



## Toward understanding the role of individual fluorescent components in DOM-metal binding

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### ABSTRACT

Knowledge on the function of individual fractions in dissolved organic matter (DOM) is essential for understanding the impact of DOM on metal speciation and migration. Herein, fluorescence excitation–emission matrix quenching and parallel factor (PARAFAC) analysis were adopted for bulk DOM and chemically isolated fractions from landfill leachate, i.e., humic acids (HA), fulvic acids and hydrophilic (Hyl) fraction, to elucidate the role of individual fluorescent components in metal binding (Cu(II) and Cd(II)). Three components were identified by PARAFAC model, including one humic substance (HS)-like, one protein-like and one component highly correlated with the Hyl fraction. Among them, the HS-like and protein-like components were responsible for Cu(II) binding, while the protein-like component was the only fraction involved in Cd(II) complexation. It was further identified that the slight quenching effect of HA fraction by Cd(II) was induced by the presence of proteinaceous materials in HA. Fluorescent substances in the Hyl fraction of landfill leachate did not play as important a role as HS did. Therefore, it was suggested that the potential risk of aged leachate (more humified) as a carrier of heavy metal should not be overlooked.

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### 1. Introduction

Occurrence and behavior of metal in aquatic environment is greatly affected by the presence of dissolved organic matter (DOM) [1]. As a ubiquitous and heterogeneous substance, DOM consists of a variety of organic compounds exhibiting diverse binding behavior toward metal ions [2,3]. Unfortunately, the function of individual DOM constituents remains poorly defined owing to the intrinsic complexity of DOM. Therefore, the role of individual DOM components needs to be further elucidated for a better understanding of their impact on metal speciation and migration.

Humic substance (HS) is a well-known metal sequester due to its abundance of functional groups, such as carboxylic and phenolic groups. It can be further fractionated into humic acids (HA) and fulvic acids (FA) based on their acid/base solubility. Compared to HS, far less attention has been paid to the binding behavior of hydrophilic (Hyl) fraction, even though this fraction often constitutes a large part of DOM [4,5]. An early study found that the Hyl fraction dominated Cu and Cr binding in soil-borne DOM, while Cd exhibited a higher binding affinity to hydrophobic acid [6]. The predominant role of Hyl fraction in Cu binding was supported by Olsson et al. [7], who identified that more than 95% of Cu bound

to the Hyl fraction in the leachate of bottom ash from municipal solid waste (MSW) incinerator. On the other hand, Ma et al. [4] demonstrated that the binding affinity of Cu(II) with DOM fractions isolated from natural water and municipal wastewater effluent followed the order:  $HA \approx FA > Hyl$ . Apart from that, no distinctive difference in the Cu binding characteristics was observed between hydrophobic and hydrophilic acids isolated from soil and surface water [5,8]. These conflicting results could be attributed to the difference on molecular structure of DOMs from various sources as well as various methods adopted.

Fluorescence excitation–emission matrix (EEM) spectroscopy is a simple, sensitive, rapid and nondestructive technique that could provide invaluable information on the molecular structure of DOM [9]. In particular, EEM quenching has been revealed as a promising tool for investigating the binding behavior between fluorescent substances and metal ions [10]. However, different types of overlapping fluorophores in EEM spectra of DOM often hamper their interpretation. Fortunately, the introduction of parallel factor (PARAFAC) analysis for EEM spectra provided a good solution to this problem. PARAFAC can decompose integrated fluorescence EEMs into several independent groups of fluorescent components and effectively reduce the interference by overlapping fluorophores among various compounds [11,12]. Recent studies have demonstrated that the combination of EEM quenching and PARAFAC analysis can be a reliable tool for determination of binding parameters between metal ions and individual components within DOM [13–15]. However, great challenges are often encountered when

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interpreting the physicochemical meaning of the components that have not yet been thoroughly categorized [14,16]. Obviously, some powerful tools widely used to extract information from separated components based on physicochemical process, such as Fourier transform infrared and nuclear magnetic resonance spectroscopy, are not appropriate for further characterization of mathematics-based PARAFAC-derived components. In our previous study, it was proved that ultrafiltration fractionation prior to PARAFAC analysis could provide additional information regarding the molecular weight distribution of the PARAFAC-derived components [17]. Herein, isolation of HA, FA and Hyl was adopted prior to PARAFAC analysis in order to draw insight into the role of individual fluorescent components, especially for Hyl fraction that has not been well understood.

Until now, landfill remains the most common alternative for waste disposal, while release of contaminants (organic and inorganic) via leachate pathway is of great concern. It has been recognized for decades that DOM played a pivotal role in metal migration from landfill to surrounding aquatic environments. However, few studies have been focused on the function of individual DOM components in metal binding. Compared to DOM from other sources, those present in landfill leachate have distinct characteristics, such as high organic content [18,19]. The objective of this study was to understand the role of individual leachate components in metal binding using PARAFAC analysis on EEMs of bulk DOM and isolated fractions. For this purpose, two types of leachates generated from young and aged MSW landfills were collected, and three fractions, HA, FA and Hyl, were then isolated by XAD-8 resin combined with cation exchange resin method. Cu(II) and Cd(II), which are common in environment and widely studied, were selected as fluorescent quenching agents for titration. The binding behavior of three isolated fractions and PARAFAC-derived components toward two heavy metals were investigated.

## 2. Materials and methods

### 2.1. Sample collection and preparation

The leachate samples were taken from two MSW landfill sites in Shanghai, China. Young leachate was obtained from landfill cells with ages of 3–5 years, while aged leachate was collected from cells with ages ranging from 10 to 15 years. Each sample was collected in a pre-cleaned brown sampling bottle, and then filtered through 0.45- $\mu\text{m}$  membrane filters. The pH values of the filtered young and aged leachate were 8.02 and 7.87, and their concentrations of total organic carbon (TOC), total carbon and inorganic carbon were 4450, 6800, 2350 mg/L, and 370, 1420, 1050 mg/L, respectively. The dissolved metal concentrations of the young and aged leachate samples were 412 and 149 mg/L for Ca, 163 and 87.7 mg/L for Mg, 1.37 and 0.449 mg/L for Al, 1.44 and 0.721 mg/L for Fe, 22.7 and 14.8  $\mu\text{g/L}$  for Cu, 1.85 and 0.844  $\mu\text{g/L}$  for Cd, respectively. The filtrates were adjusted to pH 7.0 and stored at 4 °C before further analysis.

### 2.2. Isolation procedure

The isolation method using XAD-8 resin combined with cation exchange resin is generally regarded as the state-of-art method for speciation of HA, FA and Hyl fraction [20,21]. The procedure adopted here was based on the method described by Christensen et al. [22] and He et al. [18]. Prior to isolation, the XAD-8 resin (Rohm and Haas, USA) and 732 H<sup>+</sup> cation exchange resin (Shanghai Huizhi Co., China) were pretreated. Briefly, the XAD-8 resin was soaked in 0.1 mol/L NaOH for 3 d and then flushed sequentially with methanol, diethyl ether, acetone and methanol for 8 h. Next,

the resin was air-dried, after which it was washed with Milli-Q water until the effluent TOC approached zero. The 732 resin was sequentially soaked with Milli-Q water, 2–4% NaOH and 4–5% HCl to remove the impurities. Finally, the resin was washed with Milli-Q water until the effluent pH was close to 7.0. Details of the isolation procedure are illustrated in the schematic Fig. S1 in Supporting Information. As a result, each leachate sample was separated into three fractions, HA, FA and Hyl. The TOC ratios of the HA, FA and Hyl fractions in the young and aged leachate samples were 25.5%, 35.7%, 38.8%, and 50.3%, 43.9%, 5.8%, respectively. These distribution characteristics are comparable to those of many previous studies [22,23].

### 2.3. Fluorescence titration

Prior to fluorescence titration, the bulk and isolated samples were stepwise diluted to TOC < 10 mg/L using Milli-Q water as suggested to minimize the inner filtering effects [24–26]. Aliquots of 25 mL diluted solution were titrated with either Cu(NO<sub>3</sub>)<sub>2</sub> or Cd(NO<sub>3</sub>)<sub>2</sub> using an automatic syringe in 40-mL brown sealed vials. The titration range was 0–100  $\mu\text{mol/L}$ . In an attempt to maintain a constant pH condition before and after titration, the pH values of the metal titrants were adjusted to 4.0 for Cu(NO<sub>3</sub>)<sub>2</sub> and 6.0 for Cd(NO<sub>3</sub>)<sub>2</sub> using NaOH and HNO<sub>3</sub>. In addition, no more than 0.025 mL of the metal titrant was added during the titration process. To ensure complexation equilibrium, all titrated solutions were shaken in an incubator for 24 h at 25  $\pm$  0.1 °C.

Fluorescence EEM spectra were measured on a Cary Eclipse fluorescence spectrophotometer (Varian Inc., USA) in a quartz cuvette. EEM spectra were obtained by subsequent scanning at emission wavelengths (Em) ranging from 250 to 500 nm at 2-nm increments by varying the excitation wavelengths (Ex) from 200 to 450 nm at 10-nm increments. The instrumental parameters were as follows: photomultiplier tube voltage, 700 V; excitation and emission slits, 10 nm; scan speed, 1200 nm/min.

### 2.4. PARAFAC analysis

The approach of PARAFAC analysis of EEMs has been well documented elsewhere [12,27]; therefore, only a brief description is provided here. PARAFAC is a decomposition method for reducing a dataset of EEMs into a set of trilinear terms and a residual array based on an alternating least squares procedure. In other words, if the EEM dataset is arranged in a three-way array  $X$  of dimensions  $I \times J \times K$ , where  $I$  is the number of samples,  $J$  is the number of Em, and  $K$  is the number of Ex, PARAFAC decomposes them into three matrices labeled  $A$  (the score matrix),  $B$  and  $C$  (the loading matrices) with elements  $a_{if}$ ,  $b_{jf}$ , and  $c_{kf}$ , respectively, as well as the residual element  $e_{ijk}$ , which represents the unexplained variation in the model. The PARAFAC model can be written as:

$$x_{ijk} = \sum_{f=1}^F a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

$$i = 1, \dots, I; \quad j = 1, \dots, J; \quad k = 1, \dots, K; \quad f = 1, \dots, F$$

where  $x_{ijk}$  is the fluorescence intensity of the  $i$ th sample at the  $j$ th Em and  $k$ th Ex wavelength and  $F$  is the number of fluorophores (components).

In this study, 176 EEMs were analyzed by PARAFAC model using MATLAB 7.0 (Mathworks, Natick, MA) with the DOMFluor toolbox ([www.models.life.ku.dk](http://www.models.life.ku.dk)). Prior to the analysis, a few preprocessing steps were adopted as follows. (1) The EEM of a control Milli-Q water was subtracted from each EEM of the studied samples. (2) The Rayleigh and Raman scatters were removed according to the protocol described by Bahram et al. [28]. (3) The EEMs were normalized

by dividing the spectra by the corresponding TOC concentration to reduce the impact of varying DOM concentrations among samples. (4) The EEMs were arranged into a three-dimensional data array (176 samples  $\times$  26 Ex  $\times$  126 Em). During PARAFAC analysis, a non-negative constraint was applied to the parameters and two to eight components were computed for the EEMs. Determination of the number of components was primarily based on split half analysis and examination of residual loadings.

### 2.5. Complexation modeling

The classic single-site fluorescence quenching model proposed by Ryan and Weber [29] was adopted herein for determination of the complexation parameters between PARAFAC-derived components and heavy metals. In addition, the binding parameters were obtained by using nonlinear fitting of Eq. (2).

$$I = I_0 + (I_{ML} - I_0) \left( \frac{1}{2K_M C_L} \right) \left( (1 + K_M C_L + K_M C_M) - \sqrt{(1 + K_M C_L + K_M C_M)^2 - 4K_M^2 C_L C_M} \right) \quad (2)$$

where  $I_0$  and  $I$  are the fluorescence intensities in the absence of added metal and at the metal concentration of  $C_M$ , respectively, and  $I_{ML}$  is the limiting value below which the fluorescence intensity does not decrease when additional metal is added. The conditional stability constant ( $K_M$ ), ligand concentration ( $C_L$ ) and  $I_{ML}$  were estimated by nonlinear fitting based on the Quasi-Newton algorithm. The  $C_L$  values were not reported in this study owing to their extremely small values estimated by this nonlinear model.

## 3. Results and discussion

### 3.1. EEM contours of the bulk DOMs and isolated fractions

The EEM spectra of the isolated fractions and bulk DOMs measured with (total concentration = 50  $\mu\text{mol/L}$ ) or without the addition of either Cu(II) or Cd(II) are illustrated in Fig. 1. As expected, multiple EEM peaks overlapped considerably in the bulk DOMs, especially in the young leachate. Although the isolation procedure was able to fractionate and purify specific fractions of DOM, overlapping fluorophores still existed in their

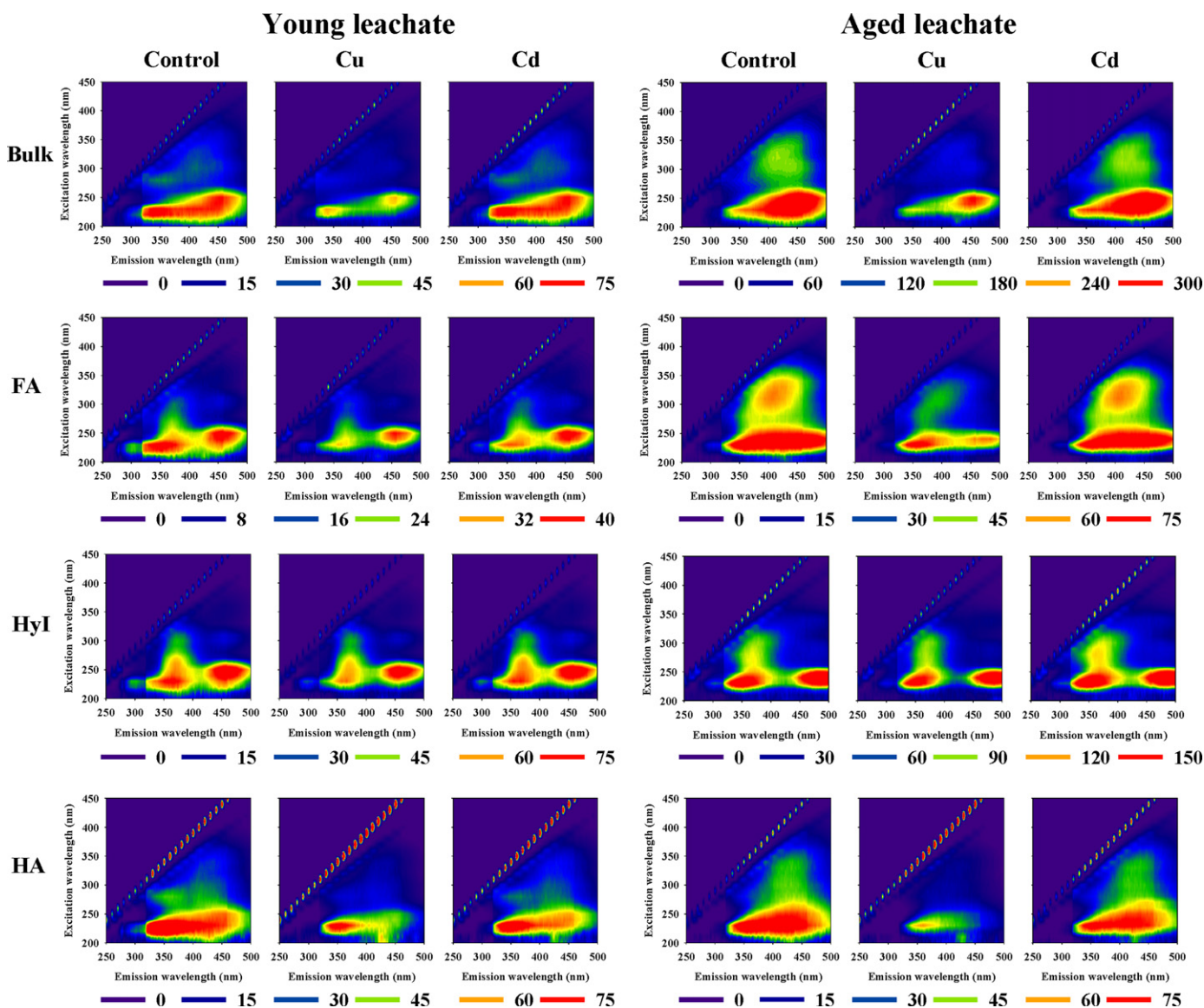


Fig. 1. Fluorescence excitation-emission matrix spectra of the isolated fractions and bulk DOMs in the absence or presence of Cu(II) and Cd(II) at a total concentration of 50  $\mu\text{mol/L}$ . Control: no added metals.



EEM spectra. Comparison of the EEM contours between various fractions and two bulk samples revealed several implications regarding the evolution of fluorescence substances in leachate during the landfill process. Overall, the fluorescence intensities per unit of TOC (FI/TOC) in the aged leachate samples (including bulk DOM and isolated fractions) were much higher than that in the young leachate, except for the HA fraction in which similar intensities were found. These results suggested that fluorescence substances would be highly concentrated during landfill process. It is worth noting the similarities and differences observed in the same fraction of two different samples or in the various fractions of the same sample. Specifically, the FA and Hyl fractions shared similar fluorophores in the young leachate, being characterized by a pair of distinctive and intense fluorescence peaks (Ex/Em: 230/356 and 250/458 nm for FA; 230/362 and 250/456 nm for Hyl), and fluorescence substances were more concentrated in the Hyl fraction. Furthermore, the Hyl fraction in the aged leachate sample also exhibited similar fluorophores (Ex/Em: 230/348 and 240/488 nm), although their emission spectra had shifted slightly. This red shift in emission wavelength from 456 nm to 488 nm illustrated the increasing complexity in structure of Hyl fraction in the aged leachate compared to the young leachate [30]. Similarly, no marked difference in the EEM spectra of HA was observed between the two leachate samples. In contrast, the EEM landscape of the FA fraction isolated from the aged leachate was quite different from that of Hyl in the aged leachate as well as that of FA in the young leachate. This observation suggested that marked changes in the composition and structure of the FA fraction occurred after long-term storage in landfill. Accordingly, the effect of these changes on heavy metal binding should be further investigated.

The addition of heavy metals induced relevant changes in the fluorescence EEM spectra that were dependent on the metal species and DOM characteristics. In particular, marked quenching effects were observed in bulk DOM, FA and HA fractions in the two leachate samples with Cu(II), while negligible effects were found in the Hyl fraction. This phenomenon was in good agreement with the results of a previous study on the binding affinity of Cu(II) by isolated fractions of HA, FA and Hyl from natural waters and municipal wastewater effluent [4]. Conversely, Cd(II) titration produced a

slight decrease in fluorescence intensity in the HA fraction, while negligible effects were observed in the bulk DOM, FA and Hyl fraction. Previous studies have shown that Cd(II) formed complexes with hydrophobic acids more preferentially when compared to hydrophilic acids [6]. This study further demonstrates the key role of the HA fraction when compared to the FA fraction. Further quantitative analysis was impeded by overlapping fluorophores.

### 3.2. PARAFAC analysis of EEM spectra

The EEM spectra of bulk DOM and fractions isolated from the two leachate samples titrated with Cu(II) and Cd(II) at ten various concentrations were analyzed by PARAFAC. The results showed that all 176 EEMs could be successfully decomposed into a three-component model by PARAFAC analysis despite differences in the fluorescence characteristics among samples, including bulk DOM and isolated fractions, and different quenching effects in response to different metals at various concentrations.

Each component identified by the PARAFAC model displayed EEM characteristics of a pair of peaks (Fig. 2). Specifically, components 1 and 2 possessed two maxima in the excitation spectra and a single peak in the emission spectra, while the fluorophores of component 3 displayed the same excitation wavelength at two different emission wavelengths. Comparison of the EEM spectra of each component with those reported in previous studies (Table 1) revealed that component 1 was assigned as HS-like component and component 2 as protein-like one. Component 3 has not been traditionally defined. Like other PARAFAC-derived components that have not been well categorized in previous studies, it is a tough issue for their further interpretation, because the extracting procedure of PARAFAC is mainly based on mathematical calculation. It is impossible to use physicochemical tools for characterizing mathematics-derived components. Therefore, an isolation procedure was employed in this study prior to PARAFAC analysis for better understanding the physicochemical characteristics of such components.

It has been shown that PARAFAC analysis could provide additional quantitative information regarding the distribution of each component in studied samples [14,15]. Fig. 3 shows the

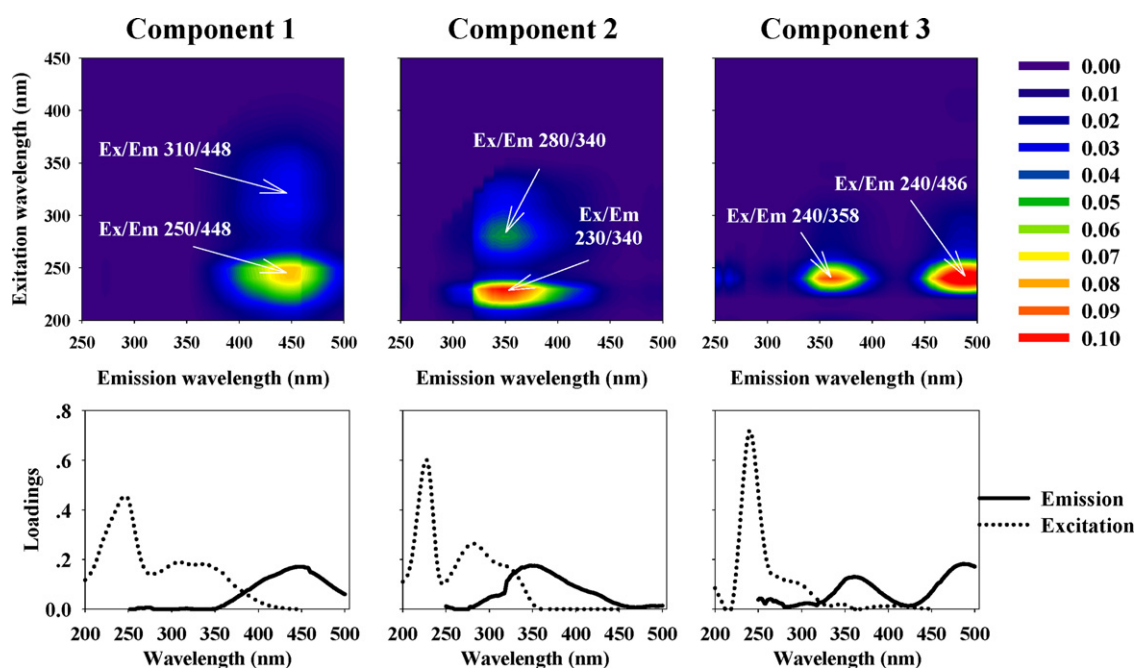


Fig. 2. Fluorescence excitation–emission matrix contours and loadings (excitation and emission) of the three components identified by the DOMFluor-PARAFAC model.

**Table 1**

Comparison of the EEM peaks of each component investigated in the present study with those of similar characteristics reported in previous works.

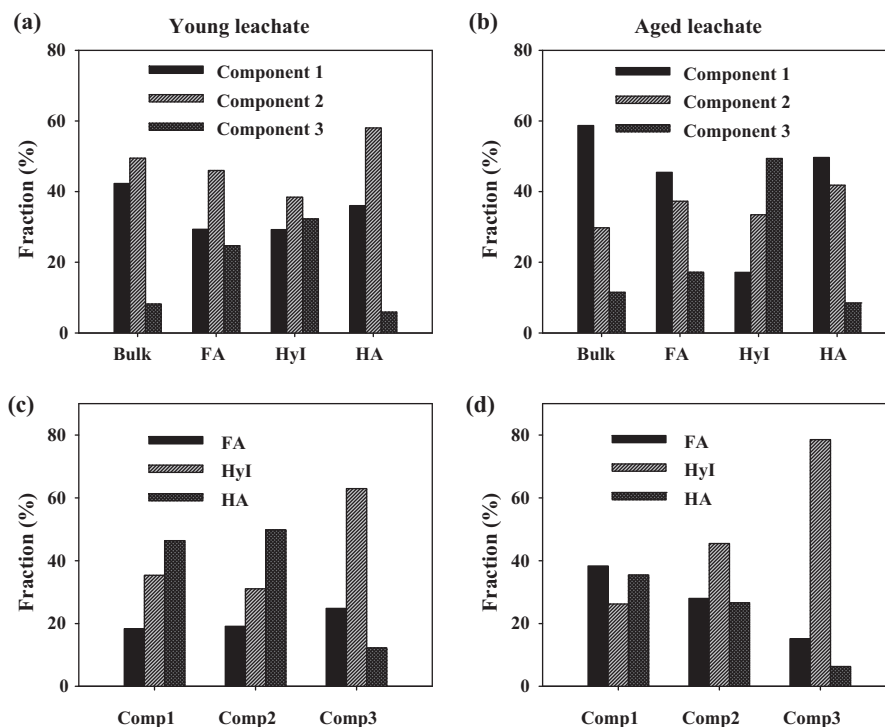
Present study		Tradition peaks [31]		Comparison with other studies		
Component	Ex/Em	Ex/Em	Fluorophore type	Ex/Em	Reference	Sources
1	250,310/448	237–260/400–500	Peak A humic-like	250,315/445	[23]	Landfill leachate (isolated FA)
		300–370/400–500	Peak C humic-like	230–250/400–440 320–360/400–470 237–249/417–429	[24] [24] [32]	Landfill leachate (bulk sample) Landfill leachate (bulk sample) Surface water (isolated hydrophobic acid)
2	230,280/340	225–237/340–381	Peak T <sub>2</sub> protein-like	225–237/340–381, 275/340	[33]	Sewage-derived DOM (bulk sample)
				220,280/340	[34]	Compost-derived WEOM (bulk sample)
		275/340	Peak T <sub>1</sub> protein-like	220–230/340–370 220,280/340	[24] [16]	Landfill leachate (bulk sample) Landfill leachate (PARAFAC-derived component)
3	240/358,486	225–237/340–381	Peak T <sub>2</sub> protein-like	225–237/345–357	[32]	Surface water (isolated hydrophilic acid)
		237–260/400–500	Peak A humic-like	225–237/357–369 250/458	[32] [15]	Surface water (isolated hydrophilic base) MSW-derived DOM (primary peak in PARAFAC-derived component)

quantitative distribution of PARAFAC-derived components and isolated fractions in the two leachates. As expected, HS-like component 1 was primarily concentrated in the aged leachate, including bulk DOM and isolated fractions excluding the HyI fraction, while protein-like component 2 was more enriched in the young leachate. In addition, the percentage of component 3 in the HyI fraction was significantly higher than that in the bulk DOM or other fractions. Interestingly, great similarity was observed in the EEM contours produced by HyI fractions isolated from the two leachate samples with that of component 3. Moreover, the dominant proportion of the HyI fraction in component 3 (Fig. 3c and d) further demonstrated their high correlation. It is interesting to note that protein-like component 2 accounted for a major part of the HA fractions (58.0% for the young leachate and 41.8% for the aged leachate).

Similar peaks with high intensity were also observed in the corresponding region of the EEM contour in the HA fraction (Fig. 1). These observations are consistent with the findings of HA fractions isolated from soil and sediment [35]. It has been reported that proteinaceous materials could be intrinsic part of humic compounds [36–38], which provides a good explanation to these observations.

### 3.3. Behavior of PARAFAC-derived components titrated with Cu(II) and Cd(II)

The fluorescence quenching curves of each component and isolated fraction with Cu(II) or Cd(II) are illustrated in Fig. 4. Specifically, a significant quenching effect was observed for Cu(II) by component 1, whereas negligible quenching effect was found for

**Fig. 3.** DOM distribution of the young and aged leachate based on the PARAFAC-derived components (a and b) and isolated fractions (c and d).

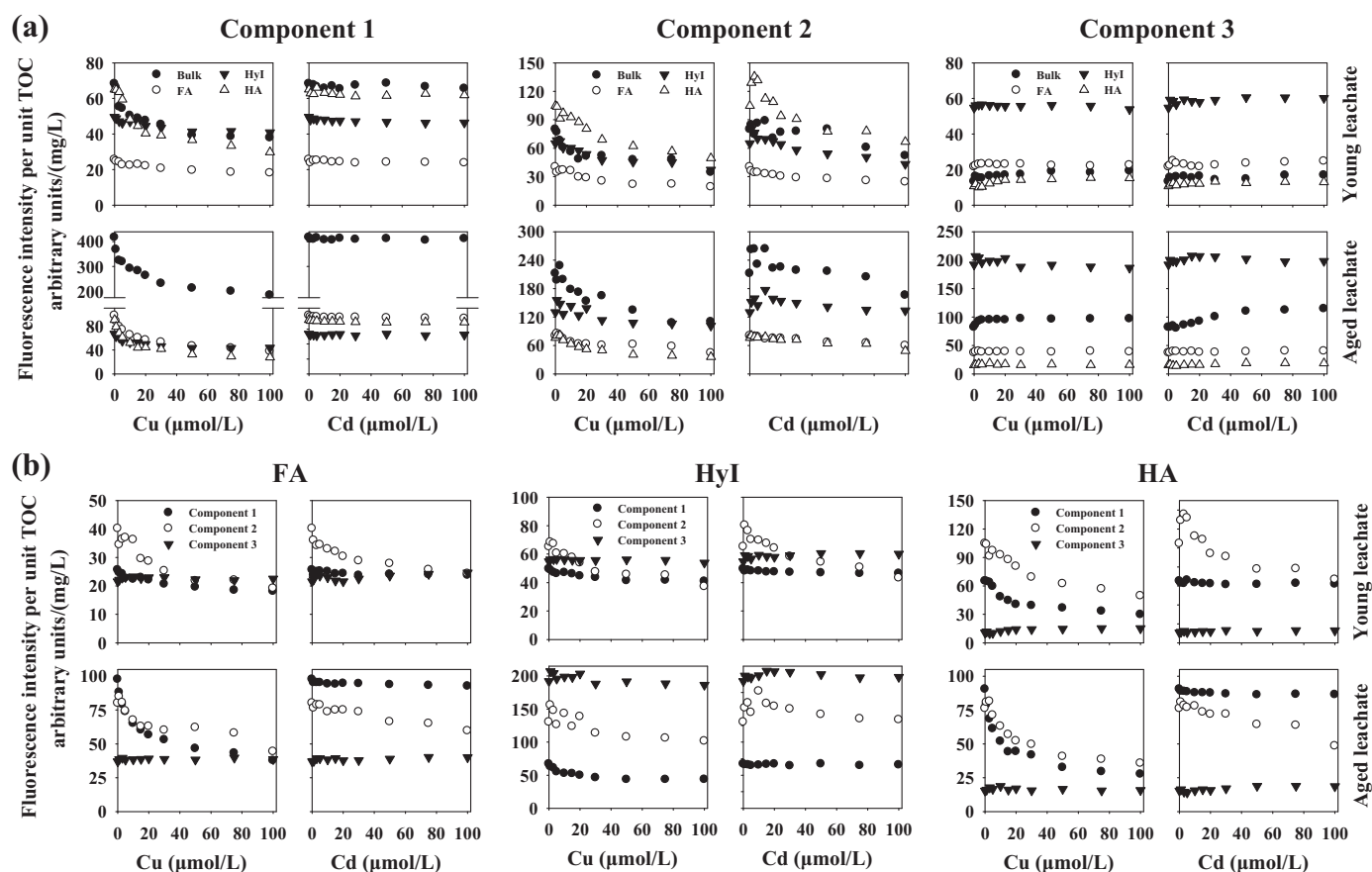


Fig. 4. Fluorescence quenching curves with Cu(II) or Cd(II) based on the PARAFAC-derived components (a) and isolated fractions (b).

Cd(II). The quenching characteristics of HS-like component 1 were more similar to those of HA than FA fractions derived from various sources [39–41]. These findings were in accordance with the dominant contribution of the HA fraction in component 1 extracted from the young leachate, while they were slightly different from that of the aged leachate (Fig. 3) (the ratios of HA and FA in component 1 were comparable). The results suggested that the FA fraction in aged leachate, which likely had a high degree of humification, shared similar binding characteristics with that of the HA fraction. On the other hand, the quenching effects of protein-like component 2 were significant in the presence of Cu(II), while a weak response to Cd(II) and larger fluctuations existed in its quenching curves. These results were consistent with the previous observations when the same method was employed [14,15], which suggests that the approach of EEM quenching-PARAFAC analysis was more appropriate for evaluation of the binding characteristics of the HS-like component than that of the protein-like component. Copper tends to be complexed with DOM to greater extents than Cd, as would be expected from their affinities for organic ligands [42,43]. And it was also reported that proteins have strong binding site for Cu and Cd, with higher affinities for Cu than for Cd [44]. Copper binding is mainly at the phenolic sites, while for Cd, the carboxylic sites are dominant [43]. By contrast, the fluorescence intensity of component 3 was not quenched in response to the addition of either Cu(II) or Cd(II). Considering the high correlation of component 3 with the HyI fraction, this observation revealed that the HyI fraction in landfill leachate may not play a large role in heavy metal (Cu(II) and Cd(II)) binding and migration.

As shown in Fig. 4(b), the specific fractions mainly responsible for Cu(II) binding in the three isolated fractions were components 1 and 2. Correspondingly, component 2 was the only fraction

that contributed to Cd(II) complexation, revealing that the slight quenching effect of HA by Cd(II) (Fig. 1) was induced by the proteinaceous materials in HA (component 2 in this study). These findings clearly demonstrated that combination of fractionation procedure with EEM quenching and PARAFAC analysis could provide a deeper insight into the binding characteristics of individual fractions within DOM.

The stability constants ( $\log K_M$ ) calculated using the Ryan and Weber Model for HS-like component 1 and Cu(II) are listed in Table 2. The  $\log K_M$  values ranged from 3.43 to 5.36, which were in the same ranges as those reported for commercial HS [45] and fractions like HA and FA extracted from various sources [40,46,47]. The binding affinities were largely dependent on structure as well as functional groups. It is interesting to note that the  $\log K_M$  values of component 1 from the three isolated fractions in the two leachates were different and followed the order: HA > HyI > FA, indicating that even the same component extracted from various fractions

Table 2

The  $\log K_M$  values for Cu(II) binding to humic-like component in landfill leachate as determined by the Ryan and Weber model.

Symbol	Cu & Comp 1		
		$\log K_M$	$R^2$
Young leachate	Bulk	4.79	0.97
	FA	3.43	0.97
	HyI	3.89	0.95
	HA	5.36	0.98
Aged leachate	Bulk	4.56	0.99
	FA	4.66	1.00
	HyI	4.87	0.98
	HA	4.91	0.99

by PARAFAC may also possess various physicochemical properties. The higher log  $K_M$  values of the HA fraction in young leachate as well as the FA and Hyl fraction in aged leachate demonstrated that the Cu(II) binding affinities of HS-like substances may be partially positively correlated with their degrees of humification.

In previous studies, heavy metals in young leachate have drawn more attention than those in aged leachate owing to their higher concentrations [48,49]. However, HS-like substances are much more concentrated in aged leachate than young leachate per unit of TOC. Recognizing the much higher binding affinities of HS compared to the Hyl fraction, aged leachate may have great potential to carry heavy metals. Therefore, its induced risk should not be overlooked.

#### 4. Conclusions

Both HS-like and protein-like compounds were identified to be responsible for Cu(II) binding, while protein-like substances was the only fraction involved in Cd(II) complexation. The slight quenching effect of HA in response to Cd(II) was attributed to the presence of proteinaceous materials within HA. Fluorescent substances in the Hyl fraction of landfill leachate did not exert much influence on metal speciation and migration as HS did. Therefore, the potential risk of aged leachate (highly humified) as a carrier of heavy metal should not be overlooked. Overall, the combined approach of chemical isolation, EEM quenching and PARAFAC analysis could provide a deeper insight into the fluorescence characteristics and binding behavior of individual fractions within DOM.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.02.075.

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