

# Excitation–emission fluorescence spectra and trihalomethane formation potential in the Tama River, Japan

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**Abstract** Three-dimensional fluorescence spectroscopy was applied to investigate the water quality in the Tama River, Japan, and the variation of the excitation–emission matrix (EEM) profiles of the river water was discussed with particular reference to trihalomethane formation potential (THMFP). The EEMs of the water downstream of a lake exhibited a small but strong peak at 225 nm (excitation)/295 nm (emission) and a weak one at 270 nm/295 nm, which seemed to be derived from algae. The EEMs of the water receiving treated sewage had a distinctive, large and broad peak at around 345 nm/430 nm and also a strong fluorescence at 240 nm/330–450 nm. From the comparison with EEMs of human urine, humic acid and a laundry detergent with fluorescent whitening agents, the peak at around 345 nm/430 nm on the EEM of treated sewage was also found on the EEM of the laundry detergent. The fluorescence intensity had a good correlation with T-THMFP for a wide range of excitation/emission wavelengths considered. The fluorescence intensity at 255–295 nm/345–385 nm correlated with T-THMFP better than E260, and the maximum value of the determination coefficient was  $R^2 = 0.90$  at 260 nm/355 nm, where no distinctive peak was found on the EEMs of the river water samples. The highest correlation coefficient between the fluorescent intensity and DOC was  $R^2 = 0.77$  at 280 nm/335 nm. Multiple regression analysis revealed that the fluorescence emission from a unit concentration of THM precursors was 1,000 times or more higher than that of the overall DOC, particularly in the wavelength range of 250–265 nm/325–480 nm, and that the THM precursors in the river water contributed to a much larger degree to the fluorescence than the other DOMs not forming THM.

**Keywords** Dissolved organic matter (DOM); excitation emission matrix (EEM); fluorescence spectrum; natural organic matter (NOM); trihalomethane formation potential (THMFP)

## Introduction

Dissolved organic matter (DOM) in drinking water resources is of significant concern since it introduces disinfection by-products, colors and odors in drinking water, and bacterial regrowth in distribution systems (Williamson *et al.*, 1999). The indices such as trihalomethane formation potential (THMFP), color, odor, assimilable organic carbon (AOC) and bacterial regrowth potential (BRP) must be measured at drinking water facilities, but they yield little information on the origin of the DOMs. Characterization of DOMs from physical/chemical/biological viewpoints is essential not only in water treatment, but also in water resource management.

Due to the complex nature of DOMs in drinking water resources, various analytical methods have been used to characterize them, e.g., the use of chemical components such as amino acids and carbohydrates, molecular weight/size distribution, pyro-chromatogram and fluorescence spectrum. The methods used to characterize natural organic matter (NOM) have been summarized recently by Croue *et al.* (2000). Fluorescence spectrometry has been widely used due to its simplicity and its requirement of minimal sample amount and pretreatment. A three-dimensional excitation–emission matrix (EEM) obtained by fluorescence spectrometry with scanning the wavelengths of both excitation and emission can be used to distinguish DOMs in natural waters (Coble *et al.*, 1990; Coble, 1996).

In this study, three-dimensional fluorescence spectroscopy was applied to investigate the water quality in the Tama River, Japan, from the upstream (drinking water resource) to

the downstream (receiving treated sewage) location, and the variation of EEM profiles of the river water was discussed with respect to DOC (dissolved organic carbon), UV absorbance and THMFP. To determine the compounds responsible for the fluorescence peaks on the EEMs of the river water, EEMs of three kinds of possible pollutants were also measured. Simple and multiple regression analyses between the fluorescence intensity and DOC or THMFP were applied: (1) to discuss the characteristics of the EEM profiles; (2) to predict the THMFP value by fluorescence intensity; and (3) to understand the contribution of THM precursors to the fluorescence.

### Materials and methods

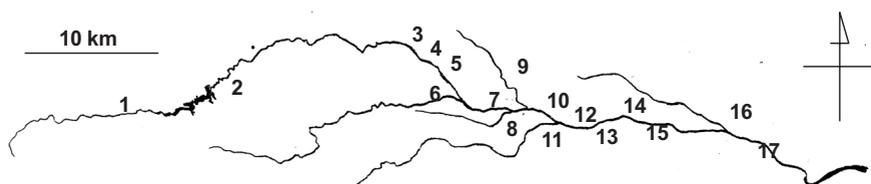
River water was collected at 17 sampling sites in the Tama River, Japan, and its tributary streams (Figure 1) in September and November 2000 and in January 2001. The collected water was filtered with pre-combusted GF/F filters for further analysis. The Tama River originates from a mountainous area and reaches Tokyo Bay, flowing from west to east. There is an artificial lake (185,400,000 m<sup>3</sup>) between sites 1 and 2, where there is less human activity and the forest is dominant. Between sites 3 and 4 there is an intake weir, where the flow rate decreases down to 0–51.2% due to the intake for drinking water resource. The lower Tama River flows through a densely populated area of more than five million people, receiving the discharges from polluted tributary streams and sewage treatment plants. Sites 6, 8, 9, 11 and 16 were located at the tributary streams and site 14 was at a discharge channel of a sewage treatment plant. Site 17 was located at the south end of the Tokyo metropolis.

Dissolved organic carbon (DOC) was determined as dissolved non-purgeable organic carbon (D-NPOC) using a TOC analyzer (Shimadzu TOC-5000). Ultraviolet (UV) absorbance at 260 nm (E260) was measured by a spectrophotometer (Hitachi U-2000) with 5 cm quartz cells. Specific UV absorbance (SUVA) was calculated, dividing E260 by DOC. THMFP was determined according to the Japanese Standard Methods for the Examination of Water (the samples were put in contact with chlorine for 24 hours at 20°C and the residual chlorine concentration was 1.0–2.0 mg/L). Total THMFP (T-THMFP) was given as a sum of concentrations (mol/L) of CHCl<sub>3</sub>, CHCl<sub>2</sub>Br, CHClBr<sub>2</sub> and CHBr<sub>3</sub>. Fluorescence intensities were measured using a Hitachi F-4500 spectrofluorometer at every 5 nm wavelength from 200 to 600 nm for both excitation and emission.

To determine the substances responsible for the fluorescence of the river water, EEMs of the following substances were also measured: human urine, a laundry detergent containing fluorescent whitening agents (FWAs), and commercially available humic acid (Wako Pure Chemical Industries Ltd.). All of these substances were dissolved into reagent-grade water and were applied to the fluorescence analysis.

### Results and discussion

The results of DOC, E260, THMFP and SUVA are shown in Table 1. The values of DOC, E260 and T-THMFP increased at site 2, due to retention of water at the artificial lake between sites 1 and 2. The values of SUVA at site 2 were relatively lower than those at the other sites and it was indicated that there might be a characteristic source of DOM at the



**Figure 1** Sampling location (Tama River, Japan)

**Table 1** DOC, E260, T-THMFP and SUVA at Tama River

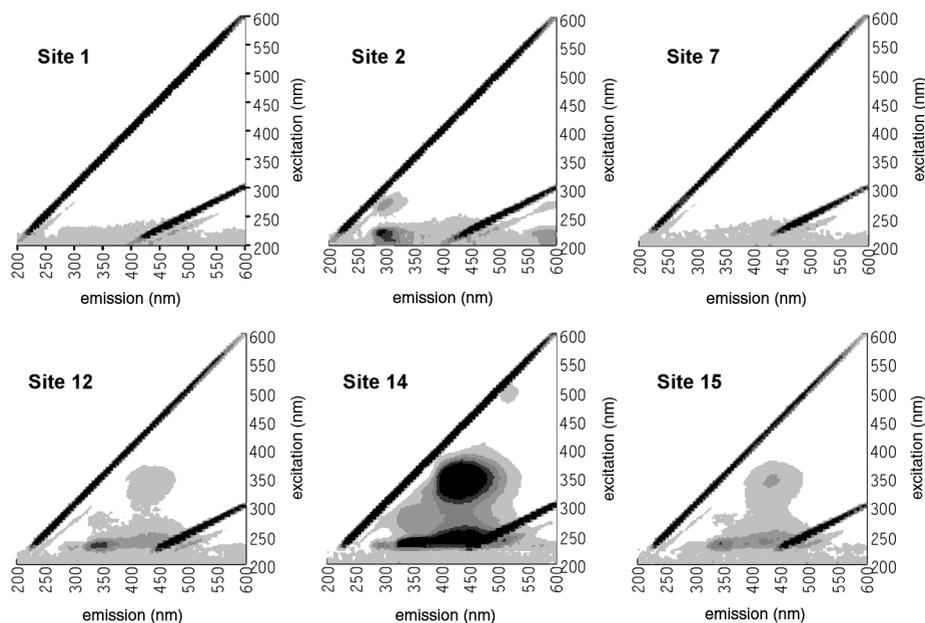
Sampling site)	DOC (mgC/L)			E260 (1/5cm)			T-THMFP ( $\mu\text{mol/L}$ )			SUVA (L/mgC/m)		
	Sept.	Nov.	Jan.	Sept.	Nov.	Jan.	Sept.	Nov.	Jan.	Sept.	Nov.	Jan.
1	1.0	0.4	0.5	0.097	0.055	0.047	0.21	0.19	0.14	1.9	2.7	1.8
2	2.2	0.8	1.2	0.107	0.057	0.064		0.26	0.17	1.0	1.4	1.0
3	1.2	0.7	0.6	0.082	0.047	0.062		0.23	0.16	1.4	1.4	2.0
4	0.8	0.4	0.7	0.081	0.049	0.066	0.20	0.20	0.22	2.0	2.6	2.0
5	0.6			0.072						2.3		
6*	0.5	0.6		0.066	0.046			0.19		2.8	1.5	
7	0.7			0.068						1.9		
8*		1.4			0.173			0.41			2.5	
9*		0.4			0.062			0.25			2.8	
10	1.4		3.5	0.163		0.260	0.28		0.54	2.3		1.5
11*	1.0		2.6	0.110		0.186			0.46	2.2		1.5
12	1.1			0.130						2.3		
13	1.5			0.136			0.35			1.8		
14**	3.6	3.5		0.381	0.372			0.77		2.1	2.1	
15	1.4			0.163			0.58			2.3		
16*	1.0			0.161						3.2		
17	1.7			0.199						2.4		

\* Tributary streams, \*\* discharge from a sewage treatment plant

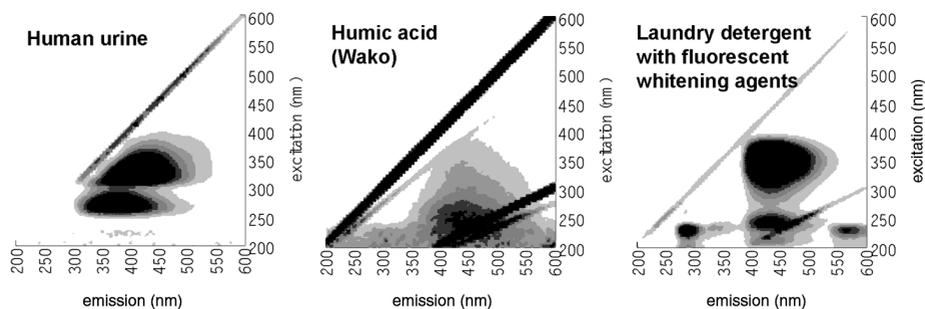
lake. From our data, algal-derived DOM had lower SUVA (about  $\leq 1$  L/mgC/m; unpublished data) and released DOM from lake sediments gave 5–15 L/mgC/m of SUVA (Nakajima *et al.*, 2001). The low SUVA at site 2 seemed to be due to the algal growth/decay. Site 10 and the lower sites had higher DOC, E260 and T-THMFP values than the higher sites, apparently affected by the inflows from polluted tributary streams and from sewage treatment plants. The determination coefficient between T-THMFP and DOC was relatively lower ( $R^2 = 0.75$ ) than that between T-THMFP and E260 ( $R^2 = 0.87$ ). As generally accepted, E260 was a good index to predict T-THMFP ( $\text{T-THMFP} = 2.10 \times \text{E260} + 0.0647$ ).

The selected EEMs of the water collected in September are shown in Figure 2. There was no distinctive fluorescence peak observed at sites 1–7 except for site 2. At site 2, or just after retention at a lake, a small but strong peak appeared at 225 nm (excitation)/295 nm (emission) and a weak one at 270 nm/295 nm. These peaks were also found on the EEMs of the algal-derived DOM (unpublished), and the algal growth/decay between sites 1 and 2 was indicated not only by SUVA but also by EEM.

The EEMs at sites 12 and 15 (Figure 2) had a distinctive, large and broad peak at around 345 nm/430 nm and also a strong fluorescence at 240 nm/330–450 nm. These peaks were much clearer at site 14, or at the discharging point of a sewage treatment plant. The appearance of the peaks was consistent throughout the sampling period. To determine the substances responsible for the fluorescence pattern, EEMs of human urine, humic acid and a laundry detergent were measured (Figure 3). The human urine had two strong peaks at 270 nm/380 nm and 320 nm/410 nm, but neither of them corresponded with the peaks at site 14. The humic acid had a broad peak at 235–255 nm/435–465 nm which was overlapped by one of the peaks detected at the sewage treatment discharge. The laundry detergent had four distinctive peaks at 230 nm/285 nm, 240 nm/435 nm, 225 nm/565 nm and 350 nm/430 nm; the latter was very broad while the others were sharp. At least, the peak at 350 nm/430 nm seemed to be caused by FWAs. It was unknown which FWAs were used in the laundry detergent, but FWAs generally have a property of UV absorbance and blue fluorescence; for example, both diaminostilbene (DAS 1) and distyrylbiphenyl (DSBP), two of FWAs, absorb UV light at 350 nm and emit blue visible light at a maximum wavelength of 430 nm. They are not biodegradable and 22% of the consumed FWAs were annually discharged to



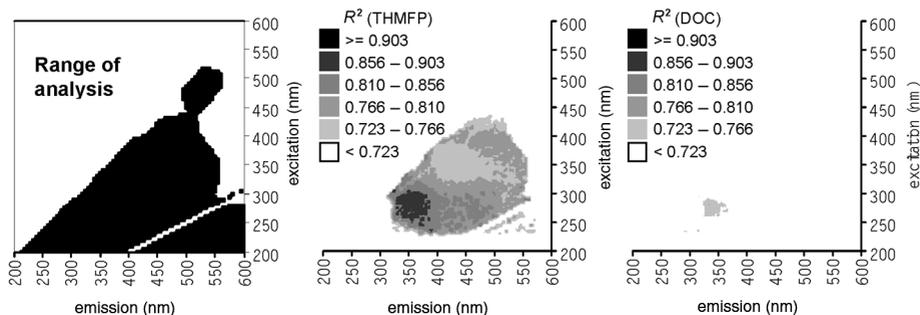
**Figure 2** Three-dimensional excitation–emission matrix (EEM) of water collected at the Tama River in September 2001. The darker color indicates a stronger fluorescence intensity



**Figure 3** EEMs of human urine, a commercially available humic acid, and a laundry detergent with fluorescent whitening agents

surface water in Switzerland (Poiger *et al.*, 1998). Two of the four peaks detected in the laundry detergent seemed to be similar to those which appeared on the EEM of site 14. This indicated that the characteristic EEM pattern at the sewage treatment discharge might be strongly influenced by discharge of detergents with FWAs.

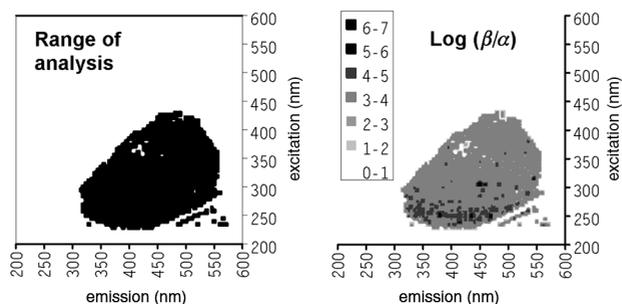
To characterize the EEMs and to find a good predictor of T-THMFP, simple regression analysis was applied to the fluorescence intensities at each grid on EEMs and two water quality indices, T-THMFP and DOC. The region of the analysis and the distribution of the determination coefficients between the fluorescence intensity and T-THMFP and between the fluorescence intensity and DOC are depicted in Figure 4. The fluorescence intensity has a good correlation with T-THMFP in the wide range of excitation/emission wavelengths. The determination coefficients between the fluorescence intensity at 255–295 nm/345–385 nm and T-THMFP were higher than the determination coefficient between T-THMFP and E260 ( $R^2 = 0.87$ ). The maximum value of the determination coefficient between the fluorescence intensity and T-THMFP was  $R^2 = 0.90$  at 260 nm/355 nm, where no distinct peak was found on EEMs of the river water samples (Figure 1). On the other hand, the correlation between the fluorescence intensity and DOC was lower than that between the fluorescence



**Figure 4** Simple regression between fluorescence intensity and T-THMFP and between fluorescence intensity and DOC: Region of the analysis (left), correlation coefficients between fluorescence intensity and T-THMFP (center) and between fluorescence intensity and DOC (right)

intensity and T-THMFP and the highest determination coefficient was  $R^2 = 0.77$  at 280 nm/335 nm. The excitation/emission wavelengths where the highest correlation coefficient was observed were not the same, but very close between T-THMFP and DOC.

To determine the contribution of THM precursors to the fluorescence, multiple regression analysis was also applied. Although THM precursor is a part of DOC and not independent, the molar concentrations of DOC and THMFP were used as independent explanatory variables in this analysis, i.e.  $\text{Fluorescence} = \alpha (\text{DOC}) + \beta (\text{T-THMFP}) + \gamma$ , because the ratio of THM precursor in DOC was as low as 0.16–0.70% (as carbon atoms). In the range of wavelengths where the determination coefficient was higher than 0.75, the value of  $\log(\beta/\alpha)$  was calculated and plotted in Figure 5. The factors  $\alpha$  and  $\beta$  have units of fluorescence intensity  $\times$  L/mol carbon, which means the fluorescence emission per unit carbon concentration, and the value of  $\log(\beta/\alpha)$  is the logarithmic ratio of the specific fluorescent emission from THM precursors and the overall DOMs. Figure 5 shows that the factor  $\beta$  was 1,000 times or more higher than the factor  $\alpha$  over almost the entire range of the analysis, and it means that THM precursors had more fluorescence than the overall DOMs. According to Senesi (1993), 40–60% of NOM is fluorescent, indicating that more than 40–60% of THM precursors seemed to be fluorescent. The relatively higher value of  $\log(\beta/\alpha)$  was observed in a broad range of wavelengths of 250–265 nm/325–480 nm (Figure 5). This range of excitation wavelengths agrees with the wavelengths used for UV absorbance measurement to predict T-THMFP. As mentioned above, the ratio of THM precursor in DOC was 0.16–0.70% and the value  $\beta/\alpha$  was more than 1,000, so then the ratio of contribution to the fluorescence  $[\beta (\text{T-THMFP})/\alpha (\text{DOC})]$  was calculated as more than 1.6–7.0. Since the DOC included the THM precursors, in other words, the contribution of



**Figure 5** Multiple regression to explain fluorescence intensity by DOC and T-THMFP, i.e.  $\text{Fluorescence} = \alpha (\text{DOC}) + \beta (\text{T-THMFP}) + \gamma$ . Range of analysis (left), and the ratio of  $\beta/\alpha$  expressed as logarithmic value (right)

the overall DOC to the fluorescence included that of THM precursors, the result indicated that the THM precursors in the river water contributed much more to the fluorescence than the other DOMs not forming THM, even though the THM precursors existed as only 0.16–0.70% of DOC.

### Conclusion

Three-dimensional fluorescence spectroscopy was applied to investigate water quality in the Tama River, Japan, and the variation of the EEM profiles of the river water was discussed with particular regard to T-THMFP. The results were as follows.

- The EEMs of the water at the downstream of a lake had a small, but strong, peak at 225 nm (excitation)/295 nm (emission) and a weak one at 270 nm/295 nm, which seemed to be derived from algae. The EEMs of the water receiving treated sewage had a distinctive, large and broad peak at around 345 nm/430 nm and also a strong fluorescence at 240 nm/330–450 nm. It was indicated that the characteristic EEM pattern at the sewage treatment discharge might be strongly influenced by the discharge of detergents with fluorescent whitening agents.
- The fluorescence intensity had a good correlation with T-THMFP in the wide range of excitation/emission wavelengths considered. The fluorescence intensity at 255–295 nm/345–385 nm correlated with T-THMFP better than E260, and the maximum value of the determination coefficient was  $R^2 = 0.90$  at 260 nm/355 nm, where no distinctive peak was found on the EEMs of the river water samples. The highest determination coefficient between the fluorescence intensity and DOC was  $R^2 = 0.77$  at 280 nm/335 nm.
- Multiple regression analysis revealed that the fluorescence emission from a unit concentration of THM precursors was 1,000 times or more, higher than that of the overall DOC, particularly in the wavelength range of 250–265 nm/325–480 nm, and that the THM precursors in the river water contributed to a much larger degree to the fluorescence than the other DOMs not forming THM, even though the THM precursors existed as only 0.16–0.70% of DOC.

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