



Characterization of dissolved organic matter fractions from Lake Hongfeng, Southwestern China Plateau

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Abstract

With XAD-series and ion exchange resins, dissolved organic matter (DOM) from Lake Hongfeng in Southwestern China Plateau was isolated into 6 fractions, i.e., humic acid (HA), fulvic acid (FA), hydrophobic neutrals (HON), hydrophilic acids (HIA), hydrophilic bases (HIB) and hydrophilic neutrals (HIN). Those fractions were characterized by high performance size exclusion chromatography, fluorescence spectroscopy and UV absorbance. Among the 6 fractions, FA was predominant and accounted for 51% of the total DOM. The weight-average (M_w) and number-averaged (M_n) molecular weight of these fractions ranged from 1688 to 2355 Da and from 1338 to 1928 Da, respectively. A strong correlation was observed between specific UV absorbance at 280 nm, E_2/E_3 (absorbance at 250 nm to 365 nm), and the molecular weight for DOM fractions. UV-Vis fulvic-like fluorescence peaks were found in all fractions. Protein-like fluorescence peaks existed in HON may indicate that microbial activity was severely in Lake Hongfeng. There was a significant relationship between fluorescence intensities and specific UV absorbance at 254 nm for those DOM fractions, suggesting their similar luminescence characteristics. The values of fluorescence index ($f_{450/500}$) indicated that hydrophobic fractions may derive from terrestrial sources, and the hydrophilic fractions from microbial and terrestrial origins. Those results suggest that there were inter-relationships between molecular weight, fluorescence and absorbance characteristics, and also subtle consistencies between the hydrophilic and hydrophobic properties and the sources for these 6 fractions from Lake Hongfeng.

Key words: dissolved organic matter; fractionation; molecular weight; UV absorbance; fluorescence spectroscopy

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Introduction

Dissolved organic matter (DOM), as an important chemical component, plays an important role in physical, chemical and biological processes in aquatic ecosystems, such as influencing the transport, toxicity and bioavailability of trace metals and organic pollutants, and participating in the formation of numerous chlorination byproducts in drinking water disinfection (Wu and Tanoue, 2001; Leenheer and Croué, 2003; Świetlik *et al.*, 2004a). DOM is generally described as heterogeneous polyfunctional polymers and can be operationally divided into humic and nonhumic organic matters. Humic substances, containing fulvic acid and humic acid, typically account for 40%–60% of the DOM in surface waters (Leenheer, 1981; Croué *et al.*, 2003). Nonhumic matter is composed of carbohydrates, proteins, amino acids and fatty acids, etc. (Thurman, 1985). The composition of DOM varied greatly in rivers (Leenheer, 1981), lakes (Wu and Tanoue, 2001),

and seawater (Coble *et al.*, 1990; Coble, 1996). The DOM composition is very complicated due to the flux of terrestrial DOM as well as the autochthonous production, especially in lakes.

To better understand the role and the composition of DOM in aquatic systems, a high veracity method, i.e., amberlite XAD resin fractionation has been applied to isolate DOM into different polar fractions, such as hydrophobic, transphilic and hydrophilic fractions (Leenheer, 1981; Aiken *et al.*, 1992; Croué *et al.*, 2003). However, most of the previous studies mainly focused on the humic substances (Aiken, 1985; Peuravuori and Pihlaja, 1997). Little is known about the composition of nonhumic substances and their environmental effects, especially hydrophilic fractions (Aiken *et al.*, 1992; Croué *et al.*, 2003).

Lake Hongfeng, a typical plateau lake, is formed by man-made reservoir in mountainous bases and located in the Southwestern China Plateau. It serves as the principal drinking water resource for Guiyang City, Guizhou Province. It has been severely polluted by wastewater from agriculture, industry, and municipal drainage recently

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(Yue *et al.*, 2006; Li *et al.*, 2008). The objective of this study was to isolate DOM in the lake using a large-volume XAD resins isolation method and to characterize those DOM fractions using high performance size exclusion chromatography, fluorescence spectroscopy, and UV absorbance. This study would help understanding of the DOM composition and its biogeochemical cycle in lacustrine environments.

1 Materials and methods

1.1 Sampling site

Lake Hongfeng (106°19′–106°28′E, 26°26′–26°35′ N) is located in the suburb of Guiyang City, Southwestern China Plateau. It is a seasonal anoxic lake with an average water depth of 10.5 m (max. 45 m), a lake surface area of 57.2 km², and a water residence time of 0.33 year. This lake is in the subtropical monsoon climate zone, covered by carbonate rocks and water is slightly alkaline (pH 7.47–8.67). Forest coverage is 8.1% in the lake watershed. Annual precipitation in the region is 1200 mm, and more than 55% of the annual precipitation occurs from May to August (Zhang, 1999).

1.2 Sampling

A 1300-L water under 0.5 m of the lake surface was collected in July 2005 at Houwu, located at the south part of Lake Hongfeng. This is one of the most seriously polluted areas in the lake. The physicochemical characteristics of the original water are pH 8.60; dissolved organic carbon (DOC) 2.92 mg/L; dissolved oxygen (DO) 10.21 mg/L; Ca²⁺ 45.49 mg/L; Mg²⁺ 14.78 mg/L; Cl⁻ 4.28 mg/L; SO₄²⁻ 57.13 mg/L; SiO₂ 3.45 mg/L; Chl-*a* 0.047 mg/L. After sampling, water sample was immediately filtered through a 0.45- μ m acetate fiber filter for further experiments.

1.3 Fractionation and characterization

1.3.1 DOM fractionation procedure

The fractionation of DOM in Lake Hongfeng was carried out based on the method by Leenheer (1981) and Aiken *et al.* (1992). Resins of XAD-8 and XAD-4 (Supelco Bellefonte, PA, USA), MSC-H (a strong acid, sulphonate, polystyrene macroporous cation-exchange resin, Dowex Marathon, 20–50 mesh) and Duolite A-7 (a weak base, phenol-formaldehyde condensation macroporous anion-exchange resin, Supelco Bellefonte, USA) were used and purified by Soxhlet extraction with methanol for 24 h before experiments.

The analytical procedure for DOM fractionation is shown in Fig. 1. Briefly, the pH of the filtered original water was acidified to 2 with HCl, and was flowed through the XAD-8/XAD-4 resin columns containing approximately 2 L of resin slurry at a rate of 2 bed volumes per hour (4 L/h). The dissolved organic carbon (DOC) concentration in the effluent was monitored. The process was discontinued and recycled, when DOC concentration was near the original water concentration. The hydrophobic acids (HOA) absorbed onto the XAD-8 resin were eluted with 0.1 mol/L NaOH. The HOA fractions including humic acid (HA) and fulvic acid (FA) were further acidified with HCl to pH = 1 and settled for 24 h to precipitate HA. After adjusting pH, the suspension was recycled through XAD-8 column and was eluted with 0.1 mol/L NaOH. The elution was FA fraction. The XAD-8 resin was dried in the air and Soxhlet-extracted with methanol. The fraction contained in methanol solution was hydrophobic neutral fraction (HON). All hydrophilic DOM fractions absorbed onto the XAD-4 were eluted with 0.1 mol/L NaOH, and pH was adjusted to 2 with HCl for further fractionation with ion-exchange resin. The adjusted solution was pumped through the MSC-H cation-exchange resin (hydrogen ion saturated) and the hydrophilic base fraction (HIB) was eluted with 1 mol/L NH₄OH. The effluent was

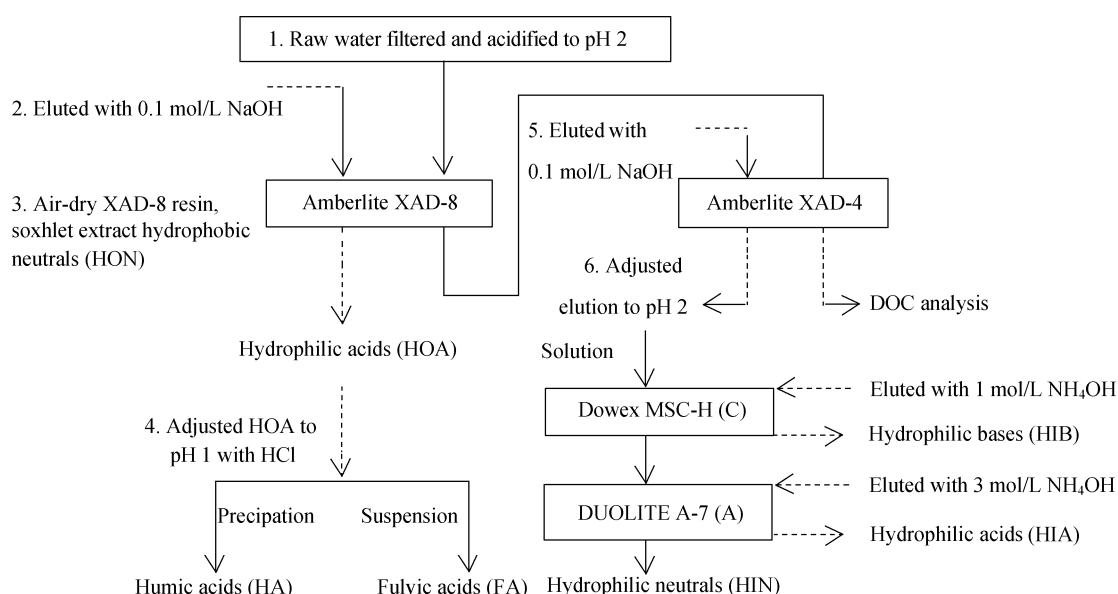


Fig. 1 Isolation scheme of DOM in Lake Hongfeng.

then pumped through a next column containing Duolite A7 anion-exchange resin (in free-base form). Hydrophilic acid fraction (HIA) was eluted with 3 mol/L NH_4OH . The resin effluent was collected as hydrophilic neutral fraction (HIN). All fractions were concentrated by rotary evaporation at 35°C, then purified with 100 Da membrane dialysis, and freeze-dried.

1.3.2 Dissolved organic carbon and molecular weight

DOC was measured by high-temperature catalytic oxidation with a High TOC II analyzer (Elementar, Germany). Potassium hydrogen phthalate was used as the standard. Molecular weight distribution was measured by high performance size exclusion chromatography (HPSEC) (Agilent 1100, PE, USA) with UV detector at 254 nm. A YMC-60 column (Waters, Milford, Japan), packed with a silica diol modified material with 5- μm gel bead in diameter and 6 nm in pore size, was used. The mobile phase (pH 6.8) was phosphate buffer (0.001 mol/L Na_2HPO_4 , 0.001 mol/L NaH_2PO_4 , and 0.03 mol/L NaCl). The flow rate was 0.5 mL/min.

The 6 DOM fraction solutions were filtered through pre-combusted GF/F glass fiber filters (5 h at 450°C, Whatman, UK) until a final concentration reach 10.00 ± 2.00 mg/L. The number-averaged molecular weight (M_n), weight-averaged molecular weight (M_w) and polydispersity (ρ) were also calculated (Zhou *et al.*, 2000).

1.3.3 UV absorbance and fluorescence spectroscopy

The 6 fraction solutions mentioned above were placed in a 1-cm quartz cuvette and scanned from 700 to 200 nm with a UV-Vis spectrophotometer. The specific UV absorptions at 254 nm and 280 nm (SUVA) (L/(mg C·m)) were measured with the Eq. (1):

$$\text{SUVA} = A/bc \quad (1)$$

where, A is the absorbance at 254 nm or 280 nm, b (m) is the cell path length, c (mg/L) is DOC concentration of the sample.

The fluorescence was determined on a spectrofluorometer (Hitachi, Model F-4500, Japan) equipped with a 150-W ozone-free xenon arc lamp as the excitation source. The excitation (Ex) wavelength ranged from 220 to 400 nm by 2-nm intervals and emission (Em) from 250 to 500 nm by a 5-nm interval. Ex and Em slits were set at 5-nm band width with a scan speed of 1200 nm per min. The spectra were blank subtracted (phosphate buffer).

The software SigmaPlot (SPSS) was employed for the three-dimensional excitation emission matrix fluorescence spectroscopy (3DEEM) contour plots and the fluorescence intensity was expressed in arbitrary. In addition, the fluorescence index ($f_{450/500}$, the ratio of fluorescence intensity at Em wavelength 450 nm to that at 500 nm at an Ex wavelength of 370 nm) was calculated (McKnight *et al.*, 2001).

2 Results and discussion

2.1 Contribution of the DOM fractions

The relative contributions of 6 DOM fractions are shown in Table 1. Based on DOC analysis, these fractions accounted for 94% of the total DOC in the original water. After further evaporative concentration and desalting, 82% of the DOC was recovered. This is similar to the results of previous studies, e.g., 81% (Leenheer, 1981), 80%–85% (Korshin *et al.*, 1997), and 65% (Świetlik *et al.*, 2004a). FA was predominately in Lake Hongfeng, accounting for 51%, while HIB was the minor fraction with a relative contribution of 3%. The hydrophobic fractions were much higher than hydrophilic fractions, contributing 77% and 23%, respectively. Similar distributions of DOM fractions were observed in Jingmi drinking water (Liu and Tao, 1994), Suwannee River (Ma *et al.*, 2001) and Mosina River (Świetlik *et al.*, 2004a). However, there were some differences. Marhaba *et al.* (2000) pointed out that FA accounted for 12% in drinking water in New Jersey, while hydrophilic fractions were up to 44%–55%. In Omega-9 wastewater, the hydrophobic fractions (49%, including hydrophobic base fraction, not list in Table 1) were slightly lower than hydrophilic fractions (51%) (Leenheer, 1981). The different percentages of DOM fractions may be related to their various origins and formation environment. Generally, allochthonous input from catchment soils may result in the increase of hydrophobic fractions (Artinger *et al.*, 2000). In sum, high precipitations and severe soil erosion may result in more terrestrial DOM input. These processes may result in the relatively high contributions of hydrophobic fractions to the total DOM in Lake Hongfeng.

2.2 Molecular weight distribution

The HPSEC chromatograms of 6 isolated fractions are shown in Fig. 2. Original water, FA, HON, HIA, HIB and HIN fractions showed broad and multimodal

Table 1 Relative contribution of DOM fractions isolated from different water bodies

Fraction	Lake Hongfeng (%)	Xin'an River ^a (%)	Suwannee River ^b (%)	Mosina River ^c (%)	Omega-9 wastewater ^d (%)	Water in New Jersey ^e (%)
HA	9	50–55*	13	19	19*	–
FA	51		68	54		8–12
HON	17	–		12	17	13–22
HIA	16	20**	17**	7	29	44–55
HIB	3			5	12	4–6
HIN	4			3	10	9–25

* HA+FA; ** HIA+HIB+HIN; –: data was not available.

^a Liu and Tao, 1994; ^b Ma *et al.*, 2001; ^c Świetlik *et al.*, 2004a; ^d Leenheer, 1981; ^e Marhaba *et al.*, 2000.

HA: humic acid; FA: fulvic acid; HON: hydrophobic neutrals; HIA: hydrophilic acids; HIB: hydrophilic bases; HIN: hydrophilic neutrals.

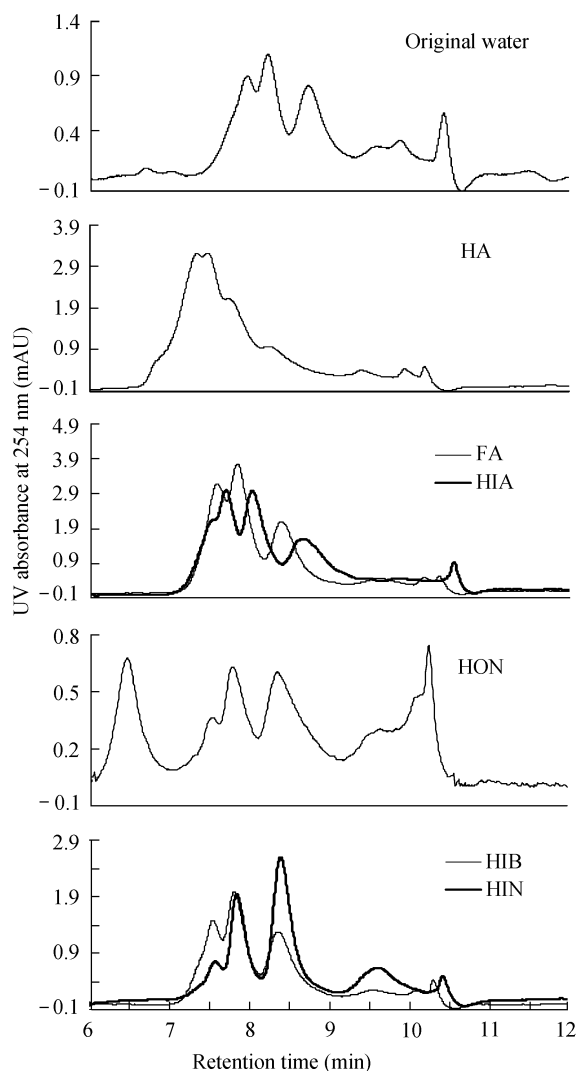


Fig. 2 High performance size exclusion chromatography (HPSEC) spectra chromatograms of 6 DOM fractions in Lake Hongfeng.

chromatograms with 4–5 sharp and well resolved peaks, whereas HA exhibited monomodal distribution. In order to characterize the molecular weight distribution of these fractions, molecular weight was further divided into four segments, i.e., large (L) (molecular weight > 3500 Da), medium-large (ML) (3500 Da > molecular weight > 2000 Da), medium (M) (2000 Da > molecular weight > 1000 Da) and small (molecular weight < 1000 Da) (Table 2). The percentage of each molecular weight segment to HON was almost the same, ranging from 19.36% to 29.24%. Medium-large and medium molecular weight were predominant in other fractions and original water with a range of 75.50% to 87.22%. Moreover, the percentage of medium-large molecular weight was much higher than medium fraction for HA (ML 64.63%; M 20.90%), HIB (ML 50.86%; M 36.36%) and original water (ML 45.34%; M 33.24%) comparing to FA, HIA and HIN. The different molecular weight distributions of DOM fractions indicate their various physicochemical properties (Zhou *et al.*, 2000). One distinct peak observed in HA may be indicative its homogeneous structure. The separation of DOM in HPSEC chromatograph seems to be better

than some previous reports for original DOM and humic acids (Peuravuori and Pihlaja, 1997; Parlanti *et al.*, 2002). This probably indicates that the isolated DOM fractions consisted of relatively simple organic compounds.

The estimated weight-averaged and number-averaged molecular weight (M_w , M_n) and polydispersity (ρ) of 6 fractions are shown in Table 2. These fractions had molecular weight range 1688–2355 Da for M_w and 1338–1928 Da for M_n , close to that of original water (M_w 1936 Da; M_n 1422 Da). Compared to the hydrophilic fractions, hydrophobic fractions had a larger molecular weight. The polydispersity (ρ) of the 6 DOM fractions and original water ranged from 1.19–1.57, and were lower than that reported by Chin *et al.* (1994) (1.5–1.9). The ρ of HA and hydrophilic fractions were lower than original water, FA and HON. This may indicate that the DOM in Lake Hongfeng had a relatively narrow molecular weight distribution and the structural composition may be more homogenous. This is consistent with the implication of molecular weight distributions of 6 fractions mentioned earlier.

DOM in the original water and 6 fractions showed relatively small molecular weight (< 3000 Da). High concentration of dissolved oxygen and strong photobleaching in surface water can make the larger molecules break-up into the smaller ones (Coble *et al.*, 1990). In particularly, soil organic matter is photodegraded intensively under strong UV light. Thus, relative smaller molecular weight fractions of terrestrial DOM was input the lake from the watershed in rainy season. The other reason was that the extracellular products of algae mass caused the increase of smaller organic molecules in the eutrophic lake (Cheng and Chi, 2003). Moreover, relatively small molecular weight DOM fractions increased the difficulty in the treatment of drinking water (Cheng and Chi, 2003).

2.3 Spectroscopic properties

UV-Vis absorbance had been widely used to characterize properties of organic matter in soils (Xing *et al.*, 2005) and waters (Chin *et al.*, 1994; Hautala *et al.*, 2000). SUVA was the specific UV absorbance at 254 nm and often used to represent the content of aromatic structures in humic samples (Peuravuori and Pihlaja, 1997). The SUVA values of the 6 fractions ranged from 1.50 to 2.88 L/(mg C·m) (Table 2). Moreover, the values of SUVA for HA, FA, HIA, HIB were higher than those of HON and HIN, indicating a lower aromaticity for the neutral fractions. The SUVA value of hydrophobic fractions and hydrophilic acids in Mosina water ranged from 3.0 to 3.9 L/(mg C·m) and 2.3 to 3.3 L/(mg C·m), respectively (Świetlik and Sikorska, 2004b). Croué *et al.* (2003) demonstrated that SUVA₂₅₄ of hydrophobic and transphilic acids, and transphilic neutrals was 2.9, 1.8, 0.7 L/(mg C·m), respectively. Comparatively, the DOM fractions in Lake Hongfeng had relatively low SUVA₂₅₄ (1.50–2.88 L/(mg C·m)), especially for hydrophilic fractions (1.50–2.50 L/(mg C·m)). This may be related to the strong ultraviolet light in the subtropical plateau zone and severe biological activities in eutrotrophic lakes. Previous studies reported that photolysis can

Table 2 Molecular weight distribution, E_2/E_3 and specific UV absorbance at 254 nm and 280 nm of the 6 DOM fractions in Lake Hongfeng

Fraction	M_w (Da)	M_n (Da)	ρ	Distribution (%)				E_2/E_3 (L/(mg C·m))	SUVA ₂₅₄ (L/(mg C·m))	SUVA ₂₈₀
				> 3500 Da	2000–3500 Da	1000–2000 Da	<1000 Da			
HA	2355	1928	1.22	5.43	64.63	20.90	9.03	2.02	2.88	2.25
FA	2045	1471	1.39	0.97	38.50	43.70	16.83	3.47	2.42	2.00
HON	2099	1338	1.57	19.36	22.71	29.24	28.68	2.93	1.70	1.02
HIA	1746	1397	1.25	0.16	37.62	40.76	21.45	4.25	2.23	1.49
HIB	1903	1599	1.19	0.00	50.86	36.36	12.77	3.82	2.50	1.61
HIN	1688	1362	1.24	2.26	30.30	45.2	22.19	5.04	1.50	1.05
Original water	1936	1422	1.36	2.60	45.34	33.24	18.82	2.63	2.72	1.93

M_n : the number-averaged molecular weight; M_w : the weight-averaged molecular weight; ρ : polydispersity; Da: the abbreviation of Dalton, i.e., the unit of molecular weight; E_2/E_3 : absorbance at 250 nm to 365 nm.

decrease the value of SUVA₂₅₄ (Moran and Zepp, 1997); and DOM originated from autochthonous sources (algae and bacteria in water) had a lower SUVA value than those from allochthonous sources (Croué *et al.*, 1999; Mcknight *et al.*, 2001).

SUVA₂₈₀ may provide important information for degree of aromaticity, sources, extent of humification and molecular weight of DOM (Chin *et al.*, 1994). E_2/E_3 is the ratio of absorbance at 250 nm to at 365 nm and often used as an indicator for humification in limnology (Peuravuori and Pihlaja, 1997). Generally, HA had a higher SUVA₂₈₀ and a lower E_2/E_3 than FA (Peuravuori and Pihlaja, 1997). There was a positive relationship between SUVA₂₈₀ and M_w for 5 fractions in Lake Hongfeng ($R^2 = 0.88$, $p < 0.05$, Fig. 3a) except for HON. E_2/E_3 was negatively correlated with M_w for 6 fractions ($R^2 = 0.96$, $p < 0.01$, Fig. 3b). Previous studies reported that strong correlations existed between molar absorptivity at 280 nm, E_2/E_3 and molecular weight of humic substances and DOM derived from different sources (Chin *et al.*, 1994; Peuravuori and Pihlaja, 1997). The results provide support that there existed a similar internal-relationship for different fractions isolated from the same DOM, and this further indicates the complexity and similarity of naturally occurring organic matter.

2.4 Fluorescence properties

3DEEM can offer important information on the source and chemical nature of DOM (Baker, 2001; Mcknight *et al.*, 2001; Chen *et al.*, 2003). There were generally three classes of fluorophores: humic-like, fulvic-like and protein-like fluorophores (Coble *et al.*, 1990; Wu and Tanoue, 2001). Figure 4 presents the 3DEEM characteristics for the 6 DOM fractions. Four distinct fluores-

cence peaks were observed, Peak A (excitation/emission (Ex/Em), Ex: 230–255 nm; Em: 424–462 nm), UV fulvic-like fluorescence; Peak C (Ex/Em, Ex: 300–305 nm; Em: 424–442 nm), visible fulvic-like fluorescence; Peak B (Ex/Em, Ex: 287 nm; Em: 358 nm) and Peak D (Ex/Em, Ex: 230; Em: 342 nm), protein-like fluorescence. Fulvic-like fluorescences existed in aquatic DOM (Coble *et al.*, 1996; Wu and Tanoue, 2001), while protein-like fluorescences were seldom reported in natural waters (Chen *et al.*, 2003). For the 6 fractions in Lake Hongfeng, protein-like fluorescence (Peaks B and D) only occurred in HON and the fluorescence intensity was strong. Protein-like fluorescence was considered to be derived from algae, bacteria, picoplankton growing in lake and municipal wastewater (Baker, 2001; Chen *et al.*, 2003). In the past years, Lake Hongfeng has been polluted by sewage and human activities and increase the degree of eutrophication (Wang *et al.*, 2001). Our results may indicate that the protein-like fluorescence of HON may be due to these living particles in Lake Hongfeng.

There were also some differences in the maximum Ex/Em wavelengths of the 6 fractions (Table 3). For Peak A, Ex/Em wavelengths shifted towards the longer wavelengths for hydrophobic fractions (Ex/Em, Ex: 240–255 nm; Em: 437–462 nm. HA, FA, HON) as compared to hydrophilic fractions (Ex/Em, Ex: 230–235 nm; Em: 424–436 nm. HIA, HIB, HIN). Red shift of Ex/Em maxima was obvious (Ex/Em, Ex: 255 nm; Em: 462 nm) for HA, while a prominent blue shift in HIN (Ex/Em, Ex: 230 nm; Em: 424 nm). For Peak C, Ex wavelength was almost similar, in the range of 300–305 nm for 6 fractions, while a longer Em for FA (Em: 442 nm) than hydrophilic fractions (Em: 424–428 nm) was found. The difference

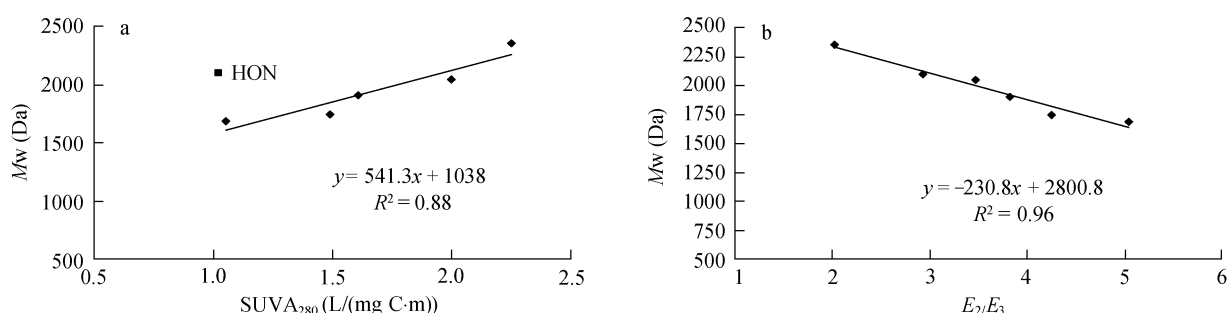


Fig. 3 Weight-averaged molecular weight (M_w) as a function of the absorbance coefficient at 280 nm (a) and E_2/E_3 (b) of 6 fractions in Lake Hongfeng.

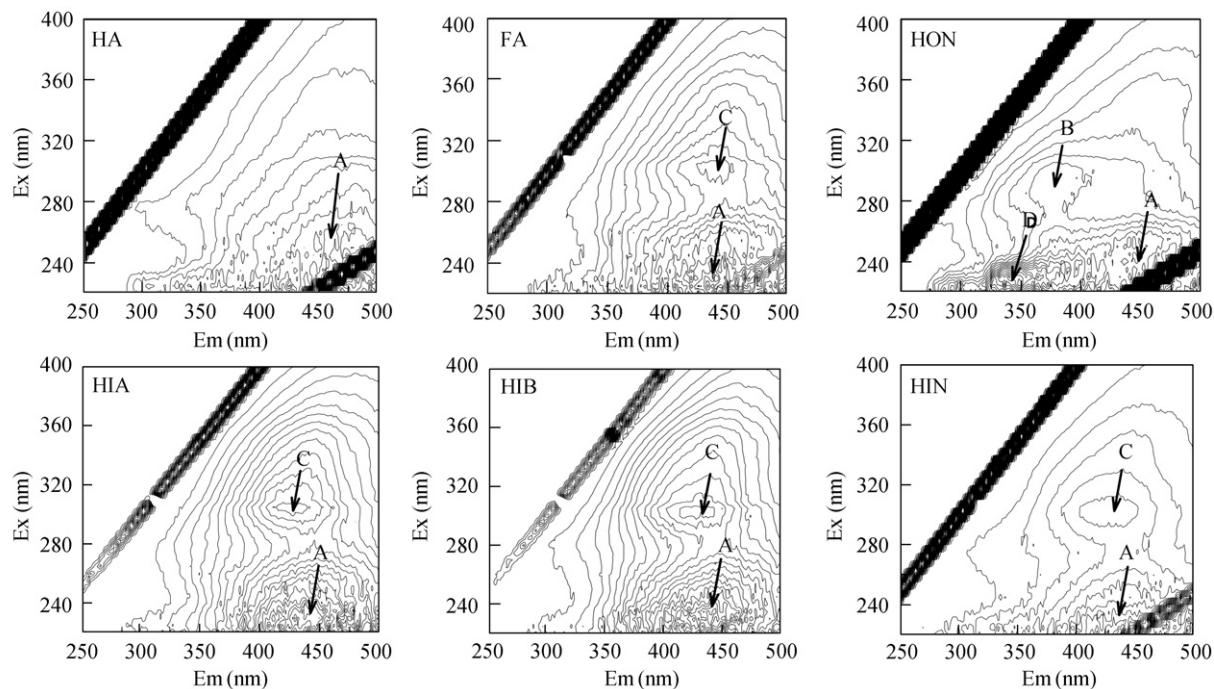


Fig. 4 The 3DEEM plots of 6 DOM fractions in Lake Hongfeng.

Table 3 Maximum fluorescence patterns and $f_{450/500}$ of the 6 DOM fractions in Lake Hongfeng

Fraction	Concentration (mg/L)	Peak A (Ex/Em) ^a	FI _A ^b (arb-L/mg)	Peak B (Ex/Em) ^a	Peak C (Ex/Em) ^a	Peak D (Ex/Em) ^a	$f_{450/500}$
HA	9.32	255/462	30.9	–	–	–	1.15
FA	10.32	240/446	47.6	–	305/442	–	1.22
HON	9.70	240/437	30.8	287/358	–	230/342	1.10
HIA	10.84	235/436	49.0	–	305/424	–	1.44
HIB	12.00	232/435	56.4	–	300/428	–	1.62
HIN	10.00	230/424	25.6	–	305/426	–	1.42

^a The units of Ex and Em are nm; ^b fluorescence intensity for peak A of per unit carbon; –: peaks can not be detected; $f_{450/500}$: fluorescence index.

in maximum Ex/Em wavelength of isolated fractions was probably associated with their different structures. Coble (1996) reported that DOM fractions of the blue shift of Ex wavelength reflected a relatively small molecular weight composition while red shift a relatively larger molecular weight composition. This is consistent with the results that the hydrophilic fractions had blue-shift Ex with a relatively small molecular weight and hydrophobic fractions red-shift Ex with a large molecular weight. The longest Ex wavelength in HA represented its largest molecular weight, and the shortest wavelength in HIN indicated its the smallest molecular weight. Furthermore, the blue-shift Em maxima in hydrophilic fractions indicate that biological activity played a role in the occurrence of these fractions (Coble, 1996; McKnight *et al.*, 2001).

Fluorescence intensity (FI) per unit carbon of 6 fractions ranged from 28.8 to 61.2 (arb-L)/mg for Peak A. It was 1.65 times in FA than that in HA, showing the higher fluorescence efficiency in the relatively small molecular weight fractions (Senesi, 1990). It was consistent with the report that aquatic humic fractions with larger molecular weight had a greater absorbance but lower fluorescence than smaller molecular weight fractions (Hautala *et al.*, 2000; Chen *et al.*, 2002). In addition, there found a significant and positive relationship between fluorescence

intensity per unit carbon and $SUVA_{254}$ for DOM fractions (except for HA) ($R^2 = 0.96$, $p < 0.01$, Fig. 5). The significant inter-relationships may suggest that fluorescence and absorbance properties reflected the similar chemical characteristics. Previous study reported that DOM fluoresced because its molecule contained such structures as aromatic or conjugate chromophores and unsaturated aliphatic chains i.e., molecules with extensive π systems (Senesi, 1990). Similarly, absorbance is mostly attributed to the aromatic chromophores and unsaturated aliphatics (Leenheer and Croué, 2003; Weishaar *et al.*, 2003).

Fluorescence index ($f_{450/500}$) can be served as a simple

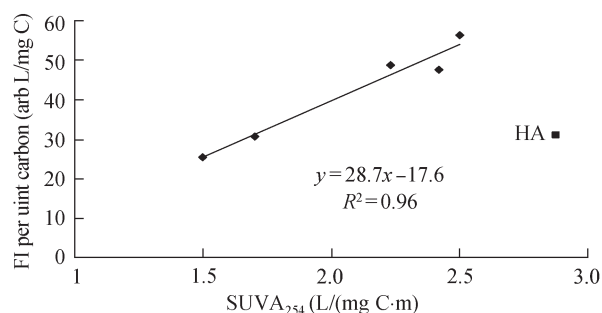


Fig. 5 Relationship between $SUVA_{254}$ and fluorescence intensity for Peak A of the 6 fractions isolated from Lake Hongfeng.

index to distinguish DOM derived from microbial and terrestrial sources (McKnight *et al.*, 2001), higher $f_{450/500}$ (ca. 1.9) may refer microbial source and lower $f_{450/500}$ (ca. 1.4) terrestrial source. The $f_{450/500}$ of 6 fractions ranged from 1.10 to 1.62 (Table 3), with hydrophobic fractions ranging from 1.10 to 1.22 and hydrophilic fractions from 1.42 to 1.62, respectively. Fluorescence index ($f_{450/500}$) indicates that hydrophobic fractions were predominantly derived from terrestrial sources and hydrophilic fractions were derived from microbial and terrestrial origins.

3 Conclusions

DOM in Lake Hongfeng was isolated into 6 fractions by XAD-8 and XAD-4 resins combined with the ionic exchange resin method. Fulvic acid was the predominant fraction. The 6 fractions were characterized by smaller molecular weight, low UV absorbance, and fulvic-like fluorescences, indicating that the ambient environment has an influence on their molecular and spectroscopic properties, photodegradation, and biological activity. Based on the strong inter-relationships among absorbance properties (E_2/E_3 , SUVA₂₈₀), molecular weight and fluorescence characteristics (patterns and intensity) for 6 fractions, this study shows the similarity and distinction in the composition, nature and origin of different fractions from the same DOM. The fractions with larger molecular weight were the relatively hydrophobic fractions, and related to both predominant fulvic-like and protein-like fluorescence, and the high SUVA₂₅₄. While the fractions with small molecular weight were the relatively hydrophilic fractions, and related to the fulvic-like fluorescence and the low SUVA₂₅₄.

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