Original Article

Comparison of Low Molecular Weight Dissolved Organic Matter Compositions in Lake Inba and Kashima River by Orbitrap Mass Spectrometry

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ABSTRACT

Lake Inba, which serves as a drinking water source in Chiba Prefecture, Japan, has not complied with the environmental standard for COD (3 mg/L) since the 1970s. The high level of dissolved organic matter (DOM) caused by eutrophication in the lake has risen the concern of water treatment. To understand the compositions and sources of DOM in Lake Inba, this study compared molecular compositions of low molecular weight DOM (LMW-DOM, <1 kDa) in the lake and a major river flowing to the lake. Water samples were collected from Lake Inba and Kashima River in June 2015. After solid-phase extraction, LMW-DOM was analyzed by Orbitrap mass spectrometry coupled with electrospray ionization. Based on the accurate mass data, 1,263 and 1,393 molecular formulae were assigned for the lake and river samples, respectively. Among them, 1,193 formulae were shared between the lake and the river, indicating that Kashima River was the representative source of LMW-DOM in the lake at the sampling occasion. Elemental composition of molecular formula demonstrated that 61% of the shared formulae were composed of carbon, hydrogen and oxygen. Hydrogen to carbon ratio and oxygen to carbon ratio of these common molecules were similar to the fingerprints of lignin and tannin.

Keywords: dissolved organic matter, eutrophic lake, molecular composition, Orbitrap mass spectrometry

INTRODUCTION

Lakes and reservoirs are major drinking water sources in Japan, accounting for 46% of the total water supply in 2006 [1]. However, eutrophication has deteriorated water quality. In particular, the compliance rate of chemical oxygen demand (COD), which is one of the environmental water quality standards for lakes and reservoirs, has remained approximately 50% for decades [2]. Dissolved organic matter (DOM) partially contributing to COD should be controlled because some components in DOM cause water quality problems such as off-flavor [3], disinfection by-products precursors [4,5], and microbial regrowth in distribution system [6]. However, understanding of DOM in lakes and reservoirs is limited because DOM is a heterogeneous mixture of complex unknown compounds from internal and external

sources [7].

Some aspects of DOM have been characterized by molecular weight distribution [5], UV absorbance [8], resin fractionation [9], and fluorescence property [10]. However, these conventional approaches are not able to elucidate the molecular compositions of DOM. Recently, non-targeted analysis through ultrahigh resolution mass spectrometry (MS) such as Fourier transform ion cyclotron mass spectrometry (FT-ICR MS) and Orbitrap mass spectrometry (Orbitrap MS) have been applied to analyze DOM in aquatic environments [11–16] as well as DOM in drinking water and wastewater effluent [17–20]. These instruments can resolve complex DOM extracted by solid-phase extraction (SPE) and assign molecular formula to each component based on accurate mass. Even though target DOM is restricted to compounds which can be extracted by SPE and easily ion-

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ized in MS analysis, molecular compositions revealed by FT ICR-MS and Orbitrap MS are informative to understand DOM. The advent of such new technologies has also shed some light on DOM in lakes. Fourier transform ion cyclotron mass spectrometry analysis revealed that DOM composition in Lake Superior was different from DOM that originated from its watershed [15]. Fourier transform ion cyclotron mass spectrometry was also applied to compare molecular fingerprints of DOM in Taifu, a eutrophic lake in China, with algal extract to specifically evaluate the contribution of algal bloom [21].

This study aimed to reveal the molecular compositions of low molecular weight DOM (LMW-DOM) in Lake Inba, Chiba Prefecture in Japan, using Orbitrap MS coupled with electrospray ionization (ESI). Although Lake Inba has suffered from severe eutrophication for decades, water is used for multiple purposes including drinking water supply. In order to discuss the sources of LMW-DOM in Lake Inba, we compared LMW-DOM of Lake Inba with that of Kashima River, one of the major rivers flowing into the lake.

MATERIALS AND METHODS

Study sites and sampling

Lake Inba, which is located in Chiba Prefecture, Japan, consists of northern and western parts. It is a hypereutrophic and shallow lake (average 1.7 m and maximum 2.5 m depth). The surface area and volume of the lake are 11.55 km² and 19.7 million m³, respectively [22]. The water retention time is approximately 22 days [23]. Watershed area of the lake covers 493.9 km² in total. The water quality has severely deteriorated since 1970's mainly because of rapid urbanization. Annual average COD of Lake Inba in 2014 was 11 mg/L, which was far above the environmental water quality standard (3 mg/L) [2]. The water from the western part of the lake is taken as drinking water source. Kashima River is a major river flowing into Lake Inba. The river length is 31.8 km and the watershed area covers 153 km² which mainly consists of urban area and forest [24]. In 2013, it was estimated that COD generation load in the watershed of Kashima River and its tributaries accounted for 55% of the total COD generation load to the western part of Lake Inba [24].

Lake water sample was collected from the center of the western part of Lake Inba (35.748385° N, 140.193832° E) on 30th June, 2015. River water sample was taken from Kashima Bridge on Kashima River (35.726814° N, 140.214643° E) on the same day. Both sampling points are registered as the regular monitoring stations for the environmental water

quality standard. All glassware and glass fiber filters used in the present study were pre-combusted at 550°C for 6 hours to eliminate residual organic matter. All samples were stored in 2-L glass bottles and filtered through 0.3-μm glass fiber filters (GF-75, Advantec, Tokyo, Japan) immediately after being transported to the laboratory within 2 hours. Thereafter, the samples were kept in a refrigerator at 4°C until further analysis.

Organic matter concentration analysis

Total organic carbon (TOC) was measured with a TOC-L_{CSH} (Shimadzu, Kyoto, Japan) after pretreatment with sonication at 200 W for 10 minutes. For the filtrated samples, dissolved organic carbon (DOC) and dissolved chemical oxygen demand (DCOD) were measured. Chemical oxygen demand analysis with KMnO₄ oxidation was based on the Japanese Industrial Standard (JIS method K 0102).

Solid-phase extraction (SPE)

The filtered water samples were concentrated and desalted by means of SPE (Bond Elut PPL cartridge, Agilent Technologies, California, USA). Polystyrene-divinylbenzene modified with nonpolar surface (PPL) can extract both hydrophobic and hydrophilic organic matter. According to a previous study, PPL showed the best recovery of DOM among different types of resins [25]. The cartridge (6 mL) contained 1 g of resin. It was first conditioned with 10 mL of methanol and was subsequently rinsed with 20 mL of 0.01 M HCl. The samples were acidified to pH 2 with 5 M HCl. Thereafter, the samples (500 mL for Lake Inba, 1,000 mL for Kashima River) were passed through the cartridges with vacuum at 70 kPa. After all samples passed through the cartridge, 20 mL of 0.01 M HCl was used to rinse the cartridge. Finally, adsorbed organic matter was eluted with 10 mL of methanol. To evaluate DOC recovery, 1 mL of the eluate was evaporated under N₂ flow at 40°C and re-dissolved in 20 mL Milli-Q water for DOC analysis. The rest of the eluate (9 mL) was stored in freezer at -20°C until Orbitrap MS analysis. Milli-Q water (1,000 mL) was treated in the same manner as an operational blank.

Orbitrap mass spectrometry

The DOM extracted by PPL cartridge was analyzed in triplicate by Orbitrap MS (Exactive, Thermo Scientific, Massachusetts, USA) coupled with negative-mode electrospray ionization (ESI). The extracted DOM in methanol (10 μL) was introduced to the ESI source by flow injection with a flow rate of 200 μL/min using Accela 600 (Thermo

Table 1 Organic matter concentration of Lake Inba and Kashima River

Parameter	Lake Inba	Kashima River
TOC (mg/L)	6.7	2.4
DOC (mg/L)	3.4	2.1
DCOD (mg/L)	2.3	2.7
DCOD/DOC	0.68	1.3

Scientific, Massachusetts, USA). No prior column separation was applied. The operational blank was also analyzed in the same manner. The analytical settings were as follows: spray voltage: 4.5 kV, sheath gas flowrate: 30 arbitrary unit (AU), auxiliary gas flowrate: 5 AU, sweep gas flowrate: 0 AU, capillary temperature: 250°C, capillary voltage: 60 V, tube lens voltage: 90 V, skimmer voltage: 30 V, resolution setting: 100,000 at m/z 200, maximum injection time: 250 ms. Mass data were collected in the range of m/z 100 – 1000.

The mass spectra were processed by software Sieve 2.2 (Thermo Scientific, Massachusetts, USA) to screen isotopologues and to check charge state of ions based on the carbon isotopologues. The sample peaks whose intensities were significantly higher than the corresponding peaks in the operational blank were selected by t -test ($p < 0.05$). Then, peaks with signal-to-noise ratio ≥ 10 and absolute intensities of more than 10,000 were extracted for further analysis. In addition, if the peak intensity was more than 10,000 in the blank, the corresponding peaks in the samples were removed. The singly charged negative ion was converted to neutral by adding a proton and further analyzed by compound identification algorithm (CIA) for molecular formula assignment [26]. Relation error and mass error in the CIA was set at 20 ppm and 5 ppm, respectively [26]. Carbon, hydrogen, oxygen, nitrogen and sulfur were considered in the configuration of molecular formula. Additional restrictions were applied in CIA to select chemically plausible formula as the following: conservative elemental ratios ($0.2 \leq \text{H:C} \leq 3.1$; $\text{O:C} \leq 1.2$; $\text{N:C} \leq 1.3$); nitrogen rule, maximum number of heteroatoms ($\text{N} \leq 3$ and $\text{S} \leq 3$); and degree of unsaturation ($-10 \leq \text{DBE-O} \leq 10$, see the following section) [27,28].

Characterization of the assigned molecular formulae

Double bond equivalence (DBE) is the measure of hydrogen deficiency in a particular molecule as calculated in Eq. (1). It indicates the equivalent double bonds or ring structures of a molecular formula.

$$\text{DBE} = \text{C} + 1 - 0.5 (\text{H-N}) \quad (1)$$

In order to exclude the contribution of carbonyl group containing carbon-oxygen bond (C=O) which was generally abundant in natural DOM, DBE-O was defined to assess the degree of unsaturation of DOM as calculated from Eq. (2) [29].

$$\text{DBE-O} = \text{C} + 1 - 0.5 (\text{H-N}) - \text{O} \quad (2)$$

where C, H, O, and N denotes the number of carbon, hydrogen oxygen, and nitrogen atoms, respectively.

For oxygenated compounds consisting of only carbon, hydrogen and oxygen atoms, relationship between peak intensity-weighted average of DBE (DBE_w) and the number of oxygen atoms was evaluated to check the existence of multiple carboxyl groups [30]. Intensity-weighted average of DBE was calculated separately for each class of CHO compounds with different number of oxygen atoms as shown in Eq. (3).

$$\text{DBE}_w = \sum (\text{DBE}_i \times I_i) / \sum I_i \quad (3)$$

where DBE_i and I_i indicate peak intensity and DBE value of molecular formula i .

Peak intensity-weighted O:C and H:C were calculated to compare the overall characteristics of elemental ratios of LMW-DOM. In addition, O:C and H:C ratios of the assigned molecular formulae were plotted on van Krevelen diagram to associate with major classes of compounds. It is reported that van Krevelen diagram can qualitatively discriminate lignin ($1.0 \leq \text{H:C} \leq 1.4$, $0.35 \leq \text{O:C} \leq 0.50$), tannin ($0.75 \leq \text{H:C} \leq 1.4$, $0.35 \leq \text{O:C} \leq 0.85$), carbohydrates ($1.50 \leq \text{H:C} \leq 2.00$, $0.50 \leq \text{O:C} \leq 1.00$), peptides ($1.60 \leq \text{H:C} \leq 1.80$, $0.35 \leq \text{O:C} \leq 0.50$), and lipid ($1.75 \leq \text{H:C} \leq 2.25$, $0.02 \leq \text{O:C} \leq 0.15$) among others [12,31].

RESULTS AND DISCUSSION

Organic Matter Concentrations of Lake Inba and Kashima River

Water quality data of Lake Inba and Kashima River are presented in **Table 1**. Total and dissolved organic carbon of Lake Inba were higher than those of Kashima River probably due to the presence of algae in the lake. Though DCOD were comparable between two samples, DCOD/DOC of Lake Inba and Kashima River were 0.68 and 1.3, respectively, indicating that DOM compositions contributing to COD could be different. It was reported that DCOD/DOC of Lake Kasumigaura and rivers flowing to the lake were 1.29 ± 0.22 and 1.15 ± 0.12 , respectively [32]. Though DCOD/DOC of Kashima River was similar to the previous report for rivers,

Fig. 1 Mass spectra of LMW-DOM from a) Lake Inba and b) Kashima River. (asterisk indicates m/z 239.07941 which could not be assigned to specific formula)

DCOD/DOC of Lake Inba was nearly half of that of Lake Kasumigaura. It is possible that DOM contributing to COD could be different between two lakes.

Ultrahigh Resolution Mass Spectrum

Dissolved organic carbon recovery of SPE extraction was 46.3% and 49.4% for Lake Inba and Kashima River, respectively. This range of DOC recovery was similar to the previous study [25]. **Figure 1** shows the mass spectra of Lake Inba and Kashima River samples after blank subtraction. The most abundant peak (m/z 239.04971) was identical in both samples. However, the algorithm failed to assign molecular formula to this peak. It is likely that the molecule may contain other heteroatoms which are not considered in this study. Compared with the spectrum of Lake Inba, several prominent peaks were detected in the spectrum of Kashima River. The number-averages of m/z were 308.31610 and 320.32108 for Lake Inba and Kashima River, respectively. Both spectra were composed of peaks below m/z of approximately 500. Other studies analyzing DOM in aquatic environments with

FT-ICR MS also reported that the detected peaks were below m/z 1,000 [15,16,21,33]. Though DOM molecular size distribution of Lake Inba sample was not determined, larger than 5 kDa accounted for 45% of the total DOC in Lake Biwa, Japan [34]. It is likely that DOM molecules larger than 500 Da constitute DOM in Lake Inba. The reason for the exclusive detection of molecules less than 500 Da by Orbitrap MS was probably because; 1) LMW-DOM can be ionized more easily than large DOM or 2) ESI can break loosely aggregated large DOM to small constituents [35].

Molecular Compositions of LMW-DOM

Molecular formulae assignment

For Lake Inba sample, 1,263 molecular formulae were assigned out of 1,349 extracted peaks. For Kashima River sample, 1,393 molecular formulae were assigned out of 1,540 extracted peaks. The unassigned ions would possibly be the compounds which did not satisfy the assignment criteria or they contained other heteroatoms such as phosphorus. Number-average DBE-O for the lake and river samples were

Fig. 2 Composition of assigned molecular formulae.

0.72 and 0.43, respectively, indicating that LMW-DOM in Lake Inba was composed of more unsaturated compounds. As shown in **Fig. 2**, the assigned formulae were summarized based on the elemental compositions: formulae consisting of only C, H and O (CHO) and formulae containing N and S (CHON, CHOS, and CHONS). Formulae consisting of only C, H and O were the most abundant group (59% for Lake Inba sample and 57% for Kashima River sample) followed by CHON, CHOS, and CHONS classes for both samples. The dominance of CHO formulae was commonly reported in other studies analyzing DOM in aquatic environments with ultrahigh resolution MS [15,36]. **Table 2** shows the number- and intensity-weighted averages of hydrogen to carbon ratios (H:C) and oxygen to carbon ratios (O:C) of the assigned formulae. These values were quite similar between the lake and the river sample, which were also consistent with H:C and O:C data reported for other lakes and rivers [15,21].

Comparison of major components

Molecular formulae assigned to top 10 intense peaks for Lake Inba and Kashima River samples are listed in **Table 3**. All top 10 intense peaks were assigned to CHO formulae and CHOS formulae for the lake and river samples, respectively.

According to H:C and O:C ratios of top 10 compounds in the lake sample, they were similar to lignin and tannin fingerprints (lignin $1.0 \leq \text{H:C} \leq 1.4$, $0.35 \leq \text{O:C} \leq 0.50$; tannin $0.75 \leq \text{H:C} \leq 1.4$, $0.35 \leq \text{O:C} \leq 0.85$) [12,31]. On the other hand, most of the formulae in river sample were suspected to be sulfophenyl carboxylic acids (SPC) and dialkyl tetralin sulfonate intermediates (DATSI) such as $\text{C}_{10}\text{H}_{12}\text{O}_5\text{S}$ (C_4 -SPC), $\text{C}_{11}\text{H}_{14}\text{O}_5\text{S}$ (C_5 -SPC), $\text{C}_{12}\text{H}_{16}\text{O}_5\text{S}$ (C_6 -SPC), $\text{C}_{13}\text{H}_{18}\text{O}_5\text{S}$ (C_7 -SPC), $\text{C}_{14}\text{H}_{20}\text{O}_5\text{S}$ (C_8 -SPC), $\text{C}_{15}\text{H}_{22}\text{O}_5\text{S}$ (C_9 -SPC), $\text{C}_{13}\text{H}_{16}\text{O}_5\text{S}$ (DATSI), $\text{C}_{14}\text{H}_{18}\text{O}_5\text{S}$ (DATSI), and $\text{C}_{15}\text{H}_{20}\text{O}_5\text{S}$ (DATSI). Sulfophenyl carboxylic acids are known as biodegraded products of linear alkylbenzene sulfonates (LAS) and DATSI are major coproducts of LAS. These LAS-related chemicals are more easily ionized and found from wastewater effluent [18] and reclaimed water [19,20]. Though most of wastewater in the watershed of Lake Inba is transported outside of the watershed, Kashima River still receives effluent from Jyokaso system which is the possible source of LAS-related compounds. Because LAS-related compounds were not found as the major components in the lake sample, it is likely that they were rapidly degraded after discharge. In this analysis, only ESI-negative mode was applied. In order to cover comprehensive molecules in DOM, ESI- positive mode should be considered in future analysis. Furthermore, the results cannot provide any structural information of the assigned formulae. Therefore, additional survey such as MSⁿ analysis is required to exactly identify the compounds.

Molecular-level matching of LMW-DOM between Lake Inba and Kashima River

All the assigned formulae were compared between Lake Inba and Kashima River samples to extract common compounds and compounds only detected in either of the samples. **Table 4** shows the result of molecular-level matching. Large number of formulae (1,193 was shared between Lake Inba and Kashima River, indicating that Kashima River was one of the representative sources of LMW-DOM in Lake Inba.

Table 2 Number-average and intensity-weighted average of elemental ratios for the assigned molecular formulae.

		This study		Minor et al. (2012) [15]		Zhang et al. (2014) [21]	
		Lake Inba	Kashima River	Lake Superior	Brule River	Lake Taifu (non-algal bloom)	Lake Taifu (algal bloom)
Number-average	H:C _n	1.13	1.15	1.17	1.20	1.08	1.16
	O:C _n	0.47	0.48	0.36	0.41	0.43	0.42
Intensity-weighted average	H:C _w	1.21	1.23	1.22	1.28	-	-
	O:C _w	0.47	0.47	0.38	0.42	-	-

Table 3 Molecular formulae assigned to top 10 peaks in Lake Inba and Kashima River samples.

Rank	Lake Inba				Kashima River			
	Assigned formula	Theoretical Mass (Da)	Observed mass (Da)	Error (ppm)	Assigned formula	Theoretical mass (Da)	Observed mass (Da)	Error (ppm)
1	C ₁₂ H ₁₆ O ₆	256.0947	256.0952	2.11	C ₁₃ H ₁₈ O ₅ S	286.0875	286.0880	1.86
2	C ₁₁ H ₁₆ O ₆	244.0947	244.0950	1.21	C ₉ H ₁₂ O ₃ S	200.0507	324.1761	0.74
3	C ₁₃ H ₁₈ O ₆	270.1103	270.1115	4.39	C ₁₂ H ₁₆ O ₅ S	272.0718	272.0724	2.05
4	C ₁₁ H ₁₄ O ₆	242.0790	242.0799	3.66	C ₁₁ H ₁₄ O ₅ S	258.0562	258.0562	0.13
5	C ₁₀ H ₁₄ O ₆	230.0790	230.0793	0.93	C ₁₄ H ₂₀ O ₅ S	300.1031	300.1036	1.59
6	C ₁₂ H ₁₈ O ₆	258.1103	258.1103	0.25	C ₁₅ H ₂₂ O ₅ S	314.1188	314.1194	2.02
7	C ₁₂ H ₁₆ O ₇	272.0896	272.0902	2.28	C ₁₀ H ₁₂ O ₅ S	244.0405	244.0408	1.18
8	C ₁₁ H ₁₄ O ₅	226.0841	226.0847	2.62	C ₁₄ H ₁₈ O ₅ S	298.0875	298.0875	0.06
9	C ₁₄ H ₂₀ O ₆	284.1260	284.1266	2.05	C ₁₅ H ₂₀ O ₅ S	312.1031	312.1037	1.63
10	C ₁₂ H ₁₆ O ₅	240.0998	240.1001	1.34	C ₁₃ H ₁₆ O ₅ S	284.0718	284.0723	1.75

Table 4 Molecular-level matching of the assigned formulae between Lake Inba and Kashima River.

Molecular formula groups based on the elemental composition	Common between Lake Inba and Kashima River	Only detected in Lake Inba	Only detected in Kashima River
CHO	722	24	78
CHON	291	23	43
CHOS	152	10	66
CHONS	28	13	13
Total	1,193	70	200

This is also consistent with the fact that Kashima River and its tributaries accounted for 55% of the total COD generation load to Lake Inba (western part) [23]. Among them, 722 were CHO formulae. On the other hand, only 70 and 200 formulae were unique in LMW-DOM in Lake Inba and Kashima River, respectively. The unique compounds detected only in the lake were probably derived from other internal or external sources. It is likely that the unique compounds detected only in the river were rapidly degraded biologically or photochemically in the lake. Alternatively, they were diluted to undetectable concentration. As shown in **Table 1**, DCOD/DOC of Lake Inba and Kashima River were different. Considering the high similarity of LMW-DOM composition between the two samples, the difference in DCOD/DOC could be attributed to larger fractions of DOM which were out of the analytical window of Orbitrap MS (m/z 100 – 1,000).

Characterization of CHO compounds by van Krevelen diagram

Because CHO compounds are dominant among the assigned formulae (**Fig. 2**), the CHO compounds were further

characterized on van Krevelen diagrams. The diagrams are presented for Lake Inba (**Fig. 3a**) and Kashima River (**Fig. 3b**) as well as the common compounds between two samples (**Fig. 3c**) and unique compounds in Lake Inba (**Fig. 3d**) or Kashima River (**Fig. 3e**). The dominant common CHO compounds were mostly located in the region of lignin and tannin. Lignin and tannin fingerprints were also prominent for DOM in oligotrophic Lake Superior [15]. Although the number of compounds which were uniquely detected from only Lake Inba was small, they were generally located outside of the known chemical classes. In contrast, the unique compounds only detected from Kashima River were mostly associated with lignin, tannin, and carbohydrates.

Characterization of functional group of oxygenated compounds (CHO formulae only)

Formulae consisting of only C, H and O were further characterized to assess the existence of multiple carboxyl groups. The plots between DBE_w and the number of oxygen atoms of CHO formulae with the same number of oxygen atoms for Lake Inba and Kashima River are shown in **Fig. 4**. For both

Fig. 3 van Krevelen diagram of CHO formulae.

(a) Lake Inba, (b) Kashima River, (c) common formulae between Lake Inba and Kashima River, (d) unique formulae only detected in Lake Inba, (e) unique formulae only detected in Kashima River.

samples, R^2 were 0.96 and 0.93 for Lake Inba and Kashima River, respectively, indicating the strong correlation between two parameters. The slopes of the lake and river samples were 0.45 and 0.42, respectively, which were close to 0.5. If all oxygens are present in the form of carboxylic group, two oxygen atoms account for one DBE, resulting in the slope of 0.5. High degree of linearity between DBE_w and the number of oxygen atoms with slopes close to 0.5 demonstrate the existence of multiple carboxylic groups in CHO molecules [30]. The slopes which were slightly less than 0.5 suggest that

some of the oxygen atoms are present in other forms such as hydroxyl group. In fact, it is reasonable to find the abundance of multiple carboxyl groups since most of the CHO compounds are similar to lignin and tannin fingerprints. It is known that carboxyl functional groups are dominant in aquatic humic and fulvic acids which are closely related to lignin and tannin structures [37].

Fig. 4 Plots between DBE_w and the number of oxygen atoms of CHO formulae with the same number of oxygen atoms. (a) Lake Inba, b) Kashima River

CONCLUSIONS

Low molecular weight dissolved organic matter samples extracted from Lake Inba and Kashima River were analyzed by Orbitrap MS in this study. The following conclusions were obtained based on the molecular characterization of LMW-DOM.

1. Among the assigned molecular formulae (1,263 for Lake Inba and 1,393 for Kashima River), 1,193 formulae were shared between the two samples. This indicated that Kashima River was one of the representative sources of LMW-DOM in Lake Inba.
2. CHO formulae accounted for 61% of the shared molecules. They showed the fingerprints of lignin and tannin with respect to H:C and O:C information on van Krevelen diagrams.
3. The multiple carboxyl groups were suspected as the major form of oxygen in the CHO formulae in the lake and river samples.

Molecular compositions of DOM revealed by Orbitrap MS are informative to track the source and fate of LMW-DOM in lake. This study evaluated only Kashima River as an external source of LMW-DOM in Lake Inba. However, there are other external sources and internal sources, for example, DOM derived from primary production, sediment, and other rivers. Therefore, other possible sources and their seasonal variations should be considered to understand the dynamics of LMW-DOM in lake.

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