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Fluorescence characterization of dissolved organic matter in an urban river and its complexation with Hg(II)

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Abstract

Dissolved organic matter (DOM) in the Nanming River, an urban river in Guiyang City in SW China, and its complexation behavior with Hg(II) were investigated using fluorescence spectroscopy and the quenching titration technique. Three major fluorophores, two humic-like and one protein-like fluorescence, were observed in most of the DOM samples. Significant correlations were observed between the humic-like and protein-like fluorescence intensities, as well as, between them and other water quality parameters such as dissolved organic carbon, PO_4^{3-}, chemical oxygen demand and NH_4^+ concentrations, suggesting that agricultural and municipal wastewaters may be the source for both protein-like and humic-like fluorescence materials in the river. The fluorescence quenching titration resulted in similar values for the conditional stability constants for Hg(II) complexes with the humic-like and protein-like fluorophores, likely due to the dominance of Hg binding with O-containing function groups at the high Hg(II) concentrations used in the titration. Effects of Cl^-, Ca^{2+}, Mg^{2+} and Cu^{2+} ions on the binding between Hg(II) and three different fluorophores were also studied. The fluorescence index from the Nanming River was further found to be controlled by pH and Hg(II), cautioning its use in discriminating the sources of DOM.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of aromatic and aliphatic organic compounds, which can be divided into two main categories, (a) biochemically defined compounds such as proteins, carbohydrates, and lipids, and (b) humic substances (which could be further divided into fulvic and humic acids based on their solubility) (Thurman, 1985; Leenheer and Croué, 2003). DOM has been widely studied because it plays a significant role in a variety of biogeochemical processes in the aquatic environment (Leenheer, 1981; Thurman, 1985; Coble, 1996; Thomas, 1997; Frimmel, 1998). For example, DOM could bind with metal ions, thus controlling the concentration, speciation, and bioavailability of metals in the aquatic environment (Thurman, 1985). Unfortunately,

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only approximately 25% of DOM has been characterized so far (Baker and Spencer, 2004), which has greatly limited our understanding of water quality in general.

Fluorescence spectroscopy has been widely used to investigate the chemical property and source of DOM in natural waters (Senesi, 1990; De Souza et al., 1994; Ferrari and Mingazzini, 1995; Coble, 1996; Yamashita and Tanoue, 2003; Jaffé et al., 2004). With the development of fluorescence technology, synchronous fluorescence spectroscopy (SFS) (Cabaniss and Shuman, 1987; Senesi et al., 1991; Miano and Senesi, 1992; De Souza et al., 1994; Pullin and Cabaniss, 1997; Jaffé et al., 2004) and especially 3-dimensional excitation emission matrix (3DEEM) fluorescence spectroscopy have been successfully used to probe the chemical structure of DOM because they can distinguish among different classes of DOM of different origins (Coble et al., 1990; Mopper and Schultz, 1993; Coble, 1996; McKnight et al., 2001; Baker, 2001; Wu et al., 2003; Baker and Spencer, 2004). SFS (Ahmad and Reynolds, 1995; Reynolds and Ahmad, 1997; Galapate et al., 1998) and 3DEEM (Baker, 2001, 2002a,b; Baker et al., 2003) have also been used to investigate waste waters and river pollution. However, the exact relationship between fluorescence properties and biogeochemical structure of DOM remains unknown (Baker and Spencer, 2004).

Mercury pollution is a global problem with both ecological and human health implications. Previous studies have shown that DOM plays a key role in the fate and biogeochemical cycling of Hg in the aquatic environment (Miskimmin et al., 1992; Watras et al., 1995; Yin et al., 1997; Cai et al., 1999; Wu et al., 2004). For example, the reduction of Hg(II) to Hg^0 can be initiated by humic substances (Allard and Arsenie, 1991), which would reduce the bioavailability of Hg for methylation and subsequent biological uptake (Miskimmin et al., 1992). Ravichandran (2004) reviewed the role of DOM in the direct and photochemical reduction of Hg, and the effect of DOM on Hg methylation and bioaccumulation. However, it is unclear to what extent DOM controls the availability of Hg compounds due to the complex nature of DOM in different waters. Lu and Jaffé (2001) investigated the interaction between Hg(II) and DOM from Florida Everglades estuarine waters using emission and synchronous fluorescence methods. Wu et al. (2004) used steady-state and kinetic fluorescence to evaluate the quenching of DOM in headwater streams in Ontario and the structural changes of DOM caused by the addition of Hg(II), and the possible effect of cationic, dissolved organic carbon (DOC) concentrations and UV irradiation on the Hg–DOM complexation.

In this study, a small urbanized catchment in Guiyang City, SW China was chosen to investigate the fluorescence properties of the urban river DOM and its biogeochemical role. 3DEEM was used to characterize the Hg(II)–DOM complexation. The influence of Cl^-, Cu^{2+}, Ca^{2+} and Mg^{2+} on the Hg(II)–DOM complexation, and the influence of pH and Hg(II) on the fluorescence index were also investigated.

2. Materials and methods

2.1. Sampling

Water samples were collected from 10 sites along the Nanming River (Fig. 1) on January 8, 2003. The Nanming River is the source river of the Qingshui River which flows into the Wujiang, a tributary of the Changjiang (Yangtze River), China. With a small catchment area of 1433 km^2, much of the Nanming River flows through Guiyang, the capital city of Guizhou Province, China.

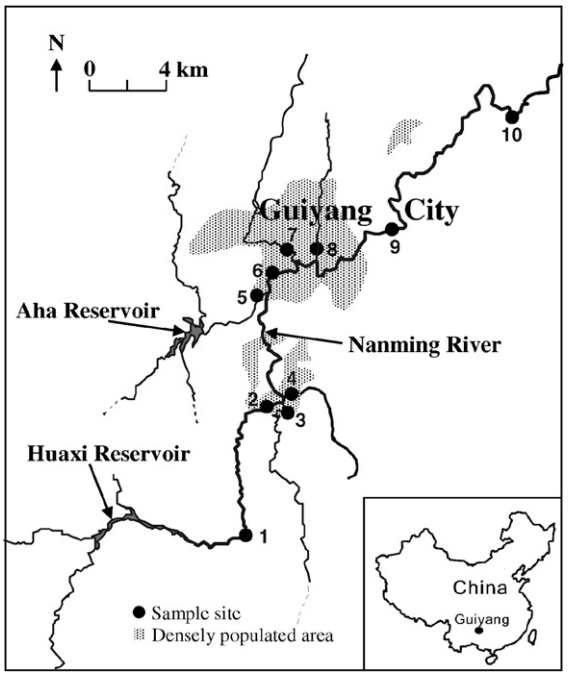

Fig. 1. Location of the sampling sites in the Nanming River catchment, Guiyang City, SW China.

The water quality at the two upstream sites (Sites 1 and 2) is believed to be good. Farms and small towns are around Sites 3 and 4. The water quality at Site 5 is also relatively good, as Aha Lake is one of the drinking water sources for Guiyang City. From Site 6 downstream the river flows through the Guiyang City and is contaminated by industrial and domestic sewerage, as well as by farming activities nearby.

At each site, a 5 L water samples was collected in polyethylene bottles, and returned to the laboratory within 6 h. The samples were filtered through precombusted 0.45 μm GF/F filters (450 °C for 5 h, Whatman, UK) and kept at 4 °C in the dark until analysis.

2.2. Chemical analyses

All chemicals used were AR grade, and Milli-Q water (18.2 MΩ cm, Millipore) was used in all experiments. Dissolved inorganic C (DIC), DOC, and total dissolved N (TDN) concentrations of the water samples were simultaneously measured with a High TOC/N analyzer (Elementar, Germany). The carbon analyzer was calibrated with potassium hydrogen phthalate. UV–Vis absorbance (A_λ) of the water samples were measured at various wavelengths ($\lambda = 250$, 300, and 400 nm, respectively) on a Shimadzu UV-3000 double beam spectrophotometer with 1-cm quartz cells at room temperature (20 °C), Milli-Q water was used as the reference. Other ancillary physical and chemical parameters (pH, Chl *a*, nutrients and chemical oxygen demand) were measured using standard methods (SEPA, 2002).

2.3. Fluorescence analysis

The fluorescence of GF/F filtered DOM samples was determined on a Model F-4500 fluorescence spectrophotometer (Hitachi, Japan) with a 150-W Xe arc lamp. The fluorescence index, $f_{450/500}$ (Battin, 1998; McKnight et al., 2001) was determined at an excitation wavelength of 370 nm. The fluorescence emission spectra were obtained at Ex = 340 nm. The synchronous fluorescence spectra were obtained at offset values between excitation and emission wavelengths ($\Delta\lambda = 20$, 40 and 60 nm). The 3DEEM data were collected for 1 nm wavelength of emission scans at every 5 nm wavelength of excitation. The bandpass width was 5 nm for excitation and 10 nm for emission, and the scan speed was 1200 nm min^{-1}. The scan range was 240–400 nm for excitation and 250–550 nm for emission. The spectra were blank (Milli-Q water) subtracted and the fluorescence intensity was expressed in arbitrary units.

2.4. Fluorescence quenching titration and data treatment

Fluorescence quenching titration technique was used to study the complexation between DOM and Hg(II). Experiments were carried out by adding 0.01 M or 0.05 M $Hg(NO_3)_2$ solutions to a series of glass bottles (National Scientific, USA) that contained 20.0 mL DOM with 0.01 mol/L $KClO_4$. pH was adjusted to 8.00 ± 0.05 by adding 0.10 mol/L $HClO_4$ or NaOH solution. In order to evaluate the effect of Cu^{2+}, Ca^{2+} and Mg^{2+} ions on the Hg–DOM complexation, the water sample was passed through a H^+-saturated cation-exchange resin (BioRad AG Dowex 50W-X8) column to remove trace metal ions and major cations. Competition between Cu^{2+} and Hg(II) for DOM complexation was investigated using fluorescence emission spectra at an excitation wavelength of 335 nm. All samples were shaken in the dark for 24 h at room temperature to ensure complexation equilibrium.

In order to evaluate the stability constants and complexing capacity, the binding sites causing the fluorescence quenching are assumed to form 1:1 complexes between the DOM and Hg(II). The complexing parameters were estimated by fitting the titration results with both the modified Stern–Volmer equation (Esteves da Silva et al., 1998) and the nonlinear model (Ryan and Weber, 1982).

The nonlinear model results in the following equation (Ryan and Weber, 1982):

$$I = (I_0 - I_{res})[(KC_L + KC_M + 1) - 1/(2KC_L)\sqrt{(KC_L + KC_M + 1)^2 - 4K^2C_LC_M}], \quad (1)$$

where I_{res} is the limiting value below which the fluorescence cannot decrease with the addition of Hg(II), C_L is the total ligand concentration, C_M is the total metal ion concentration, and K is the conditional stability constant. K and C_L were solved by a nonlinear regression analysis with the software SigmaPlot (SPSS).

The modified Stern–Volmer equation takes the form of (Esteves da Silva et al., 1998)

$$I_0/\delta I = 1/(fK[\mathrm{Hg}]) + 1/f, \quad (2)$$

where I and I_0 are the fluorescence intensity of the DOM sample with and without the addition of Hg(II), respectively. $\delta I = I_0 - I$, f is the fraction of the initial fluorescence that corresponds to the binding fluorophores, and K is the conditional stability constant, which could be solved by plotting $I_0/\delta I$ against 1/[Hg].

3. Results and discussion

Physical and chemical parameters of DOM samples collected from the Nanming River are shown in Table 1. From the measurements of chemical O_2 demand (COD), dissolved O_2 (DO) and nutrients (N, P), it is evident that the water quality at Sites 1, 2, and 5 was relatively good (legislated limits: COD < 15.0 mg/L, DO > 6.00 mg/L), whereas other sites were contaminated to various extent.

3.1. DOC and UV–Vis absorbance of the Nanming River DOM

DOC concentrations in the Nanming River ranged from <2 mg/L at Sites 1, 2 and 5 to between 4 and 7 mg/L at the downstream sites. The highest DOC concentration (7.83 mg/L) was found at Site 4. DON concentrations showed a similar trend (Table 1).

UV–Vis absorbance spectroscopy is a common method for the characterization of DOM. Generally, the UV–Vis spectra of DOM show an increasing absorption with decreasing wavelength without characteristic features (Chin et al., 1994; Artinger et al., 2000). This is true for samples studied here. As shown in Table 1, there were strong correlations between UV absorbance at 250 nm and 300 nm, and DOC concentrations in the river samples ($r^2 = 0.91$ and 0.92, respectively, $p < 0.05$, $n = 10$).

3.2. Fluorescence properties of the Nanming River DOM

The fluorescence emission spectra (Ex = 335 nm) of DOM samples in the Nanming River were similar, showing a broad band of overall intensity with a maximum intensity occurring at an emission wavelength around 430 nm. Senesi (1990) reported that those similar fluorescence features were probably attributed to the presence of unsaturated carbonyl and carboxylic functional groups in DOM

Table 1
Basic characteristics of water samples from the Nanming River[a]

Site	pH	COD (mg/L)	DO (mg/L)	DIC (mg/L)	DOC (mg/L)	NH_4^+ (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)	TDN (mg/L)	DON (mg/L)	PO_4^{3-} (mg/L)	C/N	Chlα (μg/L)	A_{250} (m^{-1})	A_{300} (m^{-1})	A_{400} (m^{-1})	E3/E4	Flu	$f_{450/500}$	$r_{(A,C)}$
1	8.05	5.00	6.86	40.1	1.50	0.44	0.03	1.62	2.31	0.22	0.17	6.82	1.36	2.99	1.61	0.46	3.50	64.9	1.49	1.68
2	7.86	6.06	5.70	37.7	1.76	0.66	0.08	2.24	3.23	0.25	0.56	7.04	2.05	4.38	2.42	0.58	4.20	153	1.53	1.65
3	7.88	20.5	4.19	32.4	3.88	3.83	0.06	0.84	5.75	1.01	1.31	3.84	2.73	10.6	5.76	1.27	4.55	332	1.67	1.35
4	7.89	39.4	4.19	60.5	7.83	10.2	1.28	0.04	15.7	4.21	0.94	1.86	4.78	16.2	10.5	4.38	2.39	506	1.58	1.55
5	8.26	4.61	6.99	26.9	1.36	0.06	0.01	0.96	1.18	0.16	0.03	8.50	6.14	1.84	1.04	0.23	4.50	57.3	1.52	2.00
6	8.03	11.9	6.34	38.9	3.47	4.31	0.15	2.75	7.49	0.28	0.62	12.4	2.05	8.75	4.84	1.15	4.20	315	1.62	1.61
7	8.03	51.3	2.40	37.8	6.77	13.5	0.39	1.05	17.5	2.65	2.23	2.55	0.18	11.7	6.56	1.50	4.38	608	1.68	1.75
8	7.89	30.8	1.70	43.6	4.93	13.0	0.16	0.09	14.7	1.41	2.13	2.31	2.73	12.1	6.68	1.96	3.41	568	1.71	1.87
9	7.92	36.2	2.98	46.6	5.10	12.0	0.14	0	19.4	7.30	2.55	0.70	2.05	11.4	6.33	1.50	4.23	527	1.66	1.70
10	7.90	29.4	3.54	46.1	4.67	12.1	0.13	0.61	17.9	5.05	1.84	0.92	1.36	10.4	6.10	1.61	3.78	437	1.69	1.38

[a] C/N: DOC/DON; E3/E4: A_{300}/A_{400}; Flu: fluorescence emission intensity (Ex = 335 nm) in arbitrary units; $f_{450/500}$: the ratio of fluorescence intensity at the emission wavelength 450 nm to that at 500 nm at an Ex = 370 nm; $r_{(A,C)}$: the fluorescence intensity ratios of humic-like peaks A to C.

structures. McKnight et al. (2001) introduced a fluorescence index, $f_{450/500}$, which is the ratio of fluorescence intensity at the emission wavelength 450 nm to that at 500 nm at Ex= 370 nm, to discriminate the source of DOM. It has been reported that $f_{450/500}$ is ~1.90 for aquatic and microbial sources, and ~1.40 for the terrestrial and soil sources (Battin, 1998; McKnight et al., 2001). As shown in Table 1, DOM samples at Sites 1 and 5 of the Nanming River had $f_{450/500}$ of 1.52 and 1.49, suggesting a dominating terrestrial source. At the mainstream sties 1, 2, 6, 9 and 10 in the Nanming River, $f_{450/500}$ gradually increased from 1.52 to 1.70, indicating that more DOM in the downstream sites were of microbial origin owing to the municipal wastewater. This is supported by a previous study by Ogawa et al. (2001) that bacterial activity may produce refractory DOM.

Typical synchronous fluorescence spectra (SFS) (offset values, $\Delta\lambda = 20$, 40 and 60 nm) of DOM from the Nanming River gave similar spectra featuring a major peak at about 280 nm, and 3 shoulder peaks at about 340, 370 and 480 nm (data not shown, synchronous fluorescence spectra are just "slices" through the 3DEEM). Ferrari and Mingazzini (1995) reported that SFS of DOM showed the presence of 4 relatively broad peaks which were assigned to: (1) mono-aromatic and proteinaceous materials (270–300 nm, Peak I), (2) compounds of two condensed ring systems (310 and 370 nm, Peak II), (3) fulvic acids (370–400 nm, Peak III), and (4) humic acids and other humic-like substances ($\geqslant$460 nm, Peak IV). Other studies have suggested that the peak at 280 nm is attributable to the presence of proteinaceous materials, probably derived from recent biological activities (Lombardi and Jardim, 1999), and the shoulder peaks at about 340, 370 and 480 nm were due to the presence of humic substances (Miano and Senesi, 1992). The synchronous peak at 280 nm was also found in sewage samples and was primarily attributed to biodegradable aromatic acids present both in the DOM and suspended solids in sewage (Ahmad and Reynolds, 1995). It has been shown that sewage water contains many types of aromatic amino acids and a host of imponderable high molecular weight polycyclic and simple aromatic hydrocarbon compounds (Ahmad and Reynolds, 1995).

3DEEM of DOM samples from the Nanming River exhibited 3 fluorescence peaks (peaks A, B and C) (Fig. 2a). In terms of the Ex/Em maxima, peak A (Ex/Em = 330/426 nm) and peak C (Ex/Em = 245/436 nm) can be attributed to humic-like fluorescence, and peak B (Ex/Em = 275/346 nm) to protein-like fluorescence (Coble, 1996; Wu and Tanoue, 2001; Leenheer and Croué, 2003; Chen et al., 2003). Strong protein-like fluorescence has often been reported in rivers that receive effluents (Baker, 2001, 2002a,b; Baker et al., 2004). Similar to the results from fluorescence emission spectra and SFS, the 3DEEM results also show that downstream water samples at Sites 6, 9 and 10 had higher fluorescence intensities for both protein-like and humic-like fluorescence than upstream sites. Their maximum fluorescence intensity wavelengths remained constant among all the samples. Fig. 3 shows the fluorescence intensities at peak A, B and C vs. DOC for all the samples. Significant correlations were found between DOC and all of the fluorescence peaks. When considering the relation-

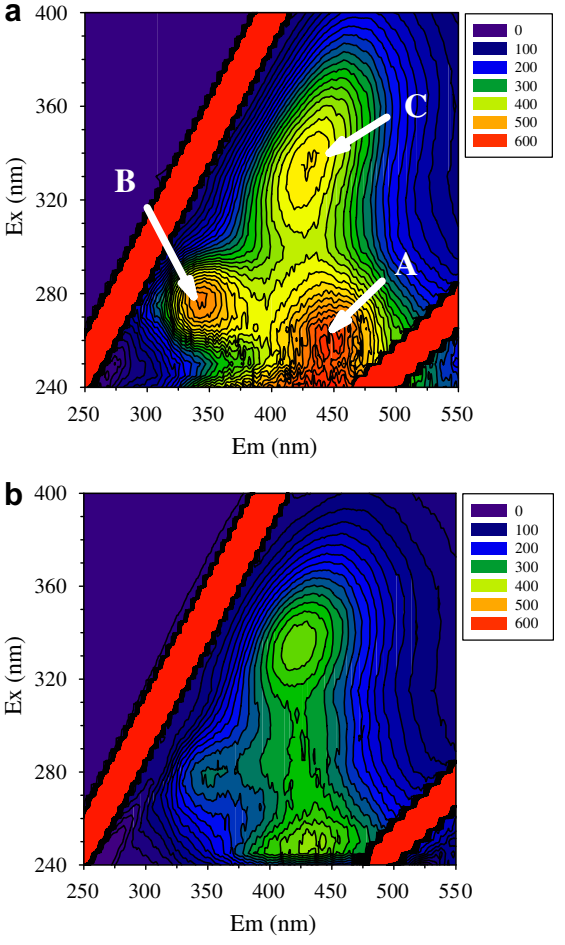

Fig. 2. 3DEEM contour plots of DOM and Hg(II)–DOM complexes. (a) Original, and (b) with 32 μM Hg(II) added. The DOM sample was collected at Site 9 on January 8, 2003.

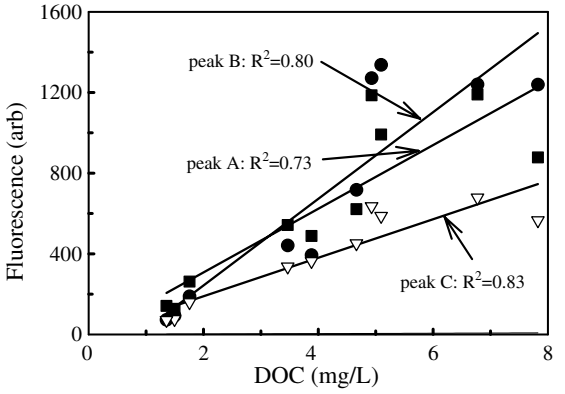

Fig. 3. Relationship between fluorescence intensities at different fluorescence peaks and DOC concentrations (peak A: ■; peak B: ●; peak C: ▽).

ship between protein-like fluorescence intensity for peak B and humic-like fluorescence intensity for peaks A and C, significant correlations were observed ($r^2 = 0.93$, $p < 0.05$, $n = 10$) (Fig. 4). This suggests that both humic-like and protein-like fluorescence substances played a significant role in the DOC. Furthermore, this indicates that farm wastewater and municipal wastewater may act as the origin not only of the protein-like fluorescence substance but also of the humic-like fluorescence substance. This has been demonstrated in wastewater effluent elsewhere by HPSEC (Imai et al., 2002), NMR (Ma et al., 2001), MS (Réveillé et al., 2003), FTIR and fluorescence spectroscopy (Duarte et al., 2003).

The fluorescence intensity ratios of humic-like peaks A to C, $r_{(A,C)}$, ranged from 1.35 to 2.00 (Table 1), which is similar to the values reported for other rivers, coastal and marine waters (Coble, 1996; Patel-Sorrentino et al., 2002). Patel-Sorrentino et al. (2002) reported that $r_{(A,C)}$ were strongly pH-dependent; a correlation was also found in the present samples between $r_{(A,C)}$ and pH ($r^2 = 0.42$, $p < 0.05$, $n = 10$).

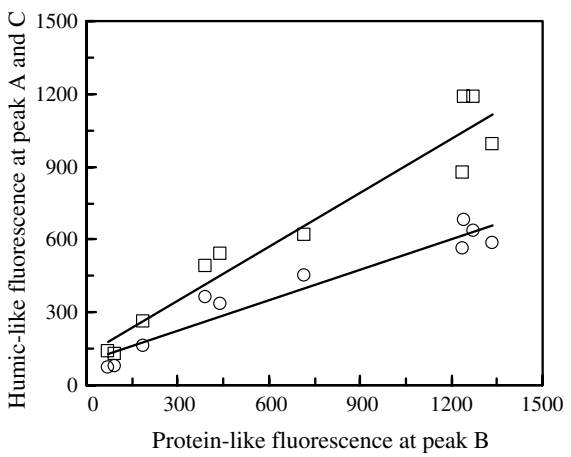

Fig. 4. Relationship between protein-like and humic-like fluorescence of DOM samples in Nanming River (peak A: □; peak C: ○).

3.3. Correlation between fluorescence intensity and PO_4^{3-}, COD and NH_4^+ concentrations

In natural waters, PO_4^{3-} concentration is relatively low (usually <0.10 mg/L) (Motomizu and Li, 2005; Jin et al., 2006). However, PO_4^{3-} concentrations in the Nanming River water were as high as 2.55 mg/L, indicating that the downstream water was polluted by the upstream agricultural activities and municipal wastewaters. The high concentrations of COD and NH_4^+ (Table 1) also support this. Reynolds (2002) demonstrated that there were strong correlations between normalized fluorescence intensity and COD, biological O_2 demand (BOD) and TOC of wastewaters. Baker and Curry (2004) found that the fluorescence intensity correlated well with NH_4^+ concentration and BOD in landfill leachate. Similar correlations were also observed between the fluorescence intensity of the different peaks and the concentrations of PO_4^{3-}, COD and NH_4^+ in the Nanming River (Fig. 5). This further suggests that, similar to the changing trend of these chemical parameters, both protein-like and humic-like fluorescence materials may originate from both farm and municipal wastewaters.

3.4. Complexation of DOM by Hg(II)

Because of the significant role of humic-like and protein-like substances in the DOC from the Nanming River, further studies were carried out to investigate the binding properties of the different fluorescent peaks with metal ions. If there is a structural relevance between humic-like and protein-like fluorophores, they would show relatively similar complexation with Hg(II).

The binding properties of the fluorescent peaks can be calculated based on the fluorescence quenching titration according to Eqs. (1) and (2). Fluorescence emission spectra of DOM showed a successive decrease in the overall fluorescence intensity as Hg(II) was added. A decrease in intensities of peak A, B and C was also observed with increasing Hg(II) concentration (Figs. 2b and 6). The plot of

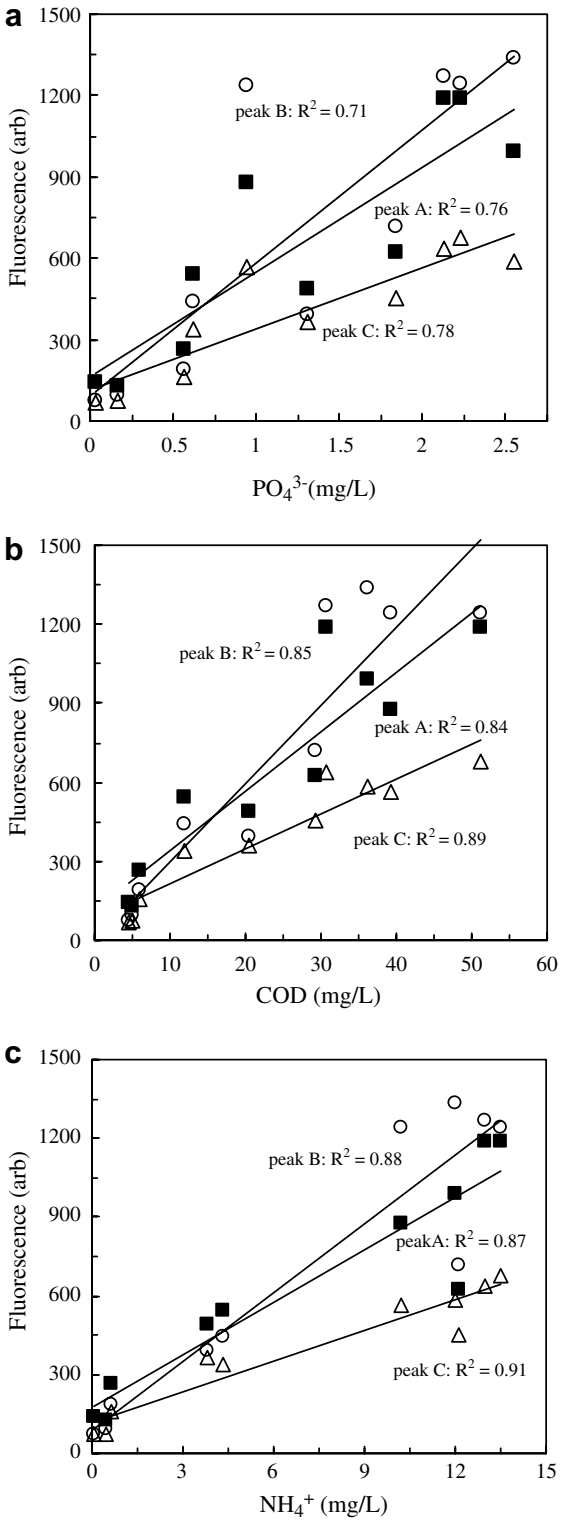

Fig. 5. Relationships between fluorescence intensity and PO_4^{3-}, COD and NH_4^+ concentrations (peak A: ■; peak B: ○; peak C: □).

$I_0/\delta I$ vs. 1/[Hg] was strongly linear for each of these 3 fluorophores (Fig. 7). The nonlinear model also fit well with the data (Fig. 8). The conditional stability constants calculated by the nonlinear model were similar to those by the linear model (Table 2). The values of $\log K$ ranged from 5.0 to 5.7 for the 3 fluorophores.

Although the conditional stability constants were in agreement with those reported in the literature (Yin et al., 1997; Lu and Jaffé, 2001; Wu et al., 2004), they were much lower than the stability constants for Hg complexes with many other ligands such as S- and N-containing ligands. While natural DOM often contains "strong" Hg-complexing sites (e.g., –SH, $–NH_2$ groups), their concentrations are much lower than the "weak" Hg-complexing sites (e.g., –OH, –COOH groups). Since the Hg(II) used

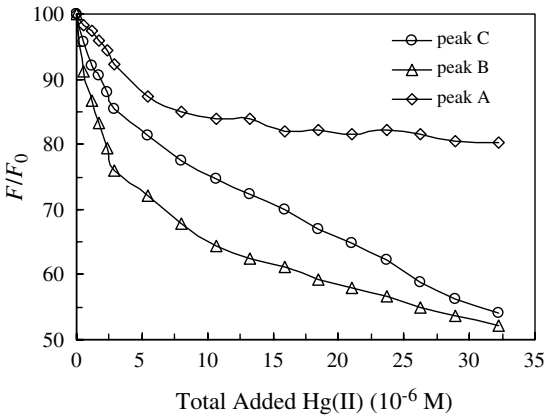

Fig. 6. Fluorescence quenching of DOM at different fluorescent peaks titrated with a Hg(II) solution.

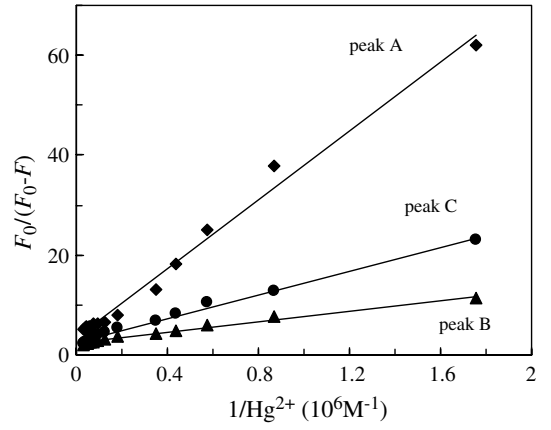

Fig. 7. Modified Stern–Volmer plots for the DOM sample.

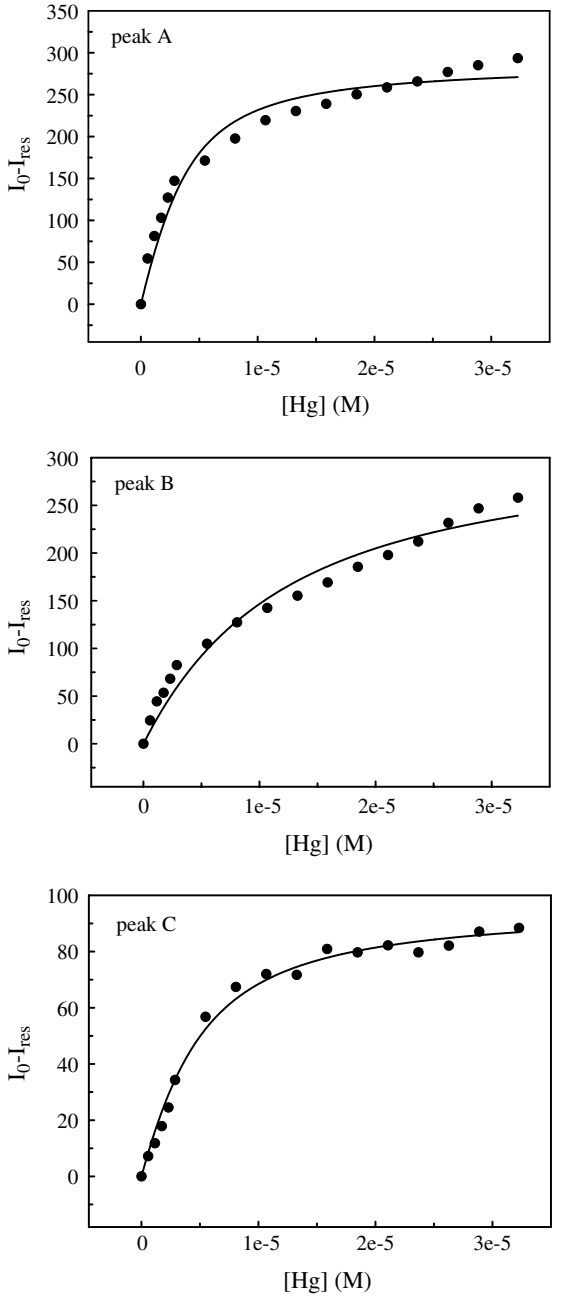

Fig. 8. Nonlinear regression analysis of the Hg–DOM complexation.

in the fluorescence quenching titration was very high (in micromolar levels), it is expected that the strong binding sites were readily saturated and the majority of Hg(II) was bound to the weak sites. Therefore, the conditional stability constants determined by the fluorescence quenching technique can only be considered as overall averaged constants. This is further supported by the fact that all 3 fluorophores showed relatively similar stability constants with Hg(II) (Table 2).

3.5. Relationships between Cl^-, cation and Hg complexation

In natural waters, DOC, Cl^- and OH^- ligands, among others, are co-present. The Cl^- ion is a ligand that can complex Hg(II) to form $HgCl_n^{2-n}$, depending on the concentration of Cl^- (Lu and Jaffé, 2001). Fig. 9 shows that the addition of Cl^- to the Hg–DOM complexes resulted in the increase in the fluorescence emission intensity at both pH 7.50 and 9.50 due to the competition between DOM and Cl^- for Hg(II).

Other metal cationic ions in natural waters may also compete with Hg(II) for the available binding sites. The competition of Ca^{2+} and Mg^{2+} for complexation of Cu(II) and Al(III) by fulvic acid has

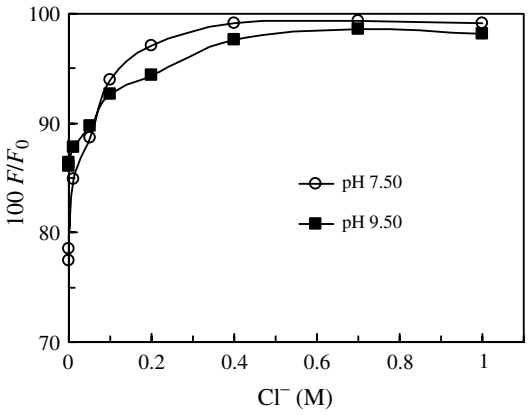

Fig. 9. Influence of Cl^- on Hg–DOM complexation (Hg(II) = 15 μM; various pHs).

Table 2
The calculated complexation parameters of different fluorescence peaks for the DOM sample collected at Site 9 on January 8, 2003

DOM sample	Peak	Ex/Em (nm)	Modified Stern–Volmer model			Nonlinear model	
			log K	f (%)	r^2	log K	r^2
Nanming River	A	245/432–438	5.62	44.2	0.98	5.74	0.97
	B	275/340–346	5.33	39.6	0.99	4.99	0.97
	C	330–335/426–434	5.01	28.2	0.99	5.50	0.99

been reported (Cabaniss and Shuman, 1988; Cabaniss, 1992). In this study, the removal of divalent and trivalent metal ions from a DOM sample (Site 10) with a Dowex 50W-X8 cation exchange resin resulted in approximately 30% decrease in the fluorescence intensity of the DOM sample, suggesting the presence of metal ions in the sample that enhanced the fluorescence. Although the binding affinities of Ca^{2+} and Mg^{2+} to DOM were much weaker than that of Hg(II), the competing effect of Ca^{2+} or Mg^{2+} may be significant owing to their high concentrations in most natural waters. The results (Fig. 10) show that the addition of Ca^{2+} strongly enhanced the fluorescence intensity of the DOM sample, especially for humic-like fluorescence, while the protein-like fluorescence was only slightly enhanced. In contrast, no obvious change in fluorescence was found in the Hg–DOM complexes after the addition of Mg^{2+}, suggesting that the competition between Hg(II) and Mg^{2+} for DOC was minimal under the condition studied.

A decrease in fluorescence emission intensity was found after the further addition of Cu^{2+} to the Hg–DOM complexes (Fig. 11). This may be due to the fact that Cu^{2+} acted as a stronger quencher for

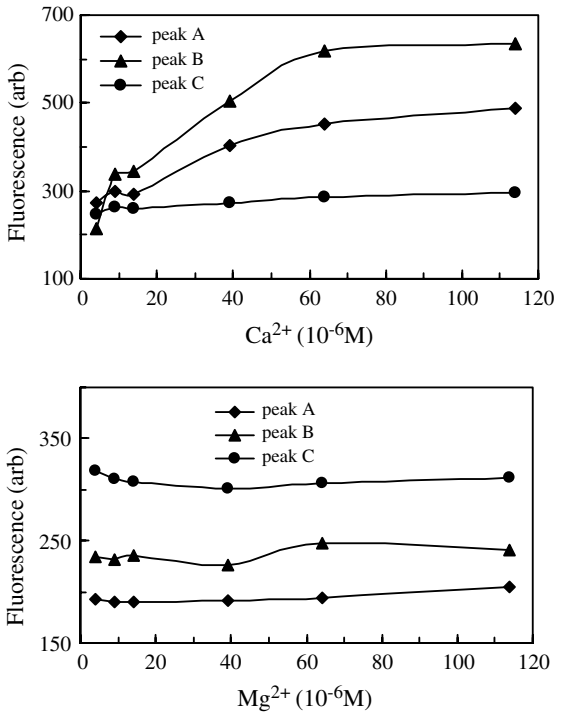

Fig. 10. Influence of Ca^{2+} and Mg^{2+} on Hg–DOM complexation ([Hg(II)] = 15 μM, pH 7.50).

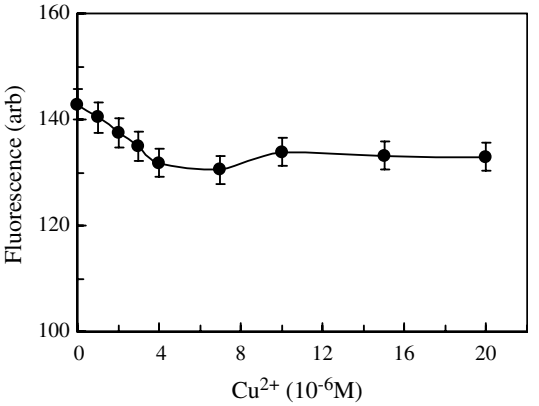

Fig. 11. Influence of Cu^{2+} on Hg–DOM complexation ([Hg(II)] = 15 μM, pH 7.50).

DOM than that of Hg(II). Another reason is that there were different fluorescent sites in the DOM sample, which were quenched by Hg^{2+} and Cu^{2+} ions, respectively. Under the natural water conditions, the majority of Cu complexation by DOM was attributed to phenolic sites instead of more acidic carboxyl sites (Lu and Allen, 2002), while Hg(II) may mainly bind to O-, N-, and S-containing sites of DOM (Zhang et al., 2004; Smith et al., 2002).

3.6. Effects of pH and Hg(II) on the fluorescence index of DOM

The fluorescence index was used in combination with other limnological and water quality data to infer the DOC sources in lakes and streams (Battin, 1998; McKnight et al., 2001). For example, Battin (1998) used the fluorescence index, along with measurements of absorbance and DOC concentrations, to differentiate between autochthonous and allochthonous colored DOM in blackwater rivers from the Orinoco Basin. However, the quantitative use of fluorescence index to discriminate the source of organic substances in natural waters may be compromised by many factors (McKnight et al., 2001). In this study, the effects of pH and Hg^{2+} on the fluorescence index of DOM from the Nanming River were investigated.

Fig. 12 shows that the fluorescence index decreased as pH increased. When the fluorescence was quenched by Hg(II), pH showed less impact on the fluorescence index. In addition, the fluorescence index seemed to have an increasing trend with the addition of EDTA on the Hg(II)–DOM system.

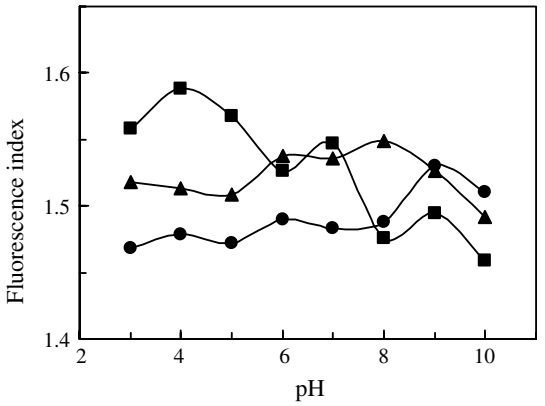

Fig. 12. The influence of pH and Hg(II) on the fluorescence index (■: original DOM sample; ▲: DOM + Hg(II); ●: DOM + Hg(II) + EDTA).

This indicates that the fluorescence index may be affected by many factors such as pH, the coexisting metal ions and ligands, which cautions the use of fluorescence index as an indicator of the source of DOM in natural waters.

4. Conclusions

This study demonstrates the usefulness of fluorescence spectroscopy to investigate urban river DOM. Both humic-like and protein-like fluorophores were revealed by SFS and 3DEEM, and were strongly associated with pollution from farm and municipal wastewaters, suggesting that the fluorescence technique provides a powerful tool to study water quality in the aquatic environment. This study also shows that humic-like fluorescence, usually reported as terrestrially originated, had a significant correlation with protein-like fluorescence, and both humic-like and protein-like fluorescence had significant correlations with the DOC, PO_4^{3-}, COD and NH_4^+ concentrations in the river waters, suggesting that the agricultural and municipal wastewaters may be the source for both protein-like and humic-like fluorescence materials. The Hg(II)–DOM complexation behavior and the fluorescence index of DOM were mainly controlled by the water chemistry such as pH, major ions and other ligands.

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