Characterization of humic acids extracted from the sediments of the various rivers and lakes in China

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Abstract

The humic acids (HAs) isolated from the sediments of the various rivers, lakes, and reservoirs in China were studied using elemental analyzer, fourier transform infrared (FT-IR), and CP/MAS ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The results showed that the HAs were characterized by some common chemical and physicochemical properties, but they also pose some differences in the C-containing functional groups. The C/N, C/H, O/C, and O/H ratios differ widely for the various HAs, showing that the elemental composition of the HAs from the various sediments was different due to the different environmental conditions. All HAs show similar spectra with different intense absorbance in the region of 4000–2000 cm^{-1}, suggesting that they have very similar structures and functional groups. The absorbance region between 1900 and 400 cm^{-1} also showed similar spectra with different intense absorbance, different relative intensities, and some relevant differences for the various HAs. The total aromaticities for the six HAs varied from 23.1% to 41.8%. The differences in the elemental composition and functional groups for the various HAs were attributed to their different biogeochemical origins. The HAs in the sediments from Taihu Lake (one freshwater shallow lake with heavy eutrophication), the Pearl River (the tropical river), and the Liao River (located at the joint of the temperate zone and cold-temperate zone) showed different structural properties due to their different geographical and climate zones. These different properties for the six HAs were expected to affect the sorption, distributions, and fates of heavy metals and organic chemicals.

Key words: CP/MAS ^{13}C NMR spectroscopy; elemental analysis; fourier transform infrared spectroscopy; humic acids; surficial sediment; structural characteristics

Introduction

Humic substances are ubiquitous organic materials in the terrestrial and aquatic ecosystems, which have important influences on the sorption, distributions, transports, and fates of environmental pollutants (Xing, 2001; Chefetz et al., 2000; Chin et al., 1997). The predominant fractions of humic substances are humic acids (HAs), which are very active in interacting with organic and inorganic contaminants. These interactions are well known to depend on the chemical structures and compositions of humic macromolecules (Chiou et al., 1998).

The information on the compositions and functional groups of HAs is critical for understanding their reactivity with organic and inorganic contaminants. However, due to their complexity and heterogeneity, it is difficult to determine the HAs structures. The elemental analysis and the spectroscopic methods such as infrared, electron spin resonance, and fluorescence and nuclear magnetic resonance spectroscopy (NMR) have been used to investigate HAs compositions and structures. Especially, NMR is one of the most powerful tools. The most popular solid-state ^{13}C

cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR has been used for studying the structures of organic molecules (Chai et al., 2008; Senesi et al., 2003; Lu et al., 2000). Many studies mainly focus on the characterization of HAs from the soils (Xing et al., 2005; Gu et al., 2000; Quideau et al., 2000; Rivero et al., 1998; Tao et al., 1999; Barancikova et al., 1997) and natural organic matters in the surface waters from different watersheds (Wu et al., 2005; Alberts and Takacs, 2004, 1999; Frimmel and Abbt-Braun, 1999; Frimmel, 1998; Peuravuori and Pihlaja, 1997). It is relatively less for the studies of HAs from different sediments (Calace et al., 2004; Mendonca et al., 2004; Tremblay and Gagne, 2002; Fooken and Liebezeit, 2000; Sardessai and Wahiduliah, 1998; De Paolis and Kukkonen, 1997; Golebiowska et al., 1996; Garcia et al., 1994).

There are many rivers and lakes from south to north of China. The physicochemical characteristics of the organic matters in the surface waters and sediments of the rivers and lakes might be different due to their different origins and geographical and climate zones. The purposes of this study are: (1) to investigate the detailed chemical properties of HAs extracted from the different sediments of rivers and lakes; (2) to compare their similarities and differences

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in the compositions and structures; and (3) to probe into the probable reasons causing different compositions and structures.

1 Materials and methods

1.1 Sediment sampling

In this study, six surface sediments (four rivers, one lake, and one reservoir) were collected in June 2002. They represent different aquatic ecosystems (river, lake, and reservoir) and different regions in China. The Pearl River is China's third longest river (2,200 km), second largest by volume (after the Yangtze River), and located in South China. It flows into the South China Sea between Hong Kong and Macau. The sediment from the Pearl River was sampled in the reach flowing through Guangzhou City (23°06′30″N, 113°18′30″E). The Liuyang River is situated at Hunan Province of Central China. The sediment from the Liuyang River was sampled in the reach flowing through Changsha city (28°10′37″N, 113°04′46″E). The Yangtze River is the longest river in Asia and the third longest in the world. The river is about 6,300 km long and flows from its source in Qinghai Province, eastwards into the East China Sea at Shanghai. In the Yangtze River, the sediment was sampled in the reach of Wuhan City of Central China (30°19′51″N, 114°06′50″E). The Liao River originates from Bald-head Hill of the Qilaotu Mountains and flows into the Bo Sea near Panjin City, Liaoning Province, via Hebei, Inner Mongolia, Jilin, and Liaoning Provinces, with a distance of 1,345 km. The sediment in the Liao River was sampled in the reach flowing through Liaozhong city, Liaoning Province (41°30′04″N, 122°37′54″E). Taihu Lake is a largest lake in the Yangtze Delta plain, on the border of the Jiangsu and Zhejiang Provinces in East China. With an area of 2,250 km^2 and an average depth of 2 m, it is the third largest freshwater lake in China, after the Poyang and the Dongting Lake. In recent years, Taihu Lake has been plagued by pollution as a result of rapid economic growth in the surrounding regions. The sediment was collected at site Meiliangwan, north of Taihu lake (31°41′15″N, 120°18′75″E). The Huairou Reservoir, located in the northwest of Beijing, is a typical northern reservoir with less pollution (40°18′52″N, 116°36′52″E).

All sediments were sampled using Van Veen sampler. About 10 kg of samples were collected in each sampling area by mixing several samples collected from five nearby spots. Upon reaching the laboratory, samples were air dried, ground, sieved through a 1-mm sieve, and stored at room temperature. Sediment characteristics are shown in Table 1. The pH for sediment samples from the Pearl River, Yangze River, Liao River, and Huairou Reservoir was more than 7, whereas it was less than 7 for the sediments from the Liuyang River and Taihu Lake. The TOC contents for various HAs were in the range of 10.08–34.74 g/kg. The sediment from Taihu Lake contained the highest TOC, showing high contents of organic matter.

1.2 Extraction and purification of humic acids

Humic acid was isolated from the sediment samples according to conventional methods (Wen, 1984). In brief, humic substances were extracted using 0.1 mol/L NaOH under an atmosphere of N_2 at an extractant to soil ratio of 10:1 (*V/W*). The extracted humic substances were then separated into HA and fulvic acid (FA) fractions by acidifying the extract to pH 1–1.5 using 6 mol/L HCl. The precipitate HA and the supernatant FA were separated by centrifuging at 4,500 r/min for 30 min. The HA fraction was suspended in a solution of 0.1 mol/L HCl and 0.3 mol/L HF to remove mineral impurities and then dialyzed until elimination of Cl$^-$. The HA fractions were freeze dried for chemical analysis.

1.3 Characterization of humic acids

The elemental compositions of the isolated HA were measured using an automated elemental analyzer (Vario EL spectrometer, Elementar Co., Germany). The ash content was determined by heating HAs at 740°C for 4 h.

The fourier transform infrared (FT-IR) spectra were recorded in the transmission mode by a NEXUS 670X spectrophotometer (Nicolet Instrument Co., USA) using KBr pellet containing 1% of HA.

The ^{13}C NMR spectra were acquired on a Bruker AU-300 spectrometer (Bruker Co., Switzerland) using cross-polarization magicangle spinning (CPMAS) with total sideband suppression. The spectra (about 5,000 scans per sample) were integrated into the following chemical shift regions: alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm), phenolic C, carboxyl C (160–190 ppm), and carbonyl C (190–220 ppm), respectively. The relative intensity of these regions was determined by the integration of the corresponding peak areas (Gu *et al.*, 2000).

The aromaticity of the samples was calculated by expressing the level of aromatic C as the percentage of the sum of the contents of aliphatic C (0–110 ppm) and

Table 1 Physicochemical property of the sediments from different rivers and lakes

Sample	pH	TOC (g/kg)	CEC (cmol/kg)	Grain size distribution (% dw)		
				0.15–0.02 mm	0.02–0.002 mm	< 0.002 mm
Pearl River	7.41	19.45	15.50	34	36	30
Liuyang River	5.63	11.28	11.02	19	50	31
Yangtze River	7.39	10.08	13.69	42	32	26
Liao River	7.45	14.41	25.34	22	49	29
Taihu Lake	5.56	34.74	24.33	42	40	18
Huairou Reservoir	7.87	11.33	20.93	27	43	30

CEC: cation exchange capacity.

aromatic C (110–160 ppm) Eq.(1).

$$\text{Aromaticity} = C(\delta_{110-160})/C(\delta_{0-160}) \times 100\% \qquad (1)$$

where, δ (ppm) is chemical shift, *C* is the content of C-containing functional group. The aliphaticity of the samples was calculated by expressing aliphatic C as a percentage of the aliphatic (0–110 ppm) and aromatic C (110–160 ppm).

$$\text{Aliphaticity} = C(\delta_{0-110})/C(\delta_{0-160}) \times 100\% \qquad (2)$$

2 Results and discussion

2.1 Elemental analysis and functional group composition of humic acids

The elemental compositions, ash content, and atomic ratios of the six HAs from different sediment are shown in Table 2.

The C, H, N, and O contents of the six HAs varied in limited ranges, which were 43.7%–53.8% for C, 31.1%–37.1% for O, 4.1%–5.8% for H, and 3.5%–6.2% for N. Generally, these values are a little larger for C and O and a little smaller for H and N (Golebiowska *et al.*, 1996.). The ash content of HA ranged from 1.2% to 13%, with the smallest value for the HA from the Liao River and the largest value for the HA from the Liuyang River, which suggests relatively large mineral associations for the latter HA.

The atomic ratios of C/N, H/C, O/C, and O/H are often used to identify humic substances from different sources to monitor structural changes of humic substances in different environments and to elucidate structural formulae for humic substances (Steelink, 1985). The data in Table 2 show that the C/N, C/H, O/C, and O/H ratio differs widely for the various HAs. It shows that the HAs from different sediments pose different elemental compositions due to the different environmental condition (Lu *et al.*, 2000; Ishiwatari, 1985).

The C/N ratio of HAs ranged from 8.1 to 14. These values are similar to the data given by Filip *et al.* (1988) for sedimentary HAs and plant decay humic substances (7.9–16.2). The high C/N ratio and C/H ratio of HAs from the Liao River suggest a high stability and condensation degree and an extended humification degree of organic matter in the sediment (Lu *et al.*, 2000). The O/C ratio is believed to be an indicator of the carbohydrate and carboxylic acid contents of humic substances and can be used to compare humic substances from different depositional environments. The O/C ratio varies from 0.62 to 0.78 for various HAs. The HAs from the Liuyang River

and Pearl River have relatively high O/C ratio, showing that organic material with a relatively high O-alkyl and carboxylic acid composition (Belzile *et al.*, 1997). The O/C ratio of humic substances is also representative of the degree of humification (Ibarra and Juan, 1985). A decrease in O/C ratio suggests an increase in the degree of aromatic condensation of humic substances. A relatively higher O/C ratio for HAs in the Liuyang River and Pearl River suggests that these humic substances underwent a low degree of aromatic condensation and are considerably more aliphatic in nature than humic substances from other rivers (Lu *et al.*, 2000). By comparing the O/C ratio of humic substances from different sources in this study, the maturity of humic substances increases generally in the order: Liuyang River < the Pearl River < other rivers and lakes. The C/H ratio in Table 2 varied from 8.3 to 12, larger than one, reflecting that these HAs probably originate from vascular plant material rather than algal or bacterial organic matter (Gonzalez-Vila *et al.*, 1992; Bourbonniere and Meyers, 1978). The C/N ratio is considered to be an indicator of the sources of humic substances in natural systems. Nonvascular aquatic plants have high C/N, typically between 2.0 and 10, whereas vascular land plants, which contain cellulose, have C/N ratios of 20 and higher. The C/N ratio in this study was in the range of 8.1 to 14, relatively low, indicating that aquatic plants might be the dominant contributors to these humic substances (Meyers and Ishiwatari, 1993). Especially, the C/N ratio for HAs from Taihu Lake was low. It may be related to the freshwater shallow lakes with heavy eutrophication (Zhang *et al.*, 2008).

2.2 FT-IR spectroscopy of humic acids

The FT-IR spectra of the six HAs studied are shown in Fig.1. Their interpretation is based on the work of Ena and Katharina (2007), Hafidi *et al.* (2005), Senesi *et al.* (2003), Mansuy *et al.* (2001), Garcia *et al.* (1992), and Piccolo *et al.* (1992). The following are the main absorption bands: a broad band at 3100–3600 cm^{-1} (H-bonded OH and NH groups), a slight shoulder at 3000–3100 cm^{-1} (aromatic C–H stretching), a band at 2800–3000 cm^{-1} (aliphatic C–H stretching), a shoulder or a peak at 1710 cm^{-1} (C=O stretching of COOH and ketonic carbonyls), a peak or a shoulder at 1590–1700 cm^{-1} (aromatic C=C stretching, H-bonded C=O, NH$_2$ deformation), a peak or a shoulder at 1490–1590 cm^{-1} (NH$_2$ deformation of amides), a band at 1300–1500 cm^{-1} (aliphatic CH deformation), a strong band at 1660–1630 cm^{-1} (mainly attributed to the C=O stretching of amide groups, amide I band), an absorption at 1540–1510 cm^{-1} (assigned to N–H deformation and C=N

Table 2 Elemental contents, ash content, and atomic ratios of humic acids

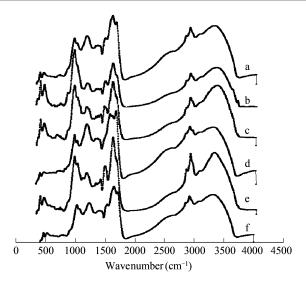| Sample	C (%)	H (%)	N (%)	O (%)	Ash (%)	C/N	C/H	O/C	O/H
Pearl River	47.9	4.7	3.5	35.7	8.2	14	10	0.75	7.6
Liuyang River	43.7	4.8	5.0	33.8	13	8.7	9.1	0.78	7.0
Yangtze River	47.5	4.9	5.0	31.1	12	9.5	10	0.66	6.4
Liao River	53.8	4.1	3.8	37.1	1.2	14	13	0.69	9.1
Taihu Lake	50.2	5.8	6.2	31.2	6.7	8.1	8.3	0.62	5.4
Huairou reservoir	51.7	4.6	4.9	34.6	4.2	11	11	0.67	7.5

Fig. 1 FT-IR spectra of the humic acids in the sediments from different rivers and likes. (a) Pearl River; (b) Liuyang River; (c) Yangtze River; (d) Liao River; (e) Taihu Lake; (f) Huairou reservoir.

stretching of amides, amide II band), and a peak at 1420 cm^{-1} (due to C=N stretching of primary amides, amide III band).

The results indicate that all sediment HAs exhibited generally similar IR spectra (Fig.1), suggesting that they have very similar structures and functional groups. In the region of 4000–2000 cm^{-1}, the six HAs show similar spectra with different intense absorbance. The absorption peaks in range of 3400–3300 cm^{-1} are due to the stretching of H-bounded OH groups of alcohols, phenols, and organic acids, as well as H-bounded N-H groups. All HAs show absorbance peak at 2925 cm^{-1} due to symmetric and asymmetric stretching vibrations of aliphatic C–H bonds in CH$_3$ and CH$_2$ groups (Santos and Duarte 1998), and the HA from Taihu Lake has the highest absorbance.

As shown in Fig.1, the absorbance region between 1900 and 400 cm^{-1} (the "fingerprint" region) also show similar spectra with different intense absorbance, different relative intensities, and some relevant differences for various HAs. The HAs from the Liao River and Huairou reservoir have the absorption peak around 1725 cm^{-1}, which was attributed to the C=O stretching vibration mainly caused by COOH groups but also to other carbonyl groups such as ketones and aldehydes (Santos and Duarte 1998; Peshel and Wildt 1988). Because the Liao River and Huairou Reservoir, located in North China, have long icebound time in winter, the reductive sediment environment is favorable for the form of carbonyl groups. All HAs show a peak

around 1650 cm^{-1}. The HAs from Taihu Lake show intense absorbanc peak, indicating the more aromatic structures or amides (Hafidi et al., 2005). The result corresponds with the C/N ratio due to freshwater shallow lakes with heavy eutrophication for Taihu Lake (Zhang et al., 2008). The absorbance centered at 1550 cm^{-1} appears only in the HAs from the Liuyang River, Yangtze River, Taihu Lake, and Huairou Reservoir. A band at this wavenumber has been attributed to amide groups and may indicate the presence of polypeptides-derived structures (Peshel and Wildt 1988). Additional bands for the HAs from the Pearl River, Liuyang River, Liao River, and Huairou Revervoir were also observed at 1260 cm^{-1}, which correspond to the C–O stretching of aryl ethers and phenols and C–O, O–H bending of carboxylic groups. The HA from the Liao River exhibited highest absorbance peak. Around 1030 cm^{-1}, all HAs show absorbance, which is attributed to the C–O stretching of polysaccharides. The HA from the Pearl River has high absorbance peak, whereas the absorbance peaks for other HAs are relatively low. In the range of 900–650 cm^{-1}, the HAs from the Pearl River shows some absorbance, which are attributed to the CH bending of the substituted aromatic groups. The spectra of HA from Pearl River show the same positions of bands as those spectra reported for HAs from sediments of tropical river (Garcia et al., 1994).

2.3 CPMAS ^{13}C NMR of humic acids

The CPMAS ^{13}C NMR spectra of the HAs samples and the C-containing functional group contents are presented in Fig.2 and Table 3, respectively. The spectral area integration (0–50 ppm, 50–110 ppm, 110–160 ppm, 160–190 ppm, 190–220 ppm) was carried out using program Win NMR software.

The CPMAS ^{13}C NMR spectrum of the six HAs all exhibited major peaks at 30 ppm (alkyl C), 55 ppm and 71 ppm (alkyl-O carbons), 130 ppm and 152 ppm (aromatic carbons), 173 ppm (carboxylic carbon), and 196 ppm (carbonyl carbon). But there are some differences in the C-containing functional group of various HAs due to different origins. The HA from Taihu Lake exhibited a high content of aliphatic compounds (76.9%) and had major peak at 30 ppm (alkyl carbon), 55, 71, and 105 ppm (alkyl-O carbons). It also exhibited the highest carboxyl carbon and carbonyl carbon among all HAs. The HA from Taihu Lake with lower C/H ratio is more aliphatic in nature (Table 2). The HAs from the Pearl River, Liao River, and Huairou Reservoir also had high peak at 30 ppm. The HAs from the Liuyang River and Yangtze River had relatively high

Table 3   Relative intensity of total area for the ^{13}C NMR signals of humic acids and the total aromaticity

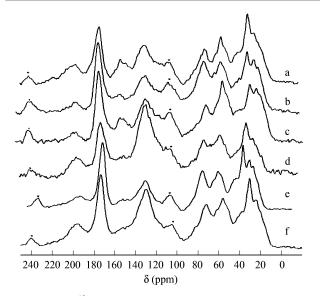|	Relative intensity of functional group (%)					Aromaticity (%)	Aliphaticity (%)
	Aliphatic 0–50 (ppm)	Carbohydrate 50–110 (ppm)	Aromatic 110–160 (ppm)	Carboxyl 160–190 (ppm)	Carbonyl 190–220 (ppm)		
Pearl River	29.9	22.2	24.9	13.9	9.0	32.4	67.6
Liuyang River	27.8	32.8	21.6	15.0	2.8	26.3	73.7
Yangtze River	28.4	28.8	25.2	15.0	2.6	30.5	69.5
Liao River	21.6	24.3	33.0	15.5	5.6	41.8	58.2
Taihu Lake	31.5	25.9	17.2	16.4	9.1	23.1	76.9
Huairou Reservoir	23.2	25.5	26.4	15.7	9.1	35.2	64.8

Fig. 2 CPMAS ^{13}C NMR spectra of the six humic acids. (a) Pearl River; (b) Liuyang River; (c) Yangtze River; (d) Liao River; (e) Taihu Lake; (f) Huairou Reservoir.

peak at 55 ppm. The NMR spectrum of the HA from the Liao River exhibited major peaks at 130 ppm, which is assigned mainly to unsubstituted and alkyl substituted aromatic carbon (Tao *et al.*, 1999). It is well in agreement with FT-IR data for the HAs from the Liao River.

The aliphaticity for the various HAs is higher than the aromaticity. The HA from Taihu Lake (76.9%) is characterized by the highest aliphaticity. The total aromaticity calculated for the six HAs was between 23.1% and 41.8%. Humic acid from the Liao River was characterized by the highest aromaticity (41.8%). The relative contents of carbonyl and carboxyl carbons for the six HAs are 2.6%–9.1% and 13.9%–16.4%, respectively. A decreasing area of oxygenated aliphatic C and an increasing area of aromatic C indicate an increasing stage of humification involved in microbial and chemical degradation (Lu *et al.*, 2000). Nuclear magnetic resonance data are well in agreement with elemental analysis data (Fig.3). The aromaticity show good positive correlation with C/N ($R > 0.05$), C/H ($R > 0.01$), O/H ($R > 0.05$), and negative correlation with H ($R > 0.05$). As mentioned above, Taihu Lake is one freshwater shallow lake with heavy pollution and eutrophication. Because of the input of a several fresh organic matter,
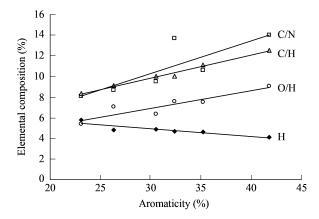
such as dead algae and organic pollutants, the humification in the sediment is low. However, the Liao River located in North China has long icebound period, the sediment is in the anaerobic environment, so the humification is high. Elemental analysis also shows a greater C/H ratio for the HA from the Liao River than the HAs from others, indicating a higher degree of aromaticity (Table 3).

3 Conclusions

The results of elemental analysis, FT-IR, and NMR, for HAs are basically in agreement with each other. The HAs from different rivers, lakes, and reservoirs generally exhibit some common chemical and physicochemical characteristics, but they pose some differences in the elemental compositions and C-containing functional groups of the various HAs due to different origins. Taihu Lake is the freshwater shallow lake with heavy pollution and eutrophication; thus, aquatic plants are the dominant contributors to the humic substances. The FT-IR spectrum for the HAs from the Pearl River is similar to those reported for the sediments of tropical rivers. The Liao River and Huairou Reservoir have long icebound time in winter; hence, the reductive sediment environment is favorable for the form of carbonyl groups. The different composition and functional group properties of the six HAs studied are expected to affect the sorption, distributions, and fates of sediment contaminants.

Fig. 3 Relationship of aromaticity and elemental composition.

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