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Multistep, microvolume resin fractionation combined with 3D fluorescence spectroscopy for improved DOM characterization and water quality monitoring

**Qunshan Wei · Changzhou Yan · Jianshe Liu ·
Zhuanxi Luo · Qiujin Xu · Xian Zhang ·
Christopher W. K. Chow · Meng Nan Chong**

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Abstract Conventionally, resin fractionation (RF) method has been widely used to characterize dissolved organic matter (DOM) found in different source waters based on general and broad DOM fractions grouping. In this study, a new refined method using multistep, microvolume resin fractionation combined with excitation emission matrix fluorescence spectroscopy (MSM-RF-EEMS) was developed for further isolation and characterization of subfractions within the primary DOM fractions separated from using the conventional RF method. Subsequently, its feasibility in indicating the occurrence of urban pollution in source waters was also assessed. Results from using the new MSM-RF-EEMS method strongly illustrated that several organic subfractions still exist within the regarded primary pure hydrophobic acid (HoA) fraction including the humic- and fulvic-like organic matters, tryptophan- and tyrosine-like proteins. It was found that by using the MSM-RF-EEMS method, the organic subfractions present within the primary DOM fraction could be easily identified and characterized. Further validation on the HoA fraction using the MSM-RF-EEMS method revealed that the constant association of EEM peak T1 (tryptophan) fraction could specifically be used to indicate the occurrence of urban pollution in source water. The correlation analysis on the presence of EEM peak T2 (tyrosine) fraction could be used as a supplementary proof to further verify the presence of urban pollution in source waters. These findings on using the presence of EEM peaks T1 and T2 within the primary HoA fraction would be significant and useful for developing a sensory device for online water quality monitoring.

Keywords Dissolved organic matter (DOM) · Water quality monitoring · Fractionation · DAX resin · 3D fluorescence

Q. Wei (✉) · C. Yan (✉) · Z. Luo · X. Zhang
Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences,
1799 Jimei Road,
Xiamen 361021, China
e-mail: qswei@iue.ac.cn
e-mail: czyan@iue.ac.cn

Q. Wei · J. Liu
College of Environmental Science & Engineering,
Donghua University,
Songjiang District, Shanghai 201620, China

Q. Xu
Chinese Research Academy of Environmental Sciences,
Chaoyang District, Beijing 100012, China

C. W. K. Chow
Australian Water Quality Centre,
South Australian Water Corporation,
250 Victoria Square,
Adelaide, SA 5000, Australia

M. N. Chong
School of Engineering, Monash University,
Jalan Lagoon Selatan,
46150 Bandar Sunway, Selangor, Malaysia

Introduction

Unpredictable rainfall patterns, in conjunction with population increases, are placing pressure on water resources in many urban areas. Consequently, water utilities around the world are developing action plans with the aim of ensuring water is managed sustainably while meeting customer demand (Henderson et al. 2009). A rapid, highly sensitive, and selective detector is urgently required to detect contamination events in source water. Fluorescence spectroscopy has been suggested as a potential monitoring tool for water quality monitoring given its high sensitivity and selectivity (Henderson et al. 2009; Hudson et al. 2007).

Dissolved organic matter (DOM) that presents in waterways normally contributes to most of the fluorescence in source water. However, DOM is usually of different natures and originated from a range of sources. Some are allochthonous, transported from the surrounding landscape to the water body, and some are derived from and influenced by the geologic land use and hydrology of their origin. Some are created in situ through microbial activity, which may provide an independent source of organic matter into the water body (Chow et al. 2004; Hudson et al. 2007). Certain types of human activities are also considered as sources of generating DOM, much of which are believed to be labile and can enter the aquatic system through direct point discharges, diffuse leaching, and aerial dispersal (Bieroza et al. 2009; Hudson et al. 2007). Whether it causes esthetic concerns such as color, taste, and odor; leads to the binding and transport of organic and inorganic contaminants; produces undesirable disinfection byproducts; provides sources and sinks for carbon; or mediates photochemical processes, the nature and properties of DOM in water are topics of significant environmental interest (Leenheer and Croue 2003). Therefore, a complete understanding on the unknown structures and components in DOM is key to better supply of source water and better treatment of drinking water (Chow et al. 2004; Leenheer and Croue 2003).

Since the 1970s, resin fractionation (RF) has been widely used in the characterization of DOM in different source waters. The RF method fractionates or isolates DOM components into a number of primary fractions depending on their affinities to different resins and back-elution efficiencies (Aiken et al. 1979; Chow et al. 2006; Imai et al. 2001; Kitis et al. 2002; Leenheer 1981; Peuravuori et al. 1997; Thurman and Malcolm 1981). Typically, the DAX-8 (or XAD-8) and XAD-4 resins are used to fractionate DOM into primary chemical hydrophobic and hydrophilic fractions, normally known as humic or nonhumic distribution or operationally grouped as hydrophobic acids (HoA), neutrals (HoN), and bases (HoB), transphilic acids or weakly (slightly) hydrophobic acids (WHoA), and other chemical fractions (Chow et al. 2006; Leenheer and Croue 2003; Wei et al. 2008a, b).

The RF method has also been widely used in the performance assessment of water treatment processes (e.g. coagulation, advanced oxidation; Fabris et al. 2004; Fitzgerald et al. 2006; Liu et al. 2008; Lu et al. 2009; Soh et al. 2008; Xue et al. 2009; Zhang et al. 2009; Zhao et al. 2009). However, each of these conventional chemical fractions is often considered as one whole (pure) matter. For example, the HoA fraction is often collected in one beaker when eluted from the XAD-8 column, and subsequently analyzed as a whole. In such a way, the HoA can only be characterized in bulk without being able to measure the potential presence of sub-fractions that contribute to the characteristics of bulk HoA (Provenzano et al. 2006; Wong et al. 2002). To date, only a few studies have focused on the further isolation of sub-fractions within the traditional primary chemical fraction (TPCF). In the most recent study, Wei et al. (2011, 2012) has investigated on the molecular size subfractions of aquatic HoA fraction (mainly humic acid). They revealed that the HoA actually consists of all size range of molecular organics and contains relatively high proportion of low size materials (<5 kDa), which is not the same as previously regarded HoA that is predominantly comprised of large molecules.

In this study, a new refined method using multistep, microvolume resin fractionation combined with excitation emission matrix fluorescence spectroscopy (MSM-RF-EEMS) was developed for further isolation and characterization of sub-fractions within the primary DOM fractions separated by traditional RF method. The EEMS is a relatively rapid, nondestructive analytical method with little sample preparation requirement. The generation of three dimension (3D) plots of fluorescence excitation wavelength, emission wavelength, and intensity allow the visualization of a range of fluorophores from the analyzed environmental water samples. An additional feature of the EEMS approach is the presence of a vast array of spectroscopic data available for further interpretation within an EEM (Hudson et al. 2007). However, the chemical (hydrophobic/hydrophilic) characteristics of these fluorophores are less of known. This new refined method will be used to assess the feasibility of using organic subfractions as urban pollution indicators in various environmental source waters.

Methods and materials

Source waters

In January 2011, two types of source water samples, including river water and reservoir water, were collected in Guangdong Province, southern China. The river water sample (SWC river), which was known to be polluted by urban sourced pollutions (such as wastewater, sewagem or runoff), was selectively sampled at the downstream (22° 53.710′N, 114° 30.430′E, H 20.0 m) of a town (ST town). The reservoir water (FT reservoir, 22° 45.919′N, 114° 31.614′E, H 28.2 m) was regarded to be unpolluted, which was located on the top of a mountain with good environmental protection and without any industrial and agricultural activities. The DOM from the river and reservoir were designated as polluted and unpolluted DOM, respectively, in the following sections. In addition, another pair of waters (river and reservoir) were also sampled in the same time and used for verifying the new MSM-RF-EEMS method. The EEMS results of another pair of waters were similar to the first pair of waters, so the results were not shown here for concision of discussion.

Each water sample was collected in a 4 L brown color borate glass bottle that had been precleaned with 10 % HCl, 1 % NaOH, and distilled water. All the samples were filtered (0.45 μm) and refrigerated immediately after collection.

RF of HoA

Isolation of HoA by traditional RF

The volume of water samples used in this study for the traditional RA fractionation procedure is 2,000 mL and the volume ratio between the water sample to wet resin is 30:1. Filtered (0.45 μm) water sample was first passed through DAX-8 resin (Supelco, USA) without any pH adjustment, resulting in the hydrophobic base and neutral fractions being retained on the resin. The pH of effluent from the DAX-8 column was then adjusted to pH 2 and poured into another DAX-8 resin column (the same size as the first column) to adsorb the HoA. Then, 10 bed-volumes of 0.01 mol/L NaOH solution was loaded into the column and the effluent was collected into a large glass container. The isolated HoA fraction was regarded as one TPCF and the solution was used for subsequent analysis and characterization (e.g., ultraviolet absorption and EEMS). More details could refer to reported methods (Wei et al. 2008a, b).

New RF—multistep micro-RF

The new MSM-RF is an innovative and refined method, at which the multiple HoA subfraction samples are recovered and collected individually in microvolume aliquots. The diagram of the MSM-RF procedures is shown in Fig. 1. After adsorption of HoA in a DAX-8 resin column with the bed volume of 10 mL, the valve at the end of column was closed. After that, a NaOH solution (0.01 mol L^{-1}, 100 mL) was loaded into the column followed by opening the valve to collect multiple aliquots (each 5 mL) of effluent with a constant flow velocity of 15 bed-volumes/h. Each aliquot sample was then analyzed by EEMS immediately. The procedure was repeated until the 3D fluorescence signal is no longer detectable (≤(1±5 %)× pure water signal).

Excitation emission matrix fluorescence spectroscopy

EEMS measurements were undertaken using an F-4600 FL spectrophotometer (Hitachi, Japan) in accordance to the published methods (Baker 2001; Liu et al. 2007) with modifications to a certain extent. The spectrometer used a xenon excitation source, and slits were set to 5 nm for both excitation and emission. To obtain the 3D fluorescence, the excitation wavelengths were incremented from 200 to 400 nm at 5-nm steps for each excitation wavelength; while the emission was detected from 280 to 500 nm at 2-nm steps. The temperature of the analysis and PMT voltage were set at 23±1.5 °C and 700 V, respectively. Quinine sulfate (QS) solution (1 μg QS/L in 0.1 mol/L H_2SO_4) was used to monitor the stability of energy emitted by the xenon lamp in the fluorometer and no

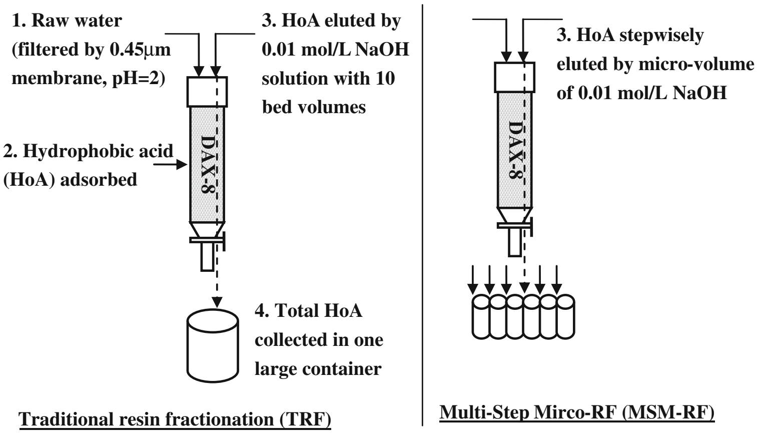

Fig. 1 Diagram of traditional and novel resin fractionation procedures

change in QS fluorescence was observed during the study. The pH of all samples was adjusted using HCl or NaOH solutions (1 mol/L) to 7 before the EEMS measurements. Finally, the 3D contour graphs were plotted by using OriginPro (7.5) software based on the raw matrix data from F-4600 instrument. The volume of HCl or NaOH solution used for adjusting sample pH was so less (<0.05 mL) that its effect on the dilution of the sample could be negligible.

Three blank water scans were run every three analyses using a sealed distilled water cell. The Raman peak of water at 345 nm (excitation) was used as a test for machine stability and Raman emission at 398 nm averaged 20.4±0.9 arbitrary fluorescence units (afu; $n=45$), with no drift through the analytical period. In addition, the stability of the Raman peak was assessed for a 10-min period at the start of each day of data collection, and analyses occurred only when the signal/noise ratio of the spectrometer was greater than 500:1 (Baker 2001).

Chemical analysis and data process

The basic water qualities of pH, conductivity and dissolved oxygen (DO) were measured using pH meter (FE20, Mettler Toledo), conductivity meter (ExStik EC400, Extech Instruments, USA) and multiparameter water quality Sonde probe (6600 V2, YSI, USA), respectively. Reagent-grade chemicals and ultrapure water (Milli-Q) were used. The filtered solution (through 0.45 μm pore size membrane) was analyzed for dissolved organic carbon (DOC; TOC-VCPH, Shimadzu, Japan), UV_{254} (UV-9600 spectrophotometer with 1 cm cuvette, Beijing Rayleigh), dissolved nitrogen (DN), and dissolved phosphorus (DP; QuikChem-8500 Flow Injection analysis instrument, Lachat, USA). The species of DN and DP were analyzed according to the QuikChem methods (10-107-04-1-C for NO_3 and NO_2,10-107-06-1-J for NH_3 and 10-115-01-1-A for orthophosphate). The measured basic water qualities are shown in Table 1.

Results and discussion

Variation of UV absorbance of micro-effluents obtained from MSM-RF

As shown in Table 1, both of the polluted water samples can be easily distinguished from the unpolluted water samples based on the basic water quality parameters. Thus, the selected waters were suitable for comparison study to test the feasibility of applying the new MSM-RF method to reveal the fractional and fluorescent characteristics in the DOM from different source waters.

The HoA fractions from both the polluted and unpolluted DOM samples were recovered and fractionated by using both the conventional RF method and new refined MSM-RF method. Subsequently, the UV absorbance measurements at 254 nm (UV_{254}) were taken from the effluents of HoA from DAX-8. By using the conventional RF method, it was measured that the UV_{254} of bulk HoA were 0.200 AU (per centimeter) for polluted DOM sample and 0.177 AU for unpolluted DOM sample, respectively. The UV absorbance results of the microvolume effluents obtained by the new MSM-RF are shown in Fig. 2.

As can be seen in Fig. 2, the UV absorbance of the effluents of polluted HoA increased significantly at the

Table 1 Basic water qualities of sampled source waters

Source waters	pH	Conductivity (µs cm^{-1})	Dissolved oxygen (mg L^{-1})	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	NH_3-N (mg L^{-1})	PO_4-P (mg L^{-1})	DOC (mg L^{-1})
Polluted	6.86	133	5.65	0.103	1.651	1.177	0.048	2.91
Unpolluted	7.75	36	10.32	0.005	0.113	0.120	0.005	1.13

sixth microvolume effluent vial (each vial volume is 5 mL) and peaked at the seventh effluent vial before dropping back at the ninth vial. It is notable that there was only one peak appeared in the UV absorbance line for each HoA samples analyzed, which suggests that the isolated HoA may be a pure chemical fraction with uniform characteristic. This is because in the conventional column eluting chromatography process, it is well known that the presence of single peak normally represents the homogeneity and uniformity in the chemical fraction properties. Owing to the lone information available from the measured UV absorbance peak, however, it is impossible to further differentiate the chemical properties between the polluted and unpolluted UV peaks. Thus, the 3D fluorescence spectroscopy method was combined to unveil the potential disparity in chemical characteristics between the polluted and unpolluted HoA as shown in Fig. 3.

Comparison between 3D fluorescence of bulk HoA by RF and microvolume HoA subfractions by MSM-RF

The bulk HoA isolated by RF method was first analyzed by EEMS and the 3D fluorescence spectrum of both the bulk polluted and unpolluted HoA are shown in Fig. 3. The most commonly accepted definition of 3D fluorescence peak positions are also given in Fig. 3a (Chen et al. 2003; Coble 1996; Hudson et al. 2007). Due to the difficulties associated with definitively identifying individual fluorescent compounds in waters these groups of fluorophores are commonly named humic-, fulvic-, and protein-like (specifically tryptophan- or tyrosine-like), so called because their fluorescence occurs in the same area of optical space as standards of these materials (Hudson et al. 2007). From the combined MSM-RF analysis with EEMS (Figs. 3 and 4), it was clearly shown that each of the polluted and unpolluted bulk HoA samples is not a pure chemical matter but consists of a mixture of at

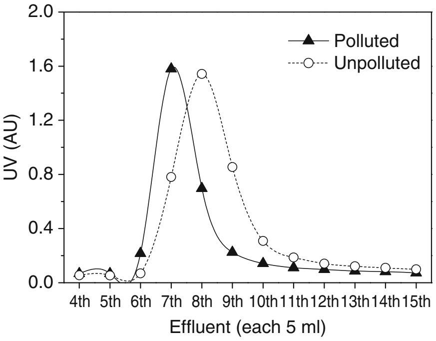

Fig. 2 Variation of UV_{254} absorbance of microvolume effluents (subfractions) of hydrophobic acid (HoA) eluted from DAX-8

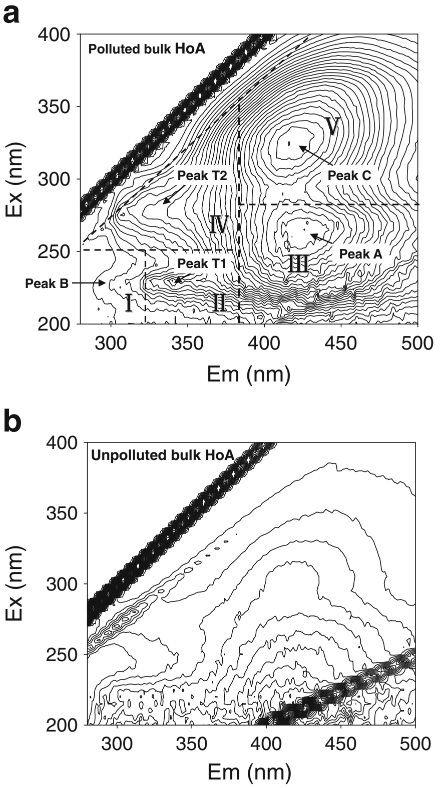

Fig. 3 EEMS (3D fluorescence spectrum) of traditional bulk hydrophobic acid (HoA) fraction and identification of 3D fluorescence peaks (contour interval is 100 afu: **a** polluted, **b** unpolluted)

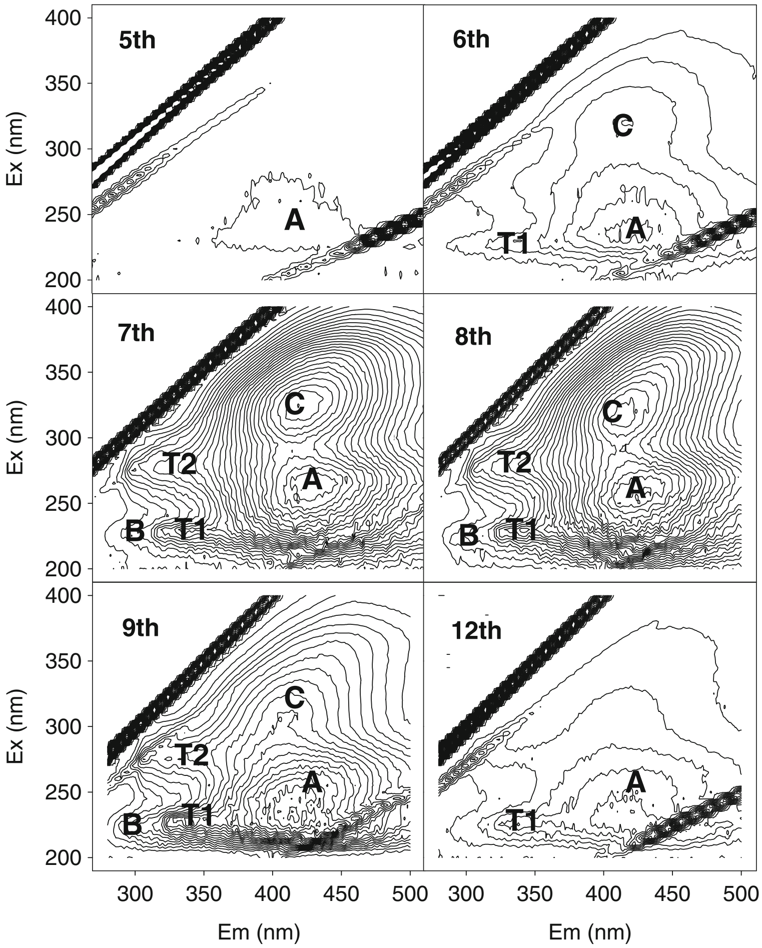

Fig. 4 EEMS (3D fluorescence spectrum) of micro-volume effluents (sub-fractions) of polluted hydrophobic acid (HoA) eluted from DAX-8 (contour interval is 100 afu)

least four fluorescent compounds—peak A (fulvic-like, Ex/Em 260/425 nm), peak C (humic-like, Ex/Em 325/420 nm), peak T (tryptophan-like, Ex/Em 230, 275/335 nm), and peak B (tyrosine-like, Ex/Em 230/300 nm).

To further utilize this combined spectroscopic information to indicate the occurrence of urban pollution in environmental source waters or assessment of the variation of source water quality, the spectroscopic data obtained from the 3D-fluorescence was scanned for representative signal. It was found that there exists a weak peak in the position of Ex/Em 275/335 nm (peak T2) for the polluted bulk HoA sample (Fig. 3a), but not in the unpolluted sample (Fig. 3b). From the rapid scanning and interpretation of all the environmental water samples in this study, it can be affirmed that the peak T2 material (tryptophan-like) at Ex/Em 275/335 nm would be a potential surrogate to indicate the presence of urban pollution in different environmental source waters.

Figure 4 (as an example) shows the DOM characterization from polluted water samples using the new refined MSM-RF-EEMS method. Results in Fig. 4 show that the fifth effluent only consisted of a dominant peak A (fulvic-like) material and then the peak C (humic-like) and peak T1 (tryptophan-like) initially appeared in the sixth effluent. Furthermore, the fluorescence intensities of both peak A and peak C in the seventh and eighth effluents rapidly became very high. Additionally, there were also peak T (T1 and T2) and peak B (tyrosine-like) presenting in the seventh, eighth, and ninth effluents. For the ninth effluent, the intensity of peak C dramatically decreased; while the intensity of peak T1 increased to the maximum value, which indicates the peak C material could be easily further separated from peak T material using the ninth effluent. It may be difficult to separate peak A from peak C materials because the fluorescence intensities for both peaks almost synchronously reached the maximum value at the seventh and eighth effluents, as shown in Fig. 5. However, it may be relatively easy to isolate peak A from peak C at the ninth effluent because of the higher intensity of peak A and lower intensity of peak C in this effluent. It was observed that the peak A always existed in all of the effluents, starting from the fifth to the 12th effluents, which indicates that the peak A (fulvic-like organic) is the most significant component in the aquatic HoA fraction.

Variation of fluorescence peak height in the micro-effluents of polluted and unpolluted HoA

The fluorescence peak heights for the five peak types from the polluted and unpolluted HoA effluents were plotted in Fig. 5. From Fig. 5, it can be shown that the peak A and peak C materials are the dominant components in each HoA samples, and they attained the highest concentration at around the eighth effluent. This means that the humic- and fulvic-like components could be easily further isolated together from the HoA fraction at the eighth effluent.

Normally, the fluorescence intensity difference between two peaks exhibits that it is easy to separate the two peak materials from each other. In this instance, it can be deduced that the larger the intensity difference between two peaks, the easier is the separation can be achieved. The absolute differential values of peak intensity among various materials (Δintensity) are also shown in Fig. 5. It was clearly shown that the peak A and C materials would be easily separated from peak T material in the polluted HoA sample since the differential intensities are very high (Δ1,681 or Δ1,491 afu). Similarly, the peak T1 material could also be further separated from peak B in the eighth effluent of the polluted HoA because of the large intensity difference of Δ822 afu.

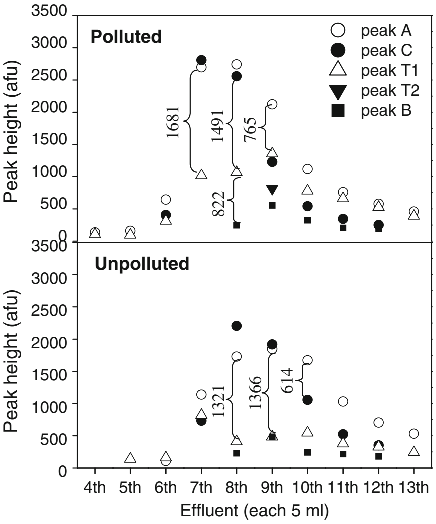

Fig. 5 Variation of 3D fluorescence peak height in microvolume effluents (subfractions) of hydrophobic acid (HoA) from DAX-8 (the peak height unit is the instrument original fluorescence intensity unit)

Overall, it can be found that there was a significant difference on the peak T height between polluted and unpolluted effluents. The peak T1 heights of most polluted HoA effluents were obviously higher than the unpolluted ones, and reached the maximum peak height at 1,500 afu; whereas, the maximum peak T1 height of unpolluted HoA effluents was only about 900 afu. This illustrated that the variation in the maximum peak T1 heights of the HoA fractions between the polluted and unpolluted effluents could probably be used to indicate the presence of urban pollution in environmental source waters. Moreover, a correlation was found between the presence of the peak T2 material with polluted water sample but not in the unpolluted HoA, which indicates that the presence of peak T2 could be used as a supplementary proof to further confirm the presence of urban pollution in environmental source waters. A consistent result has also been obtained in a recent study (Hambly et al. 2010) although the study focused on another kind of water—recycled water. The study investigated the suitability of using EEM in monitoring of recycled water affecting drinking water in a cross-connection system. The result showed that monitoring the fluorescence at peak T1 (Ex/Em 300/350 nm) was found to be the most appropriate for distinguishing recycled water from drinking water, where the intensity was found to be approximately 10 times that of drinking water and much more sensitive than common water quality parameters such as conductivity and DOC. It is anticipated that all of these works will lead to the development of an in-situ monitoring tool.

Conclusions

RF method has been widely used in the characterization of DOM in environmental source waters for several decades and need to be improved for obtaining more information in the DOM fractions. In this study, a refined RF method—MSM-RF-EEMS—was proposed and developed for further isolation and subfractionation of the primary DOM fractions to monitor the occurrence of urban pollution in different environmental source waters. As shown using the new refined MSM-RF-EEMS method, the HoA normally considered as one pure traditional chemical fraction in most of the previous researches was found to consist several sub-fractions of humic-like (peak C), fulvic-like (peak A), tryptophan-like (peak T), and tyrosine-like (peak B) organic matter. Some of these subfractions should be notable for environmental water quality monitoring research. The variation of peak T1 material in the HoA fraction was verified to be a good surrogate to indicate the occurrence of urban pollution in environmental source waters. The appearance of peak T2 could be used as a supplementary proof to further confirm the presence of urban pollution in environmental source waters. It is anticipated that the outcomes from this study will form an important basis for the future of online monitoring and sensory devices development.

The advantages of this MSM-RF-EEMS method include:(1) the isolation process was carried out on the DAX resin column (different to most of the other HPLC columns), which have higher and more flexible column capacity; (2) the isolation process could be halted at any time and the isolated solution with the required volume could be taken out for analysis with other instruments (e.g., ultraviolet absorption and EEMS), which overcomes the weakness of low resolvability and nonrecoverable sample experienced in many online measurement instruments.

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