Supporting Information

Characterizing the Interactions between Trace Metals and Dissolved Organic Matter Using Excitation-Emission Matrix and Parallel Factor Analysis

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Supporting Information Included (7 Pages):

Sampling sites (Figure S1)

FCE-PARAFAC model (Figures S2 and S3, and Table S1)

Results of triplicate Cu(II) titration experiments and Log*K* and *f* values of humic-like fluorescent components (Figure S4 and Table S2)
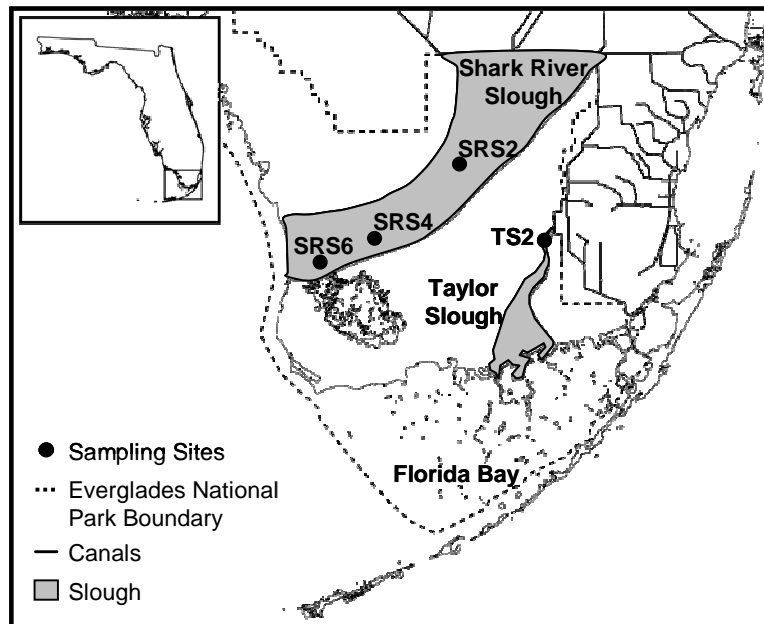
Sampling sites

Figure S1. Location of sampling sites

FCE-PARAFAC model

For the development of the FCE-PARAFAC model, a total of 1108 EEMs were obtained through monthly surface water sampling at 36 FCE sites including freshwater marshes dominated by peat and marl soils, mangrove forests dominated brackish sites, and the estuary of Florida Bay (Fig. S1). Samples collected and analyzed between October 2004 and December 2007 were modeled. The wavelength ranges used for the FCE-PARAFAC model were 260-455 nm and 290-500 nm for excitation and emission, respectively. The 1108 EEMs were randomly separated into two halves, and split half analysis was used for identical components (*14, 15*). As a result, an eight components model was found adequate to describe the data (Fig. S2). PARAFAC can decompose EEMs into different independent fluorescent groups. However, it is unlikely that each component represents a specific fluorophore because of complex nature of DOM. Thus, individual fluorescent components identified for the FCE-PARAFAC model most likely represent groups of fluorophores with very similar fluorescence characteristics and variability.
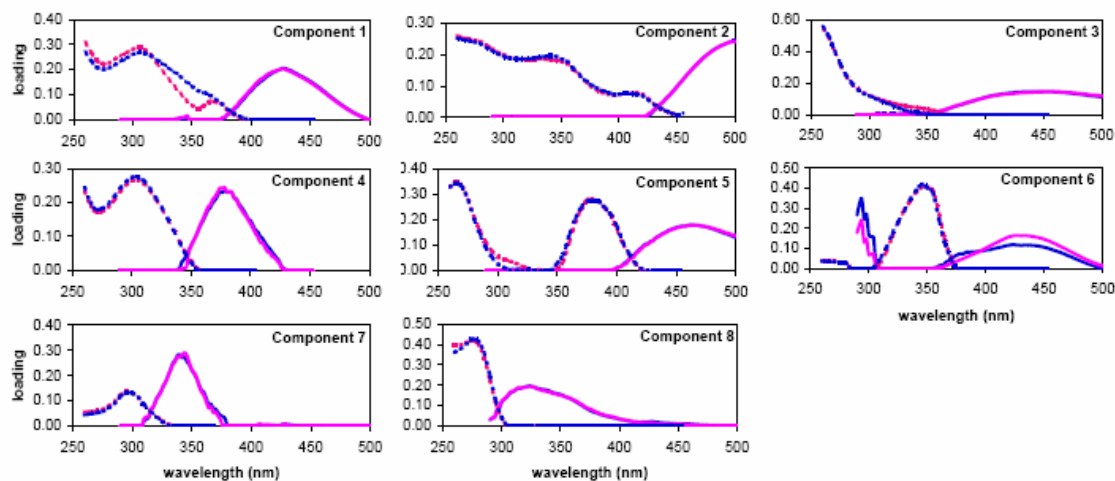

Figure S2. Validation of the eight components of FCE-PARAFAC model. The dotted and solid lines show the excitation and emission loadings, respectively. Blue and pink show the result of two independent models (split half analysis).

Component 1 was comprised of two peaks with an excitation maximum at 305 and < 260 nm with 428 nm excitation (Table S1 and Fig. 1). This peak can be categorized traditional terrestrial humic-like peak C or marine humic-like peak M (*24*), but also similar to terrestrial component 3 reported by Stedmon and Markager (*16*) and terrestrial unknown components defined by Cory and McKnight (*15*). Fluorescence characteristics of component 2 has not been traditionally defined (*24*), but was similar to a terrestrial reduced quinone-like component (SQ1, *15*), terrestrial component C3 reported by Yamashita et al. (*17*), and terrestrial/autochthonous component 2 (*16*). An intense excitation maximum for component 3 occurred below 260 nm excitation with 448 nm emission, and was similar to the traditional humic-like fluorophore in the UV region (peak A, *24*). This component was also similar to terrestrial fluorescent component identified through PARAFAC by other authors (Table S1). The spectral characteristics of component 6, characterized by a peak at 345 nm excitation and 424 nm emission

wavelengths, is similar to the traditional humic-like peak C (*24*). It also resembled terrestrial/anthropogenic component 5 (*16*), terrestrial unknown component C1 (*15*), and terrestrial component C2 (*17*). Therefore, components 1, 2, 3, and 6 determined by FCE-PARAFAC model (Table S1) could be categorized to terrestrial, i.e., higher plant derived, humic-like components.

There were two excitation maxima observed in the EEM of component 4 and 5 (Table S1 and Fig. 1). The emission maximum at 378 nm in component 4 could be categorized as the previously defined peak M (*24*). The spectral features of component 4 were also similar to a reported microbial derived component (C3 or Q3; *15* and C6; *17*), and similar to a dominant component reported in wastewater (component 6; *16*). The fluorescence characteristics of component 5 has not been traditionally defined (*24*), but were similar to terrestrial/autochthonous component 4 (*16*) as well as microbial reduced quinone-like component (SQ2, *15*). Thus, components 4 and 5 in FCE-PRAFAC model (Table S1) are likely to be microbial humic-like components.

In addition, two components were obtained in the excitation and emission wavelength range where protein-like fluorophore have been previously reported (*21, 24*). Component 7 and 8 are similar to tryptophan-like and tyrosine-like components found in previous PARAFAC studies, respectively (Table S1).

Interestingly, the 8 components identified by different PARAFAC models (Table S1) match quite well the composition of DOM from both the FCE and the Danish Bay and its catchments (*16*) even though the characteristics of the aquatic environments studied are very different. This suggests the ubiquitous occurrence of similar type of fluorophores for the wide range of aquatic systems. Further studies are necessary for clarifying the similarity and dissimilarity of fluorophores for wide range of ecosystems.

For the validation of forcing of FCE-PARAFAC model to EEMs obtained from fluorescence quenching titration experiments, residual analysis (*14, 16*) was employed. No peaks or systematic residues were found in the residue of EEMs with and without metals (Fig. S3), indicating that the FCE-PARAFAC model is properly replicating the spectral characteristics of the EEMs obtained from the fluorescence quenching titration experiments.
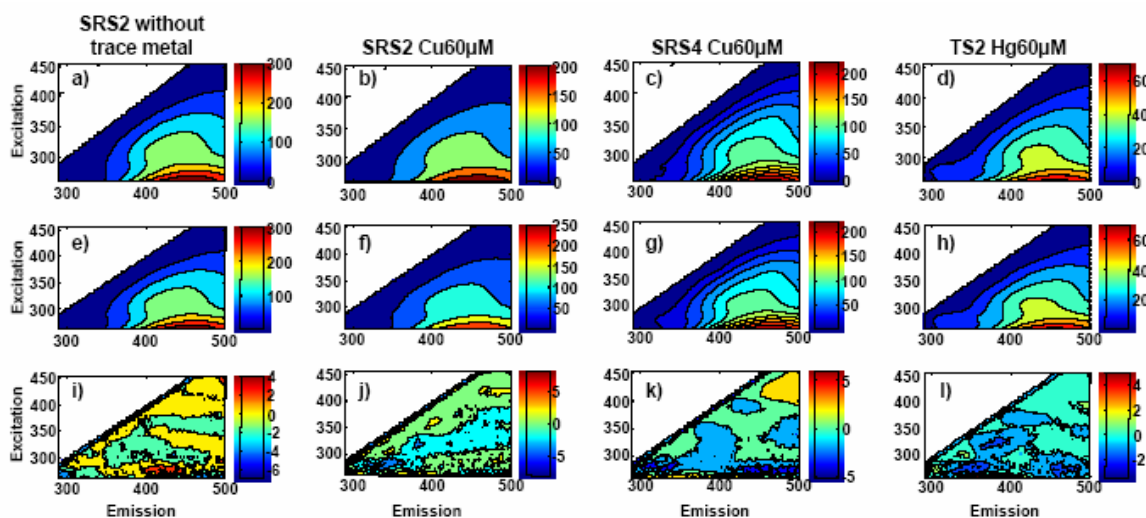

Figure S3. Examples of measured (a-d), modeled (e-h), and residual (i-l) EEMs for SRS2 in July without trace metal, SRS2 in July with 60μM Cu(II), SRS4 with 60μM Cu(II), and TS2 with 60μM Hg(II).

Table S1. Characteristics of the eight components identified by the FCE-PARAFAC model compared with those of previous studies

Component	Excitation maximum	Emission maximum	Coble (1996)	Stedmon and Markager (2005)[a]	Cory and McKnight (2005)	Yamashita et al. (2008)[a]
1	305 (<260)	428	C or M	3 (Ter)	C10	-
2	< 260 (340, 405)	> 500	-	2 (Ter/Aut)	SQ1	C3 (Ter)
3	<260	448	A	1 (Ter)	Q1 or Q2	C1 (Ter)
4	305 (<260)	378	M	6 (Ant)	Q3[b] or C3[b]	C6 (Aut)
5	265 (380)	462	-	4 (Ter/Aut)	SQ2[b]	-
6	345	424	C	5 (Ter/Ant)	C1	C2 (Ter)
7	295	340	T	7 (Trp-like)	Trp-like	C5 (Trp/N) or C4 (Trp)
8	275	324	B or T	8 (Tyr-like)	Tyr-like	C4 (Trp) or C7 (Tyr)

[a] Ter, Aut and Ant refers to origin of terrestrial, autochthonous and anthropogenic sources respectively.

[b] were identified in the Antarctic data set only (Cory and McKnight, 2005), indicating microbial origin.

Results of triplicate Cu(II) titration experiments and Log*K* and *f* values of humic-like fluorescent components
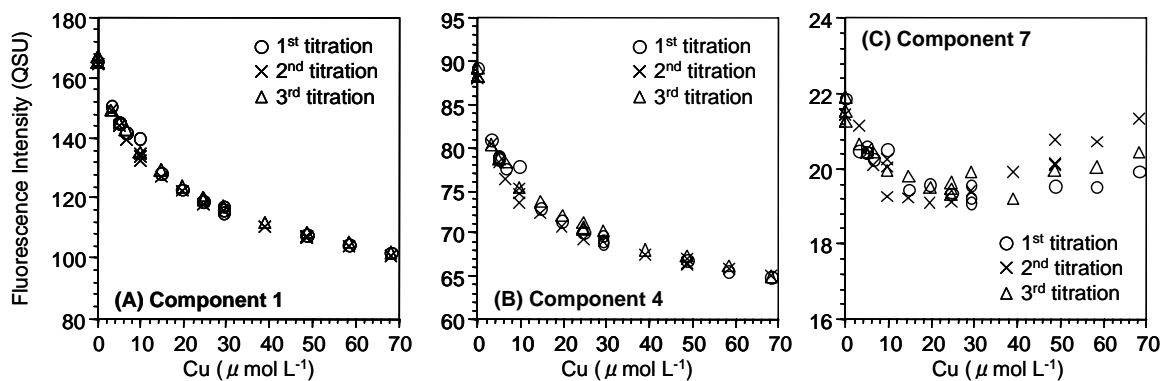

Figure S4.   Changes in fluorescence intensities of fluorescent components during triplicate titration experiments with Cu(II) at SRS2 in July: (A) Terrestrial humic-like component 1, (B) Microbial humic-like component 4, and (C) Protein-like component 7.

Table S2. The log$K$ and f values for terrestrial and microbial humic-like components with Cu(II) and Hg(II) determined by Ryan and Weber Model

Component	Site	Cu(II)			Hg(II)		
		log$K$	f (%)	R^2	log$K$	f (%)	R^2
Component 1	SRS2	4.74	52	1.00	4.93	30	1.00
	SRS4	4.49	47	1.00	5.24	18	0.99
	SRS6	4.72	41	1.00	4.17	16	0.97
	TSPH2	4.91	54	0.99	4.84	38	1.00
Component 2	SRS2	4.62	59	1.00	4.67	34	1.00
	SRS4	4.48	53	1.00	4.75	26	0.99
	SRS6	4.68	47	1.00	4.31	20	0.99
	TSPH2	4.81	61	0.99	5.01	35	1.00
Component 3	SRS2	4.68	37	1.00	4.26	33	0.99
	SRS4	5.75	14	0.98		not modeled	
	SRS6	6.32	13	0.89		not modeled	
	TSPH2	4.67	42	0.98	4.69	22	0.99
Component 4	SRS2	4.83	32	1.00	4.90	27	1.00
	SRS4	5.04	25	0.98	6.76	11	1.00
	SRS6	5.54	22	0.94	4.20	16	0.99
	TSPH2	5.08	38	0.99	4.90	38	1.00
Component 5	SRS2	4.96	32	0.98	4.76	40	1.00
	SRS4	4.71	38	0.99	5.11	27	1.00
	SRS6	4.91	35	0.99	4.09	32	0.99
	TSPH2	5.10	36	0.89	5.17	42	1.00
Component 6	SRS2	5.25	32	1.00	4.71	48	1.00
	SRS4	5.13	30	0.99	4.53	42	1.00
	SRS6	5.37	31	0.99	3.92	49	0.99
	TSPH2	5.45	30	0.99	4.97	49	1.00