

## Seasonal and spatial variations in characteristics of Lake Biwa dissolved organic matter: sorption of pyrene and its derivatives and fluorescence properties

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### ABSTRACT

The objectives of this research were to investigate seasonal and spatial variations in (1) sorption of pyrene and its derivatives onto dissolved organic matter (DOM) and (2) fluorescence properties of DOM in Lake Biwa, Japan. In the case of pyrene, sorption coefficient ( $K_{\text{doc}}$ ) of Lake Biwa DOM seasonally changed from 1,200 to 3,800 L/kgC. Vertical distribution of  $K_{\text{doc}}$  was affected by thermocline formation in summer, while it was uniformly distributed as a result of vertical mixing in winter. Functional groups affected sorption of pyrene onto Lake Biwa DOM in different manner from that onto Suwannee River fulvic acid. Three-dimensional excitation emission matrices (3D-EEMs) fluorescence spectroscopy was applied to characterize Lake Biwa DOMs and indicated the existence of at least two fluorophores. The two major peaks at Ex230/Em300 and Ex230/Em425 originated from protein-like and fulvic/humic-like substances, respectively. The peak at Ex230/Em300 showed the maximum fluorescence intensity at a depth of 5 m and could be affected by stratification of the water column in summer. On the other hand, the peak at Ex230/Em425 showed similar profiles both in summer and in winter. These results demonstrably showed that sorption of micropollutants and fluorescence properties of Lake Biwa DOMs were seasonally and spatially varied.

**Key words** | dissolved organic matter, sorption, EEM, seasonal variation, spatial distribution, Lake Biwa

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### INTRODUCTION

Dissolved organic matter (DOM) occurs ubiquitously and plays very important and crucial roles in the water environment (Thurman 1985; Hessen & Tranvik 1998). It is well known that interactions between micropollutants and DOM affect the behavior and fate of chemicals (Carter & Suffet 1982; Suffet & MacCarthy 1989). To assess and manage the environmental risk, the sorption of pollutants onto DOM has to be taken account of and to be evaluated in the consideration of intrinsic seasonal and spatial variations in characteristics of DOM for each watershed.

Fluorescence quenching method is a simple and rapid batch experiment to evaluate sorption coefficient ( $K_{\text{doc}}$ ) for

a class of organic micropollutants such as polycyclic aromatic hydrocarbons (PAHs) (Gauthier *et al.* 1986; Backhus & Gschwend 1990). To expand the versatility of understanding sorption mechanisms, the effects of functional groups should be systematically evaluated. Many researchers have investigated characteristics of DOM by means of various methods. Three-dimensional excitation emission matrices (EEMs) fluorescence spectroscopy is one of the most promising techniques to elucidate the complex chemical composition and diverse sources of DOM without elaborate pretreatments (Coble *et al.* 1990).

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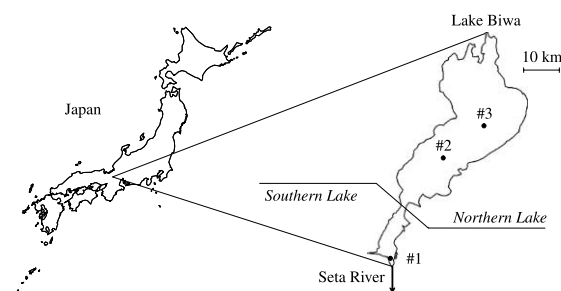
Lake Biwa is the largest lake in Japan and its watershed has been highly industrialized and urbanized for decades. The lake has been gradually eutrophicated from early 1960's. The lake supplies its water to 14 million people in the Kinki district for multi-purposes, i.e. drinking water, industry, agriculture, power generation, recreation, etc. In order to learn how to cope with the environmental risk of chemicals, elucidating variations in DOM characteristics is necessary to quantify effects of DOM on the behavior and fate of micropollutants in the lake watershed.

The objectives of this research were to elucidate seasonal and spatial variations in: (1) the sorption coefficient ( $K_{\text{doc}}$ ) of pyrene and its derivatives onto DOM and (2) fluorescence properties of DOM in Lake Biwa.

## WATER SAMPLING

### Study area

Lake Biwa, mesotrophic, is located in the center of the main island of Japan (Figure 1). The lake has a surface area and a catchment area of 674 km<sup>2</sup> and 3,174 km<sup>2</sup>, respectively. The average depth and water residence time are 43 m and about 5.5 years for the northern lake, and 4 m and about 15 days for the southern lake. More than 120 rivers are flowing into the lake, while Seta River is the only natural out-flowing river at the southernmost of the lake. In summer, the northern lake is thermally stratified, resulting in thermocline formation, and then no vertical mixing of the lake water occurs (monomictic lake). Thermocline usually appears from May to November and has a great impact on material flow and recycling in the lake.



**Figure 1** | Lake Biwa and location of sampling sites in this research: #1 RCEQM; #2 Kitakomatsu-oki; #3 Adogawa-oki.

### Sampling sites

Water collections in this research were conducted at three sampling sites (see Figure 1). In order to assess seasonal variation in the sorption coefficient ( $K_{\text{doc}}$ ), lake waters were taken at the RCEQM (#1) in April, May, June, August, October, and December, 2005. At Kitakomatsu-oki (#2) site, lake waters were sampled at depths of 0.5–m (September, 2005) and 45–m (August, 2005 and February, 2006) to assess vertical distribution of  $K_{\text{doc}}$ . To characterize fluorescence properties of DOM, 3D-EEM spectra were obtained for the lake waters collected at depths of 0.5, 5, 10, 20, 50 m at the Adogawa-oki (#3) site (August, 2005 and February, 2006). Defined thermocline formation was observed during summer season at #2 and #3 sites.

## MATERIALS AND METHODS

### Reagents

$\text{NaH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4$  (Wako Chemicals) were used as delivered for phosphate buffer (pH 7.4, 20 mM) in batch sorption experiments. Pyrene, 1-hydroxypyrene (Pyrene-OH), 1-aminopyrene (Pyrene-NH<sub>2</sub>), 1-pyrene-carboxylic acid (Pyrene-COOH) and 1-methylpyrene (Pyrene-CH<sub>3</sub>) were purchased as fluorescence probes from Aldrich and used without further purification.

### Dissolved organic matters

#### Lake Biwa DOMs

Lake waters collected at #1 and #2 sites were concentrated by reverse osmosis (RO) system (*vide infra*) and used for batch sorption experiments. For EEMs measurements, lake waters at #3 site were filtered with GF/F.

#### Suwannee River samples

Suwannee River fulvic acid (SRFA) and natural organic matter (SRNOM) were purchased from the International Humic Substances Society (IHSS) and used as delivered.

### Isolation of Lake Biwa DOM (LBDOM)

Figure 2 schematically depicts the DOM isolation system by RO (Serkiz & Perdue 1990; Kusakabe 2007). A series of pretreatment steps (filtrations and softening) was applied to lake waters before RO concentration. Suspended solids were prefiltered by a Durapore<sup>®</sup> MF module (0.45 μm, Millipore) and then the permeate line of the module was connected to Na<sup>+</sup>-form cation exchange resin (DOWEX<sup>®</sup> 50W × 8, SUPELCO) column to remove multivalent inorganic cations in particular. The pretreated water was transferred into a reservoir of RO system (Proscale<sup>®</sup>, Millipore) with a polyamide module (Nanomax-95<sup>®</sup>, Millipore). After collecting the RO isolate, the physically fouled organic matter was recovered as DOM by recirculating 1 L ultrapure water. RO system was settled and operated at 4°C to suppress any possible microbial activity and deterioration of organic matters.

### DOC analysis

Dissolved organic carbon (DOC) concentration was measured as non-purgeable organic carbon (NPOC) by a Shimadzu TOC-V<sub>CPH</sub> analyzer furnished with a high sensitivity catalyst. The samples were acidified to below pH 2 by manual addition of 2 M HCl, followed by 20-min sparging with a high purity O<sub>2</sub> gas using an ASI-V autosampler (Shimadzu).

### Fluorescence quenching method

In this research, the sorption of pyrene and its derivatives onto DOM was assessed by the fluorescence quenching

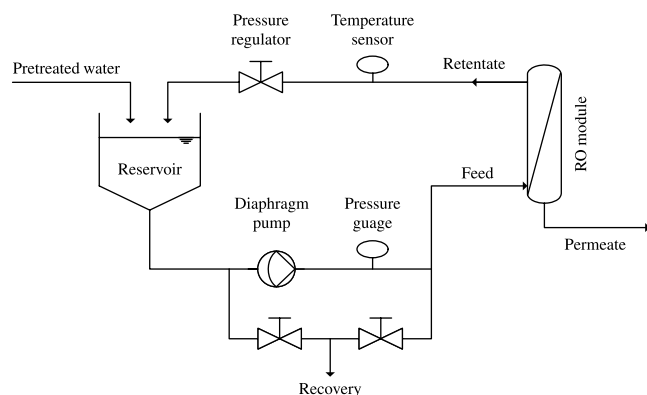


Figure 2 | RO system (Proscale<sup>®</sup>, Millipore) for DOM isolation in this research.

method. Sorption coefficients ( $K_{\text{doc}}$ ) were calculated from the Stern-Volmer equation as followed (Backhus *et al.* 2003).

$$\frac{F_0}{F} = 1 + K_{\text{doc}}[\text{DOM}] \quad (1)$$

In Equation (1),  $F_0$  and  $F$  = probe fluorescence intensities in the absence and presence of DOM, respectively;  $[\text{DOM}]$  = DOC concentration (kgC/L);  $K_{\text{doc}}$  = sorption coefficient or static Stern-Volmer constant (L/kgC). Inner filter effects (IFE) were corrected by taking into account the cell geometry of a spectrofluorometer (F-4500, Hitachi) and absorption characteristics of the solution (Shimizu & Lijstrand 1991), giving.

$$\frac{F_{\text{cor}}}{F_{\text{obs}}} = \frac{(\theta_{\beta} - \theta_{\alpha}) \ln T_{\lambda_{\text{ex}}}}{T_{\lambda_{\text{ex}}}^{\theta_{\beta}} - T_{\lambda_{\text{ex}}}^{\theta_{\alpha}}} \cdot \frac{(\omega_{\beta} - \omega_{\alpha}) \ln T_{\lambda_{\text{em}}}}{T_{\lambda_{\text{em}}}^{\omega_{\beta}} - T_{\lambda_{\text{em}}}^{\omega_{\alpha}}} \quad (2)$$

In Equation (2),  $F_{\text{cor}}$  and  $F_{\text{obs}}$  = fluorescence intensities corrected and observed, respectively;  $\theta$  = cell constants at excitation side ( $\theta_{\alpha} = 0.40$  and  $\theta_{\beta} = 0.54$ );  $\omega$  = cell constants at emission side ( $\omega_{\alpha} = 0.14$  and  $\omega_{\beta} = 1.00$ );  $T_{\lambda}$  = UV transmittance at  $\lambda$  nm. Dynamic quenching by dissolved oxygen (Danielsen *et al.* 1995) and efficiency of the static quenching by DOM (Backhus & Gschwend 1990) were almost negligible in this research.

### Three-dimensional excitation emission matrices (3D-EEMs) fluorescence spectroscopy

Fluorescence measurements were carried out by a Hitachi F-4500 spectrofluorometer. EEMs spectra were recorded by scanning both excitation (EX) and emission (Em) wavelengths from 200 to 600 nm. The unit of fluorescence intensity was normalized by comparing fluorescence intensity of 10 μg/L quinine sulfate solution at Ex350 nm/Em450 nm as 1 QSU (quinine sulfate unit). Blank spectrum was recorded with Milli-Q water and then was subtracted from all EEMs spectra of DOM. No IFEs correction was done for EEMs measurements.

**Table 1** | Results of DOM isolation by RO in this research

Date	Depth (m)	Volume (L)	DOC (mgC/L)	Concentration rate ( - )	DOC recovery (%)
<b>#1 RCEQM</b>					
16 Apr. 2005	-*	312	1.5	64.3	86.9
28 Apr. 2005	-	328	1.3	76.1	94.8
05 Mar. 2005	-	352	1.9	69.9	76.6
07 Jun. 2005	-	348	1.5	N.D.**	N.D.**
21 Aug. 2005	-	376	2.0	81.4	86.6
26 Oct. 2005	-	383	2.5	80.0	81.0
16 Dec. 2005	-	371	1.7	75.2	85.1
<b>#2 Kitakomatsu-oki</b>					
28 Aug. 2005	45	376	1.1	87.9	78.0
21 Sep. 2005	0.5	352	1.5	83.6	74.5
23 Feb. 2006	45	345	1.3	70.9	78.4

\*Sampling depth at RCEQM is about 2 m from bottom of the southern lake.

†no data.

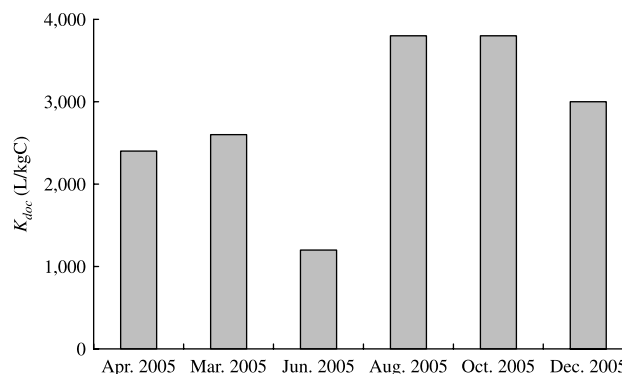
## RESULTS AND DISCUSSION

### Recovery of DOC

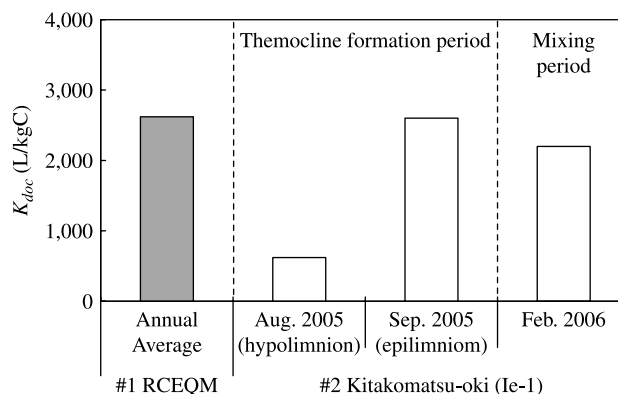
Table 1 shows the results of DOM isolation by RO system in this research. Average recovery of DOC was about 82.4% ( $n = 9$ ) and DOMs isolated in this research accounted for more than 80% of the whole DOM in the lake waters. The DOM isolation system by RO used could be applied to isolate representatives of Lake Biwa DOM (LBDOM) with relatively high recovery of DOC, without undesired fractionation, and without heat and chemical exposures.

### Seasonal variation in sorption coefficients ( $K_{doc}$ ) of pyrene

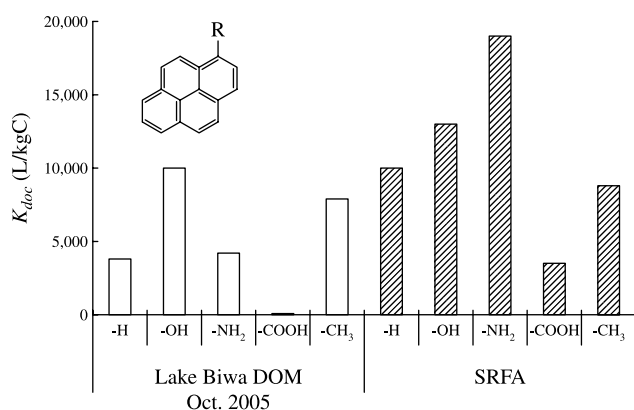
Figure 3 shows seasonal variation in  $K_{doc}$  values of pyrene onto Lake Biwa DOM at RCEQM (#1) site. During this research period,  $K_{doc}$  value of pyrene seasonally varied from 1,200 to 3,800 L/kgC. Logarithmic mean value of pyrene's  $K_{doc}$  was 2,600 L/kgC. These values of LBNOM are relatively low through the year compared to those of SRFA (10,000 L/kgC) and SRNOM (24,000 L/kgC). This indicates that more pyrene freely dissolves in water column of Lake Biwa than calculated using  $K_{doc}$  values of SRFA and SRNOM. There is emerging concern that using  $K_{doc}$  values



**Figure 3** | Seasonal variation in sorption coefficient ( $K_{doc}$ ) of pyrene onto Lake Biwa DOMs at RCEQM (#1).



**Figure 4** | Spatial variation in sorption coefficient ( $K_{doc}$ ) of pyrene onto Lake Biwa DOMs.



**Figure 5** | Effects of functional groups on sorption coefficient ( $K_{doc}$ ).

of Suwannee River samples results in underestimation of the environmental risk of a chemical of interest.

### Vertical change of sorption coefficients ( $K_{doc}$ ) of pyrene

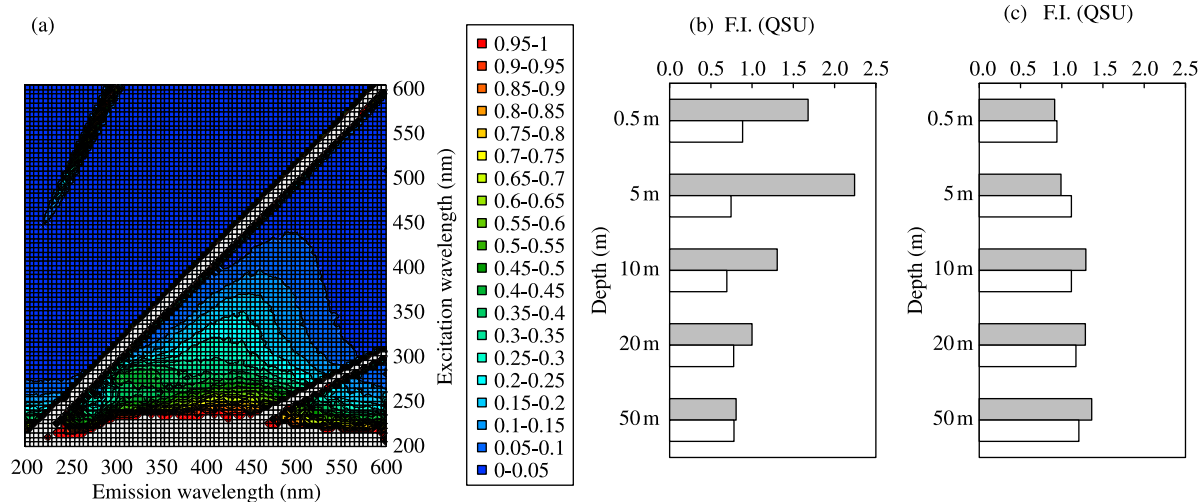
Figure 4 shows vertical differences of  $K_{doc}$  values of pyrene onto Lake Biwa DOM at Kitakomatsu-oki (#2) site between summer and winter.  $K_{doc}$  of pyrene onto Lake Biwa DOMs in summer were 620 and 2,600 L/kgC in hypolimnion and epilimnion, respectively. After mixing of lake water,  $K_{doc}$  in winter became a mean value. This result suggested that dynamic variation in sorption of micropollutants onto DOM annually occurs in Lake Biwa.

### Effects of functional groups on sorption coefficient ( $K_{doc}$ )

To elucidate the effects of functional groups on  $K_{doc}$ , pyrene and its derivatives were used as a probe for sorption experiments. Their  $K_{doc}$  values for LBNOM and SRFA were shown in Figure 5. In the case of SRFA, Pyrene-NH<sub>2</sub> and Pyrene-COOH were interacted with negatively charged SRFA stronger and weaker, respectively, due to electrostatic interactions. For Pyrene-OH, hydrogen bonding could contribute small increment of  $K_{doc}$ , while methyl group did not affect the sorption onto SRFA. In the case of LBNOM, functional groups might affect  $K_{doc}$  in different manner from SRFA. Although this reason is not clear at the present moment, these results in this research indicate that LBNOM consists of substances in different quality and quantity, and differently interacts with organic micropollutants. Further detailed component analyses should be needed to shed light upon these phenomena.

### EEMs and vertical profile of fluorescence properties of DOM in Lake Biwa

EEMs and vertical profile of fluorescence properties of DOM at Adogawa-oki (#3) site were shown in Figure 6. A few peaks, accordingly fluorophores, were detected for



**Figure 6** | Fluorescence properties at Adogawa-oki (#3) site: (a) EEMs spectrum of Lake Biwa DOM (0.5m); vertical profiles of fluorescence intensity at (b) Em230/Ex300 and (c) Em230/Ex425 (□, Aug. 2005; □, Feb. 2006). Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from [www.iwaponline.com/wst](http://www.iwaponline.com/wst).

Lake Biwa DOM by means of 3D-EEMs spectroscopy without tedious pretreatment in this research. Vertical profiles of peaks at Ex230/Em300 (protein-like) and Ex230/Em425 (fulvic/humic-like) showed individual trends. The peak at Ex230/Em300 showed maximum fluorescence intensity at a depth of 5 m in summer due to an algal activity, while no variation was shown in winter (mixing) period. On the other hand, there was no difference in profiles of the peak at Ex230/Em425 between stratified and mixing periods in this research. It is suggested that fulvic and/or humic-like substances in Lake Biwa do not originate from these autochthonous source and might have been accumulated in the lake water. The origin of the recalcitrant dissolved organic matter in Lake Biwa has been discussed and is controversial even today. Further holistic survey should be necessary to come up with an answer to this issue.

## CONCLUSIONS

In this research, sorption of pyrene and its derivatives and fluorescence properties were assessed to elucidate seasonal and spatial variations in characteristics of Lake Biwa DOMs. The following conclusions can be drawn: (1) our DOM isolation system can be used to obtain representatives of the whole DOM in Lake Biwa; (2)  $K_{\text{doc}}$ s determined by the fluorescence quenching method seasonally changed from 1,200 to 3,800 L/kgC; (3) The annual mean  $K_{\text{doc}}$  (2,600 L/kgC) was relatively low compared to SRFA (10,000 L/kgC) and SRNOM (24,000 L/kgC); (4) Vertical distribution of  $K_{\text{doc}}$  was affected in summer due to thermocline formation, *i.e.* stratification. In this research, Lake Biwa DOM in hypolimnion showed lower  $K_{\text{doc}}$  value contrary to the expectation; (5) sorption experiments to assess the effects of functional groups on pyrene indicated that Lake Biwa DOM could consist of different components from SRFA; (6) EEMs could be applied to characterize DOM in Lake Biwa. Fluorescence intensities of the peaks at Ex230/Em300 (protein-like) and Ex230/Em425 (fulvic/humic-like) varied in the different manner. It was indicated that the protein-like fluorescence peak associated with an algal activity in epilimnion in summer, and the fulvic/humic-like fluorescence peak showed no difference in vertical profile between in summer and in winter.

## ACKNOWLEDGEMENTS

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