of dissolved MCs, but it seems to have limitations in evaluating the overall contamination levels of MCs in aquatic ecosystems, because MCs mainly exist in the particulate form in water.

3.4. Comparison of MC accumulations between POCIS and oyster samples

MC concentrations adsorbed per unit mass in the HLB of POCIS was compared with MC concentrations that accumulated in oysters (Fig. 4 and Table S9). MCs were detected in all oyster samples. MC concentrations at Sites G2 and G3 were 0.36–7.9 μg g^{-1} dw and 0.5–1.2 μg g^{-1} dw, respectively. MC concentrations in oysters were relatively higher at G2, which is located closer to the estuary dam, compared to G3. Concentrations of MCs in oysters collected from G2 and G3 increased over 3 weeks, and then tended to decrease. MC concentrations that accumulated in POCIS were relatively lower than those in oyster samples (Fig. 4). This trend could be explained by the different uptake mechanisms of compounds between oysters and POCIS. In POCIS, an adsorbent exists between the microporous diffusion-limiting membranes (Alvarez, 1999). Consequently, the passive diffusion of pollutants in the water environment to POCIS is a multi-step mass transfer process. The compounds first transfer from bulk water to the water-bound layer, and then biofilm, water-filled membrane pores through membrane matrix transfer by molecular diffusion, and finally enter the adsorbent in POCIS (Seethapathy et al., 2008). In contrast, MC accumulation in organisms is affected by various factors, including exposure pathway, feeding habit, food source, exposure time, and concentration (Ibelings and Chorus, 2007). Oysters are filter feeders and can actively consume cyanobacteria as a food source (van Egmond and Jonker, 2004). Filtering rate of oysters was ~44 L d^{-1} (Cho et al., 2010), which is very high compared to

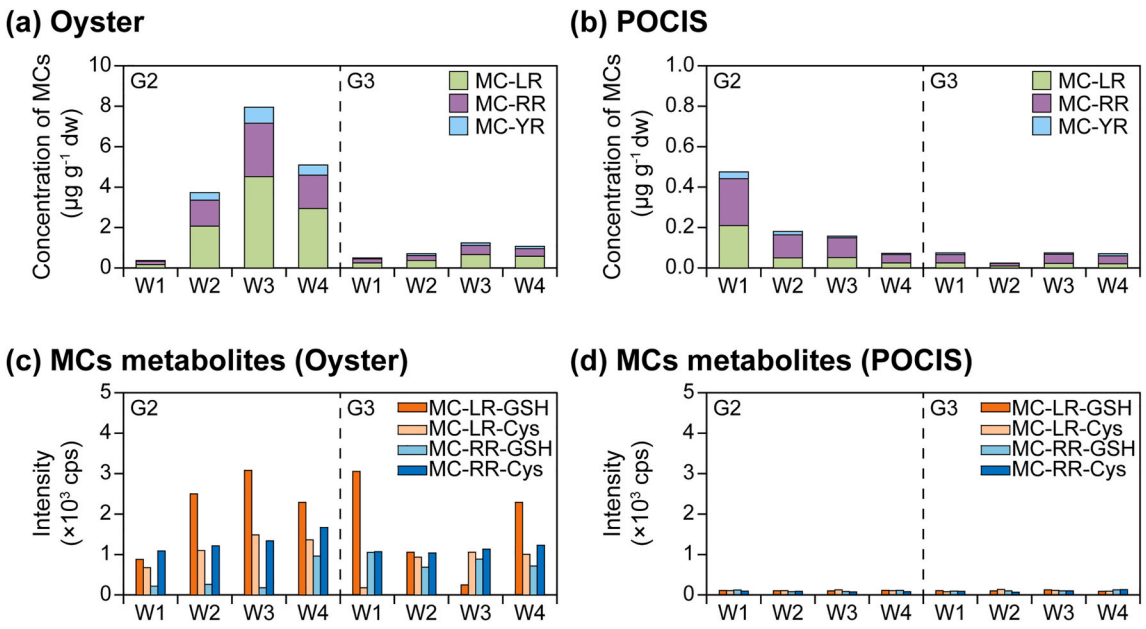

Fig. 4. Concentrations of MCs in **(a)** oysters and **(b)** POCIS. Intensity of MCs metabolites in **(c)** oyster extracts and **(d)** POCIS extracts in the Geum River Estuary.

the sampling rates of POCIS (0.006–0.71 L d^{-1}) used in the present study.

The MC concentrations that accumulated in both oysters and POCIS showed no significant relationship with MC concentrations in environmental media (water, SS, and sediments) (Fig. 3b). This result was obtained for MCs accumulation, metabolism, and excretion in the oysters, as well as compositional changes of MCs in the estuarine environments. The average composition of MCs in oyster and POCIS were MC-LR (54%) > MC-RR (36%) > MC-YR (10%) and MC-RR (49%) > MC-LR (43%) > MC-YR (8%), respectively, with no difference between G2 and G3. The contribution of MC-RR in oyster samples was relatively lower compared to that of POCIS. This difference was attributed to the hydrophilicity of MC-RR. MC-RR has amino acid arginine in X and Z positions. This specific structure makes MC-RR more hydrophilic than other MC variants (Diez-Quijada et al., 2019), with more hydrophilic MCs being more easily excreted than hydrophobic MC variants (Gupta et al., 2003). Oysters showed a relatively low MC-RR proportion compared to particulate MCs, despite being able to directly ingest suspended solids, including cyanobacteria cells in the aquatic environment. Thus, MC-RR was considered to be relatively rapidly degraded compared to other variants.

To understand the difference between the composition of MCs in POCIS and oysters, a qualitative analysis of MCs metabolites was conducted. All parent MCs and their metabolites (MC-LR-GSH, MC-LR-Cys, MC-RR-GSH, and MC-RR-Cys) were detected in several oyster samples (Fig. 4 and Table S10). POCIS has no biological function, and the sorbent is surrounded by a 0.1 μm membrane, biotransformation of the compound or degradation by bacteria may not occur (Wang et al., 2007, 2008). Organisms exposed to MCs have their own detoxification mechanisms to mitigate MCs (Schmidt et al., 2014). Glutathione (GSH) contributes to the metabolic process of MCs in various aquatic organisms, with GSH conjugate being used as the first step of MCs detoxification, which is then decomposed into cysteine (Cys) conjugate (Fig. S4) (Kondo et al., 1992, 1996). The formation of the GSH conjugate by the enzyme, glutathione-S-transferase (GST), is one of the most common types of xenobiotic modification. A previous study reported that GST activity increased in various organs of *Mytilus galloprovincialis* exposed to MCs (Vasconcelos et al., 2007), suggesting that MCs metabolism and detoxification may occur in invertebrates. The presence of MC metabolites (GSH and Cys conjugates) was confirmed based on the signal-to-noise ratio (S/N) and was detected in both G2 and G3 oyster samples (Fig. S5). MC-LR-GSH was found in all samples at relatively higher intensity compared to other metabolites. The detection frequency of MC-RR-Cys was relatively higher compared to that of MC-LR-Cys, and was observed in both G2 and G3. Because MC-RR is relatively hydrophilic, it is metabolized relatively faster compared to MC-LR. Interestingly, MCs metabolites were not detected in all POCIS samples. These results could explain the difference in the tendency of accumulation of MCs between POCIS and oysters.

4. Conclusions

MCs originate from toxic cyanobacteria, exist mainly in particulate form in aquatic ecosystems, and are heterogeneously distributed in water; consequently, it is very difficult to collect samples to determine contamination accurately. POCIS could be used to monitor dissolved MCs present in trace amounts in the aquatic environment that could not, otherwise, be detected in grab water samples. However, because most MCs existed in particulate form, there was a limit to monitoring using POCIS, which only collects freely dissolved forms. Thus, in future studies, it is necessary to develop alternative sampling techniques for the accurate and efficient monitoring of MCs in aquatic ecosystems.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118222.

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