

# Chemical and spectroscopic characterization of dissolved humic substances in a mangrove-fringed estuary in the eastern coast of Hainan Island, China\*

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**Abstract** Mangrove-derived dissolved organic matter (DOM) has an important effect on estuarine and coastal area on a large scale. In order to improve the understanding of origin, composition, and fate of DOM in mangrove-fringed estuarine and coastal areas, dissolved humic substances (DHS) were isolated from one mangrove pore-water sample and one near-shore seawater sample downstream the mangrove pore-water site in the eastern coast of Hainan Island, South China. Fulvic acids, humic acids and XAD-4 fractions were obtained from the two water samples by using a two-column array of XAD-8 and XAD-4 resins. Chemical and spectroscopic methods were used to analyze the features of these DHS. Compared to the mangrove pore-water DHS, the near-shore seawater DHS were found rich in <sup>13</sup>C with lower C/N ratios and more aliphatic compounds and carbohydrates, but less aromatic structures and carboxyl groups. As for the three fractions of the two DHS, XAD-4 fractions contain more aliphatics, carbohydrates, carboxyl groups, and enrich in <sup>13</sup>C with respect to both fulvic and humic acids. Photo-oxidation transformation and contribution from marine-derived DOM were considered as the main reasons resulted in the difference in compositional features for these DHS in this study.

**Keyword:** mangrove; fulvic acid; humic acid; XAD-4 fraction; FTIR; NMR

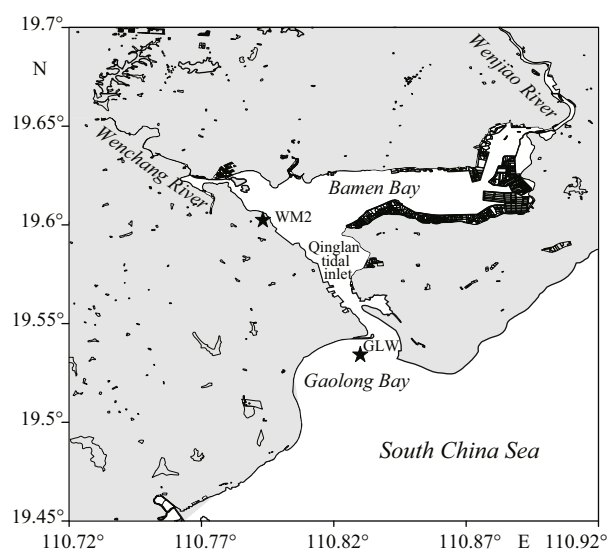
## 1 INTRODUCTION

Mangrove forests are highly productive and biodiverse ecosystems that form the fringe areas of 60%–75% of tropical coasts (Clough, 1998). The potential impact of mangroves on the dynamics of carbon and nutrients in tropical coastal waters has been a subject of intense debate in recent decades. The hypothesis of “outwelling”, first proposed by Odum (1968) and Odum and Heald (1972), suggests that a large fraction of organic matter produced by mangroves is exported to the coastal ocean, where it forms the basis of the food chain, supporting secondary heterotrophic production, and then coastal fisheries. More recently, Dittmar et al. (2001, 2006) and Tremblay et al. (2007) also suggested that the outwelling of mangrove-derived dissolved organic matter (DOM) that occurs through tidal flushing of

pore-water influenced the estuarine and coastal area on a large scale. Among the contributions of mangrove, terrestrial and marine-derived organic matter to DOM pool in a mangrove estuary in North Brazil, it was found that mangrove-derived DOM contributed much more than terrigenous input (Dittmar et al., 2001). On a global level, although mangroves cover only 0.1% of the continents’ surface, they can account more than 10% of the terrestrial-derived refractory DOM transported to the oceans (Dittmar et al., 2006). However, some authors also mentioned that the output of mangrove-derived

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**Fig.1** The sampling locations of the two water samples

organic matter is largely hydrodynamics and geomorphology-dependent, especially for particulate organic matter and sedimentary organic matter (SOM) (Lee, 1995; Xu et al., 2006).

DOM existed in estuarine and coastal water is involved in many dynamic processes. In addition to its importance as a major reservoir of bioactive elements, DOM also affects photochemical reactions. DOM forms complexes with a wide variety of trace elements and organic contaminants in water column, thereby affecting their chemical behavior and biological availability (Emerson and Hedges, 2008; Guo et al., 2011). DOM is heterogeneous mixtures, and only a small fraction of DOM including carbohydrates, lipids, proteins and amino acids has been chemically characterized (Zhang et al., 2009). Properties of a large fraction of DOM are still poorly understood because the extremely complicated structural composition and difficulty to obtain a representative fraction of the DOM pool (Simjouw et al., 2005). Dissolved humic substances (DHS) that operationally defined by International Humic Substances Society (IHSS) (<http://www.humicsubstances.org>) by using XAD-8 resin for extraction are hydrophobic organic acid fractions of DOM pool (Leenheer, 1981; Aiken et al., 1992). DHS usually account for ~56% of DOM in freshwater and 10%–30% of DOM in marine waters (Perdue and Ritchie, 2005; Mopper et al., 2007; Perdue and Benner, 2009). Moreover, by employing XAD-4 resin in the series after XAD-8 to extract the hydrophilic organic acids, which constitute so-called XAD-4

fraction, can increase the DOM recovery by 7%–25% (Aiken et al., 1992) depending on the nature of different aquatic environments.

This work is a part of a comprehensive study on DHS and sedimentary humic substances (SHS) (Zhang et al., 2011) in a mangrove-fringed area in Qinglan Lagoon in the eastern coast of Hainan Island, China. In this study, DHS including of XAD-4 fraction were isolated from one mangrove pore-water sample and one near-shore seawater sample downstream the mangrove pore-water site. Elemental analysis, Fourier transform infrared spectroscopy (FTIR) analysis, and cross polarization magic angle spinning (CPMAS)-<sup>13</sup>C nuclear magnetic resonance (NMR) were used to characterize these DHS with the aim of furthering our knowledge of the origin, composition, and fate of mangrove-derived DOM in mangrove-fringed estuarine and coastal areas.

## 2 MATERIAL AND METHOD

### 2.1 Sampling sites

The sampling region is located in Wenchang estuary of Qinglan Lagoon, which is in the eastern coast of Hainan Island, China. Qinglan Lagoon is famous for its abundant mangroves, coral reef and fringing reef (Liu et al., 2011), and composed by the internal Bamen Bay and the outer Gaolong Bay (Wang et al., 2006). The Bamen Bay connects to Gaolong Bay through Qinglan tidal inlet and joins to the coastal of South China Sea. The mangrove reserves were established from Wenchang River to the northern part of Bamen Bay and Qinglan tidal inlet (Liu et al., 2011). The dominant mangrove species present are *Rhizophora*, *Avicennia*, *Lumnitzera*, *Ceriops*, *Kandelia*, *Bruguiera* and *Sonneratia* (Mao et al., 2006).

The sampling sites are shown in Fig.1. WM2 site (110.793 2°E, 19.602 6°N) was located in the Wenchang estuary within the mangrove reserves, where ~400 L pore-water (salinity 20; pH 8.31) was collected. The method to acquire pore water was according to the procedures provided by Koch et al. (2005) and Dittmar et al. (2006). Briefly, 10 to 12 holes of 50–80 cm in diameter and 80–100 cm in depth were carefully dug into the mangrove sediment in a square field of approximately 20 m×20 m. After the holes have filled with water for 2–3 h, water samples were collected in acid-cleaned carboys. Approximately 30–40 L of pore water was collected from each hole. The other one GLW site (110.830 0°E,

19.531 4°N) is situated in the near-shore of Gaolong Bay which is in the downstream of the mangrove pore-water site, where ~1 000 L seawater (salinity 32; pH 8.3) was collected in acid-cleaned carboys. After collection, water samples were kept in dark and transported to land laboratory immediately. The well-mixed water samples from each carboy of the two sampling sites were used to further salinity and pH measurement as well as DHS extraction.

## 2.2 Extraction and purification of DHS

DHS were isolated by the methods provided by Aiken (1985) and Aiken et al. (1992). Briefly, water samples were filtered through 0.45  $\mu\text{m}$  pore-size membrane filters, then acidified to pH 2.0 with HCl and passed through an XAD-8 and XAD-4 resin tandem. Each column was separately back-eluted with 0.1 mol/L NaOH. The eluate from XAD-8 resin was re-concentrated with a smaller XAD-8 column to make the eluated DOC to contain more than 500 mg C/L. Acidified the eluate to pH 1.0 with HCl to produce humic acids (HAs) precipitation. Then the HAs were separated from fulvic acids (FAs) by centrifugation. The HAs were washed with water until the wash is negative to the  $\text{AgNO}_3$  test for chloride. Sufficient 0.1 mol/L NaOH was added to dissolve HAs and the HAs were acidified by passing through an  $\text{H}^+$  saturated cation exchange resin. FAs were re-adsorbed on XAD-8 resin and washed with one void volume of water to remove salt. The alkaline eluate was passed through  $\text{H}^+$ -saturated cation exchange resin. The eluate from XAD-4 resin was purified and concentrated by being adsorbed on a smaller XAD-4 column; the NaOH eluate was then passed through an  $\text{H}^+$ -saturated cation exchange resin. The subsamples of FAs, HAs and XAD-4 fractions were freeze-dried for further analysis.

## 2.3 Chemical and spectroscopic characterization of DHS

### 2.3.1 C/N ratios and stable isotopic composition

Elemental compositions (C and N) of FAs, HAs and XAD-4 fractions were determined by using a Vario EL 3 Elemental Analyzer (Elementar, Germany). Carbon ( $^{13}\text{C}$ ) stable isotope was measured on a Delta plus XP isotope ratio mass spectrometer (Thermo Finnigan, USA). Carbon isotopic values were reported in the standard delta notation using standards of Pee Dee Belemnite ( $^{13}\text{C}/^{12}\text{C}$ , PDB) according to the

following equation:

$$\delta^{13}\text{C value} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1\,000, \quad (1)$$

where  $R$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$ .

### 2.3.2 Fourier transform infrared spectroscopy (FTIR) analysis

Each sample was prepared as KBr discs where a 1.0 mg sample was mixed with 100 mg KBr and then ground and homogenized to reduce light scattering. FTIR spectra were obtained by collecting 200 scans with Nicolet 8700 USB FTIR spectrometer. Spectra were acquired from 4 000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  resolution. Omnic 8.1 spectroscopy software (Thermo Fisher Scientific Inc.) was used to analysis these spectra. Second-derivative spectra were obtained with a 5-points Norris derivative method for further comparison of each sample.

### 2.3.3 Solid-state $^{13}\text{C}$ nuclear magnetic resonance (NMR)

Solid-state  $^{13}\text{C}$  NMR spectra were acquired using a Bruker Advance DSX 300 MHz spectrometer equipped with a 4-mm magic angle spinning (MAS) probe. Each of FAs, HAs and XAD-4 fractions was packed into a zirconia rotor and spun at 8 kHz during data acquisition (Cook and Langford, 1998). Spectra were acquired using a ramp-cross-polarization (Ramp-CP) pulse sequence with composite pulse TPPM15 proton decoupling and magic angle spinning (MAS). The contact time and delay time were 1.5 ms and 2 s, respectively (Sihombing et al., 1996). The  $^{13}\text{C}$  chemical shifts were externally referenced to the glycine resonance at 176 ppm.

## 3 RESULT

Although the carbon recovery of the two water samples is not determined in our work, the typical yield for DOM by using a two-column array of XAD-8 and XAD-4 resins in series was between approximately 60% for freshwater and 30% for seawater (Perdue and Ritchie, 2005; Mopper et al., 2007; Perdue and Benner, 2009). The mass proportion of each fraction in the two DHS samples was different. The freeze-dried XAD-4 fraction, FA, and HA in a mass ratio was 2.5:4:1 for GLW DHS and 1:5.75:2 for WM2 DHS, respectively. The content of FAs and HAs decreased but the XAD-4 fractions increased among the DHS isolated from mangrove pore-water to near-shore seawater samples.

**Table 1 Elemental compositions and percentage distribution of the different types of carbon for the different fractions of DHS in the two water samples**

Sample	C/N (atomic)	$\delta^{13}\text{C}$	Aliphatic C 0–45 ppm	Methoxyl C 45–65 ppm	HCOH 65–90 ppm	C-O-C 90–115 ppm	C=C/Ar-C 115–145 ppm	Phenolic C 145–160 ppm	Carboxyl C 160–190 ppm	Carbonyl C 190–20 ppm
GLW XAD-4	16.42	-22.4±0.2	54.92		15.99	6.12	3.97	0.48	16.71	1.81
GLW FA	24.08	-23.5±0.2	54.24		13.55	4.93	9.48	1.15	13.72	2.93
GLW HA	12.25	-23.8±0.1	39.38	15.04	13.99	5.61	8.43	1.82	13.19	2.54
WM2 XAD-4	19.65	-25.2±0.1	48.26		14.59	6.16	7.59	2.00	17.97	3.43
WM2 FA	26.48	-26.1±0.2	46.22		10.85	5.81	15.96	4.46	14.85	1.85
WM2 HA	12.55	-26.1±0.1	31.88	15.96	12.86	5.44	11.93	4.36	14.24	3.33

### 3.1 C/N ratios and stable carbon isotopic composition

Table 1 exhibits the C/N ratios and  $\delta^{13}\text{C}$  values for each fraction of DHS in the two sampling sites. The combination of elemental and isotopic compositions is often used to distinguish between marine and terrestrial plant sources of natural organic matter (Meyers, 1994; Hedges et al., 1997). Marine algae and plankton typically have lower atomic C/N (5–9) and enrich in  $^{13}\text{C}$  ( $\delta^{13}\text{C} \approx -22$  to  $-19$ ). In contrast, terrestrial organic matters usually have higher atomic C/N ratios range from 15 to 30. The land plants which using  $\text{C}_3$  Calvin pathway to incorporate carbon into organic matter have an average  $\delta^{13}\text{C}$  value of  $\sim -27$ . By comparison of the two parameters in the two sites, WM2 DHS show typically  $\text{C}_3$  terrestrial organic matter signature, while GLW DHS enrich in  $^{13}\text{C}$  and have slightly lower C/N ratios than WM2 DHS, suggesting that certain potential contribution from marine source and/or some modifications of the mangrove-derived DHS in GLW site. It should be noted that, the C/N ratios can only be used as a reference value for DHS samples, because solid-phase extraction (including XAD resins and  $\text{C}_{18}$  adsorbents) usually has a higher selectivity for N-poor compounds as organic nitrogen is often charged and polar and therefore is not easy retained by nonionic XAD resins or apolar  $\text{C}_{18}$  adsorbents (Leenheer, 1981; Koch et al., 2005; Perdue and Benner, 2009). Moreover, the two DHS exhibit similar change trend with respect to C/N ratios and stable carbon isotopes. The C/N ratios are the highest for FAs and the lowest for HAs with the XAD-4 fractions in the middle. As for  $\delta^{13}\text{C}$  values, FAs slightly enrich in  $^{13}\text{C}$  relative to HAs, while the XAD-4 fractions are approximately one unit higher than both FAs and HAs, which give the XAD-4 fractions more signatures of marine source.

### 3.2 FTIR spectroscopy

The FTIR spectra of each examined DHS are shown in Fig. 2a. In general, those spectra are similar to another in the position of principal absorptions due to the overlapping of functional groups. Figure 2b shows the second derivative spectrum of each sample in the range of 2 000–400  $\text{cm}^{-1}$ . The second derivative allows observation of a greater number of bands because of the increase in spectral resolution.

All the spectra are characterized by a strong band at 3 600–3 200  $\text{cm}^{-1}$ , which attribute to the intra-molecular and inter-molecular hydrogen-bonded OH groups in phenol, carbohydrate and carboxylic acids compounds, as well as N-H stretching in amines and amides (Stevenson, 1994; Abdulla et al., 2010; Tremblay and Alaoui, 2011; Landry and Tremblay, 2012). The band around 2 970–2 930  $\text{cm}^{-1}$ , 2 870–2 850  $\text{cm}^{-1}$  and 1 390  $\text{cm}^{-1}$  are ascribed to the stretching of aliphatic  $\text{CH}_3$  and  $\text{CH}_2$ , respectively (Abdulla et al., 2010; Landry and Tremblay, 2012). These peak intensities are apparent decreasing, in the order, from HAs to FAs and XAD-4 fractions, indicating HAs contain more aliphatic structure than the other two fractions. In addition, DHS in GLW site exhibit more prominent signals on these bands than DHS in WM2 site, which suggest the near-shore DHS contain more aliphatic compounds than the mangrove pore-water DHS.

The adsorption around 1 720  $\text{cm}^{-1}$  and 1 220  $\text{cm}^{-1}$ , are assigned to C=O stretching as well as C-O stretching and O-H deformation of the protonated carboxylic acid functional group (Stevenson, 1994; Landry and Tremblay, 2012), which is very distinct for FAs and XAD-4 fractions but only appears as a shoulder in HAs. This phenomenon indicates that HAs contain less carboxyl groups than FAs and XAD-4 fractions. The band at 1 400  $\text{cm}^{-1}$ , which attribute to de-protonated symmetric stretching of carboxyl



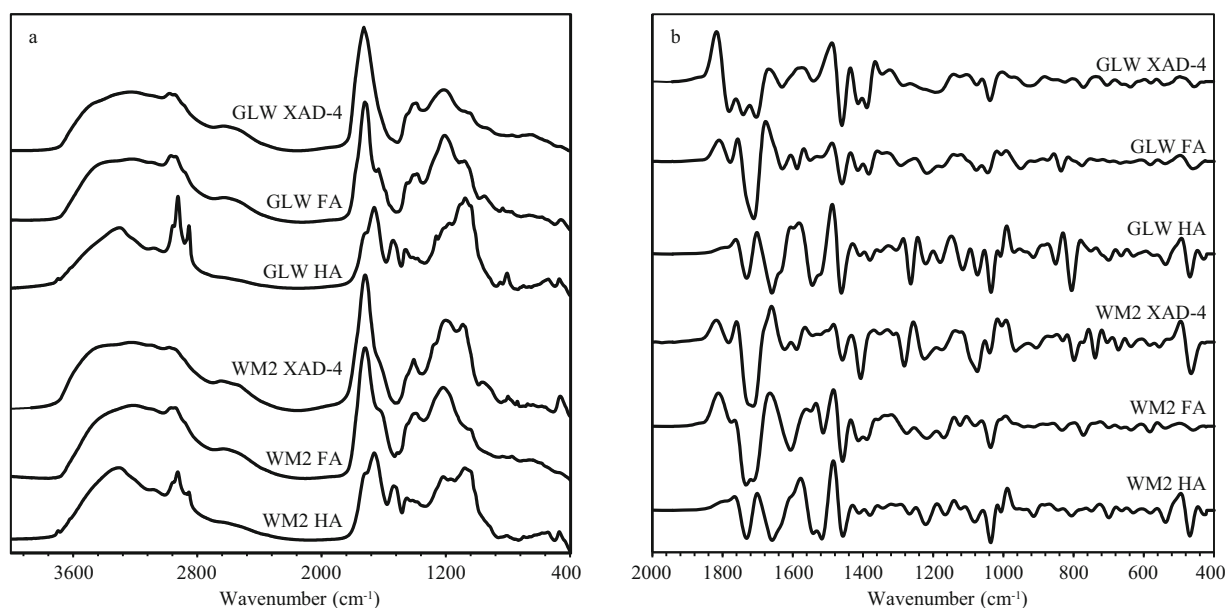


Fig.2 The FTIR spectra of the different fractions for the two DHS (a), and their corresponding second derivative spectra (2 000–400  $\text{cm}^{-1}$ ) (b)

groups, is also apparent in FAs and XAD-4 fractions, while is only presented as a shoulder in HAs for both samples can further reinforce the point that HAs contain less acidic carboxyl groups. Comparing DHS in the two sites, these peak intensities are stronger for WM2 DHS than that of GLW DHS, which imply that WM2 DHS may contain more carboxyl groups.

The band around  $1\,650\text{ cm}^{-1}$ , which attribute to C=O stretching in Amide I (Abdulla et al., 2010), is very prominent for HAs, while only shows as a shoulder in FAs and XAD-4 fractions. In the second derivative spectra of the samples, the band at  $1\,540\text{ cm}^{-1}$ , which is attributed to the vibration of N-H and C-N in amide II (Abdulla et al., 2010) is also following the same trend. Both evidences indicate HAs contain more nitrogen-containing compounds than the other two fractions, which is consistent with the C/N ratios for each fraction.

The bands at  $1\,515\text{ cm}^{-1}$  in the second derivative of FTIR spectra can be attributed to the C-O asymmetric stretching of phenol compounds and the vibration of aromatic ring (Esteves et al., 2009; Abdulla et al., 2010). As shown in Fig.2b, all of samples exhibit signals around this band, but the intensity of this band decreased from WM2 DHS to GLW DHS, which demonstrates that WM2 DHS contain more aromatic compounds than GLW DHS.

The band between  $1\,400\text{--}1\,260\text{ cm}^{-1}$  and  $1\,200\text{--}1\,000\text{ cm}^{-1}$  are attributed to the vibration of C-O bands in carbohydrate (Abdulla et al., 2010; Landry and

Tremblay, 2012), and the intensities of these bands for GLW DHS are slightly higher than WM2 DHS. As for the XAD-4 fractions, those band intensities are relatively prominent in comparison with FAs and HAs in the two DHS samples.

### 3.3 CP/MAS $^{13}\text{C}$ NMR spectroscopy

The  $^{13}\text{C}$  NMR spectra of the two DHS samples are similar with respect to the relative magnitudes of the major resonances with the exception of HAs in both sites have obvious absorptions around 45–65 ppm. The spectra, as well as the percentage of the total integrated peak in each region of the spectra, for all the FAs, HAs and XAD-4 fractions are shown in Fig.3 and Table 1, respectively. In general, those spectra are typically broad and poorly resolved bands due to extremely complex and non-repeating subunit structure of DHS (Stuermer and Payne, 1976). All the spectra could be divided into approximately four chemical-shift regions: (a) 0–65 ppm is attributed to unsubstituted alkyl carbons; (b) 65–115 ppm is assigned to carbon singly bonded to oxygen (O-alkyl C); (c) 115–160 ppm is ascribed to aromatic carbon, and (d) 160–220 ppm is attributed to carbonyl carbon (Hedges et al., 1992; Preston and Newman, 1995; Esteves et al., 2009).

As shown by Fig.3, the most noticeable feature in these spectra is the strong and broad aliphatic carbon signals for all fractions of DHS, which accounts for 46%–55% of the total carbon. The high percentage of

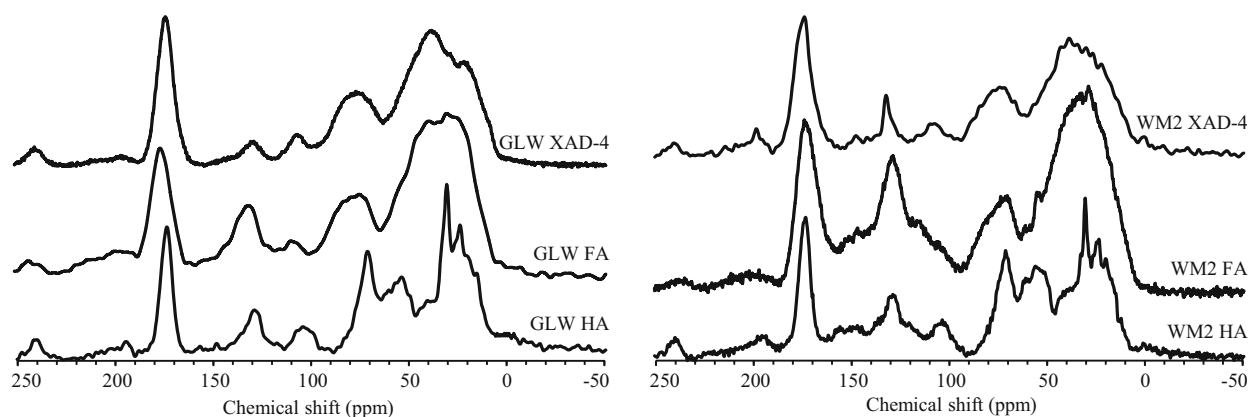


Fig.3 The CP/MAS  $^{13}\text{C}$ -NMR spectra of the different fractions for the two DHS isolated from the two water samples

aliphatic carbon is in agreement with the published data for DHS in marine environments (Wilson et al., 1983; Hedges et al., 1992; Esteves et al., 2009). The percentage of aliphatic carbon for GLW DHS is higher than that of WM2 DHS, which is consistent with the FTIR results. The central peak at 30 ppm can be attributed to the methylene carbons in long-chain aliphatic structures (Hatcher et al., 1980a, b). This band is very clear for HAs, but only shows as shoulders for FAs and XAD-4 fractions for the two DHS, which may indicate HAs contain more long-chain aliphatic structures than FAs and XAD-4 fractions in both sites. The peak at 56 ppm is assigned to methoxyl carbons that are characteristic of syringyl and guaiacyl groups in lignin (Esteves et al., 2009). Moreover, the vibration of C-N functional group (including N-alkyl and HNCH) is also found around 53 ppm (Mao et al., 2011). This peak is very prominent in HAs for both sites, which may suggest that HAs contain more lignin or lignin derived structural units, and/or contain more nitrogen-containing compounds, which is in agreement with the lower C/N ratios and FTIR results of HAs for the studied samples.

The O-alkyl C region can be subdivided into 65–90 ppm region, which is attributed to carbon singly bonded to oxygen, and 90–115 ppm region which is attributed to anomeric carbon (carbon singly bonded to two oxygens) (Esteves et al., 2009; Abdulla et al., 2010). All spectra exhibit resonance in the O-alkyl C region, being the second most prominent one in terms of area: 17%–22%. As shown in Table 1, the integration values of oxygenated aliphatic carbon in GLW site are slightly higher than that in WM2 site. By comparison of the three fractions in the same site, XAD-4 fractions contain more O-alkyl carbon (carbohydrates) than FAs and HAs.

Two fine structures can be defined in the aromatic chemical shift region. The region between 115–145 ppm can be assigned to the olefinic carbons and alkylbenzene ring carbons in which the ring is not substituted by a strong electron donating substituent. The region between 145–160 ppm can be assigned to phenolic carbon (Hatcher et al., 1980a, b). Integration of the aromatic carbon decreased from WM2 to GLW indicating mangrove pore-water DHS contains more aromatic and olefinic compounds than the near shore seawater DHS. Comparing the three fractions in the same water samples, FAs and HAs contain more aromatic and olefinic structures than the XAD-4 fractions.

In the carbonyl C region, the region of 160–190 ppm can be assigned to carboxyl, amide, and ester carbons (Hatcher et al., 1981). Ritchie and Perdue (2008) pointed out ~70% of the carbon in this region are carboxyl-like carbon. The region from 190–220 ppm can be assigned to aldehyde and ketonic carbons (Hedges et al., 1992; Preston and Newman, 1995). The content of carboxyl carbon in WM2 is slightly higher than that in GLW. As for the three fractions in the same sample, the XAD-4 fractions contain much more carboxyl groups than FAs and HAs in both sites, which is consistent with the finding by Esteves et al. (2009), who suggested that XAD-4 fractions are highly oxidized components compare to their FAs and HAs counterparts. Carboxyl groups in DHS are responsible for metal cations binding in aquatic systems (Tipping, 2002). The higher content of carboxyl groups represents more cation binding sites for these samples and fractions.

#### 4 DISCUSSION

According to the highly non-conservative behavior

of dissolved organic carbon in Wenchang estuary, mangrove-derived DOM was suggested having significant contributions to DOM pool in the estuarine area (Tian et al., 2010). In this study, DHS were isolated from a mangrove pore-water sample and a near-shore seawater sample in Wenchang Estuarine area, and three fractions were obtained for each DHS. Through chemical and spectroscopic characterization, different properties were presented for different fractions of each DHS and for DHS from different sites. Comparing DHS isolated from the two sites, WM2 DHS contain more aromatic compounds and deplete in  $^{13}\text{C}$  with respect to GLW DHS which contain more carbohydrates and enrich in  $^{13}\text{C}$ . Moreover, GLW DHS also contain more aliphatic compounds but less carboxyl groups than WM2 DHS.

Some reasons may contribute to these differences of DHS isolated from the two sites. Photo-oxidation and microbial decomposition are two major processes regulating natural organic matter transformation in the global carbon cycle (Wang et al., 2009; Feng et al., 2011). Carbohydrates in DOM are isotopically heavier than lignin due to relatively more  $^{13}\text{C}$  is incorporated into carbohydrates during biosynthesis (Benner et al., 1987). Microorganisms can utilize carbohydrates more easily than lignin, which give the remaining DOM more depleted  $\delta^{13}\text{C}$  values. However, lignin is more susceptible to photo-oxidation than carbohydrates, which can result in a shift to more enriched  $\delta^{13}\text{C}$  values in the remaining DOM (Opsahl and Benner, 1998; Opsahl and Zepp, 2001). Feng et al. (2011) reported that photo-oxidation can also increase the solubility of terrigenous organic matter by reducing the macromolecular species and by increasing lignin oxidation. In the present work, the difference of DHS between the two sites may partly attribute to the photo-oxidation as DHS have large complex macromolecular structures that are relatively resistant to direct microbial degradation (Thurman, 1985). Through photo-oxidation, lignin in initial mangrove pore-water DHS was remineralized to dissolved inorganic carbon, which may result in the enrichment of both  $^{13}\text{C}$  and carbohydrate in the remaining mangrove DHS when they reach the coastal area. In addition, Dittmar et al. (2006) reported that photo-degraded mangrove DOM contain more aliphatic compounds than the initial mangrove DOM. Tremblay et al. (2007) traced the changes of DOM as it moves from mangrove pore-waters into a Brazilian estuary by ultrahigh resolution mass spectrometry at molecular level and found that lower molecular

weights observed in the estuary DOM due to the loss of high molecular weight, highly unsaturated and/or aromatic components. They suggested that photo-degradation maybe one mechanism that removes these high-molecular-weight components once mangrove-derived DOM reaches the estuary. In addition, Gonsior et al. (2009) also suggested, photo-oxidation can transform unsaturated/aromatic compounds into much more saturated molecules. On the other hand, as for the three fractions of DHS in the same site, the XAD-4 fraction probably can be assigned to the product of photo-oxidation as this fraction contain more aliphatics, carbohydrates and carboxyl groups, as well as enrich in  $^{13}\text{C}$  but deplete in aromatics. Esteves et al. (2007) gave two hypotheses for the formation mechanism of XAD-4 fractions. One is assuming the XAD-4 fractions consist of more resistant and older organic matter among DHS, another assumption is XAD-4 fractions are younger organic matter than FAs and HAs. In our study, the content of XAD-4 fractions increased with the FAs and HAs decreased from mangrove pore-water to near shore seawater sample, which is consistent with the assumption that the near shore seawater DHS are partly the photo-oxidized product of the mangrove pore-water DHS. More FAs and HAs were converted into XAD-4 fractions through photo-oxidation reaction, which may give the evidence that the XAD-4 fractions in our studied samples constitute the older fraction among DHS.

Another possible reason of resulting in the difference between the two DHS may stem from the contribution from marine source. Seawater and freshwater DHS are distinctly different in a variety of compositional features. Marine humic substances are usually characterized by "highly branched, aliphatic carbon skeletons substitute primarily by carboxyl and singly bonded oxygen functional groups" (Hedges et al., 1992) and enriched in nitrogen and  $^{13}\text{C}$  (Meyers, 1994; Hedges et al., 1997). The slightly enriched in  $^{13}\text{C}$  and nitrogen-containing compounds, as well as the higher percentage of aliphatic carbon and carbohydrates for GLW DHS (as shown in Table 1) may also attribute to some contribution from marine source in this site. On the whole, the resulting chemical and spectroscopic properties of the DHS in the near-shore seawater should be attributed to the combined reasons, that is, the significant mixing of photo-degraded mangrove DHS and marine DHS, because either explanation cannot illustrate the whole situation.

Moreover, by comparison of the  $\delta^{13}\text{C}$  values and atomic C/N ratios of mangrove pore-water DHS with those of mangrove surface SHS that sampled from the same area as the mangrove pore-water sample in our previous study (Zhang et al., 2011), it can be found that both  $\delta^{13}\text{C}$  values (-26.1–-25.2 for DHS vs. -26.4–-25.5 for SHS) and C/N values (12.55–26.48 for DHS vs. 13.09–51.08 for SHS) are in a similar range. This phenomenon may indicate that both SOM and DOM in mangrove area are mainly from a similar source, probably from autochthonous mangrove detritus. The sedimentary rate in this area is found around 2.7 mm/a (Bao et al., 2012); therefore, it can be concluded that in our study areas, mangrove forests as highly productive ecosystems, can continuously import organic matter to sediments and pore-waters.

## 5 CONCLUSION

In this study, chemical and spectroscopic properties of DHS that isolated from one mangrove pore-water sample and one near-shore seawater sample downstream the mangrove-fringed estuary in the eastern coast of Hainan Island were analyzed by elemental composition, FTIR, and  $^{13}\text{C}$  NMR analysis. Different properties were shown for different fractions of each DHS and for DHS from different sites. By comparison of DHS in the two sampling sites, near-shore seawater DHS enrich in  $^{13}\text{C}$  and contain more aliphatic compounds, carbohydrates, but less aromatic structures and carboxyl groups than mangrove pore-water DHS. Moreover, as for the three fractions of DHS in the two sites, the XAD-4 fractions enrich in  $^{13}\text{C}$  and contain more carbohydrates and carboxyl groups, but less aromatic compounds with respect to other two counterparts. The significant mixing of photo-degraded mangrove DHS and the marine DHS were considered to the main mechanisms results in those discrepancies of the DHS.

Combined with the  $\delta^{13}\text{C}$  values and C/N ratios of SHS in our previous study, it can be found that both SOM and DOM in mangrove area are mainly from autochthonous mangrove detritus. Mangrove forests as highly productive ecosystems, continuously import organic matter to sediments and pore-waters, and the outwelling of mangrove-derived DOM can contribute to the near-shore area in a large scale, although some of the chemical and spectroscopic features of mangrove-derived DOM has been altered by photo-oxidation processes as it reaches the near shore coasts.

## References

- Abdulla H A N, Minor E C, Dias R F, Hatcher P G. 2010. Changes in the compound classes of dissolved organic matter along an estuarine transect: A study using FTIR and  $^{13}\text{C}$  NMR. *Geochim. Cosmochim. Acta*, **74**: 3 815-3 838.
- Aiken G R. 1985. Isolation and concentration techniques for aquatic humic substances. In: Aiken G R, McKnight D M, Wershaw R L, MacCarthy P eds. *Humic Substances in Soil, Sediment, and Water-Geochemistry, Isolation, and Characterization*. John Wiley and Sons, New York. p.7-38.
- Aiken G R, McKnight D M, Thorn K A, Thurman E M. 1992. Isolation of hydrophilic organic acids from water using nonionic macroporous resins. *Org. Geochem.*, **18**: 567-573.
- Bao H, Wu Y, Unger D, Du J, Herbeck L S, Zhang J. 2012. Impact of the conversion of mangroves into aquaculture ponds on the sedimentary organic matter composition in a tidal flat estuary (Hainan Island, China). *Cont. Shelf Res.*, <http://dx.doi.org/10.1016/j.csr.2012.06.016>.
- Benner R, Fogel M L, Sprague E K, Hodson R E. 1987. Depletion of  $^{13}\text{C}$  in lignin and its implications for stable carbon isotope studies. *Nature*, **329**: 708-710.
- Cook R L, Langford C H. 1998. Structural characterization of a fulvic acid and a humic acid using solid-state Ramp-CP-MAS  $^{13}\text{C}$  nuclear magnetic resonance. *Environ. Sci. Technol.*, **32**: 719-725.
- Clough B. 1998. Mangrove forest productivity and biomass accumulation in Hinchinbrook Channel, Australia. *Mangroves and Salt Marshes*, **2**(4): 191-198.
- Dittmar T, Hertkorn N, Kattner G, Lara R J. 2006. Mangroves, a major source of dissolved organic carbon to the oceans. *Global Biogeochem. Cycles*, **20**: GB1012.
- Dittmar T, Lara R J, Kattner G. 2001. River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Mar. Chem.*, **73**: 253-271.
- Emerson S R, Hedges J I. 2008. *Chemical Oceanography and the Marine Carbon Cycle*. Cambridge University Press, United Kingdom. p.261-294.
- Esteves V I, Otero M, Duarte A C. 2009. Comparative characterization of humic substances from the open ocean, estuarine water and fresh water. *Org. Geochem.*, **40**: 942-950.
- Esteves V I, Otero M, Santos E B H, Duarte A C. 2007. Stable carbon isotope ratios of tandem fractionated humic substances from different water bodies. *Org. Geochem.*, **38**: 957-966.
- Feng X, Hills K M, Simpson A J, Whalen J K, Simpson M J. 2011. The role of biodegradation and photo-oxidation in the transformation of terrigenous organic matter. *Org. Geochem.*, **42**: 262-274.
- Gonsior M, Peake B M, Cooper W T, Podgorski D, D'Andrilli J, Cooper W J. 2009. Photochemically induced changes in dissolved organic matter identified by ultrahigh resolution Fourier transform ion cyclotron resonance mass



- spectrometry. *Environ. Sci. Technol.*, **43**: 698-703.
- Guo W, Yang L, Hong H, Stedmon C A, Wang F, Xu J, Xie Y. 2011. Assessing the dynamics of chromophoric dissolved organic matter in a subtropical estuary using parallel factor analysis. *Mar. Chem.*, **124**: 125-133.
- Hatcher P G, Breger I A, Mattingly M A. 1980a. Structural characteristics of fulvic acids from continental shelf sediments. *Nature*, **285**: 560-562.
- Hatcher P G, Rowan R, Mattingly M A. 1980b. <sup>1</sup>H and <sup>13</sup>C NMR of marine humic acids. *Org. Geochem.*, **2**: 77-85.
- Hatcher P G, Schnitzer M, Dennis L W, Maciel G E. 1981. Aromaticity of humic substances in soils. *Soil Sci. Soc. Am. J.*, **45**: 1 089-1 094.
- Hedges J I, Hatcher P G, Ertel J R, Meyers-Schulte K J. 1992. A comparison of dissolved humic substances from seawater with Amazon River counterparts by <sup>13</sup>C-NMR spectrometry. *Geochim. Cosmochim. Acta*, **56**: 1 753-1 757.
- Hedges J I, Keil R G, Benner R. 1997. What happens to terrestrial organic matter in the ocean? *Org. Geochem.*, **27**: 195-212.
- Koch B P, Witt M, Engbrodt R, Dittmar T, Kattner G. 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Geochim. Cosmochim. Acta*, **69**: 3 299-3 308.
- Landry C, Tremblay L. 2012. Compositional differences between size classes of dissolved organic matter from freshwater and seawater revealed by an HPLC-FTIR system. *Environ. Sci. Technol.*, **46**: 1 700-1 707.
- Lee S Y. 1995. Mangrove outwelling: a review. *Hydrobiologia*, **295**: 203-212.
- Leenheer J A. 1981. Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. Technol.*, **15**: 578-587.
- Liu S, Li R, Zhang G, Wang D, Du J, Herbeck L S, Zhang J, Ren J. 2011. The impact of anthropogenic activities on nutrient dynamics in the tropical Wenchanghe and Wenjiaohe Estuary and Lagoon system in East Hainan, China. *Mar. Chem.*, **125**: 49-68.
- Mao J, Tremblay L, Gagné J. 2011. Structural changes of humic acids from sinking organic matter and surface sediments investigated by advanced solid-state NMR: insights into sources, preservation and molecularly uncharacterized components. *Geochim. Cosmochim. Acta*, **75**: 7 864-7 880.
- Mao L, Zhang Y, Bi H. 2006. Modern pollen deposits in coastal mangrove swamps from northern Hainan Island, China. *J. Coastal Res.*, **22**: 1 423-1 436.
- Meyers P A. 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chem. Geol.*, **114**: 289-302.
- Mopper K, Stubbins A, Ritchie J D, Bialk H M, Hatcher P G. 2007. Advanced instrumental approaches for characterization of marine dissolved organic matter: extraction technique, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Chem. Rev.*, **107**: 419-442.
- Odum E P. 1968. A research challenge: evaluating the productivity of coastal and estuarine water. In Proceedings of the Second Sea Grant Conference, University of Rhode Island. p.63-64.
- Odum W E, Heald E J. 1972. Trophic analyses of an estuarine mangrove community. *Bulletin of Marine Science*, **22**(3): 671-738.
- Opsahl S, Benner R. 1998. Photochemical reactivity of dissolved lignin in river and ocean waters. *Limnol. Oceanogr.*, **43**(6): 1 297-1 304.
- Opsahl S P, Zepp R G. 2001. Photochemically-induced alteration of stable carbon isotope ratios ( $\delta^{13}\text{C}$ ) in terrigenous dissolved organic carbon. *Geophys. Res. Lett.*, **28**: 2 417-2 420.
- Perdue E M, Benner R. 2009. Marine organic matter. In: Sensi N, Xing B, Huang P M eds. Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems. John Wiley and Sons, Hoboken, New Jersey. p.407-449.
- Perdue E M, Ritchie J D. 2005. Dissolved organic matter in fresh waters. In: Drever J I ed. Surface and Ground Water, Weathering, and Soils. In: Holland H D, Turekian K K eds. Treatise on Geochemistry, vol.5. Elsevier-Pergamon, Oxford, United Kingdom. p.273-318.
- Preston C M, Newman R H. 1995. A long-term effect of N fertilization on the <sup>13</sup>C CPMAS NMR of de-ashed soil humin in a second-growth Douglas-fir stand of coastal British Columbia. *Geoderma*, **68**: 229-241.
- Ritchie J D, Perdue E M. 2008. Analytical constraints on acidic functional groups in humic substances. *Org. Geochem.*, **39**: 783-799.
- Sihombing R, Greenwood P F, Wilson M A, Hanna J V. 1996. Composition of size exclusion fractions of swamp water humic and fulvic acids as measured by solid state NMR and pyrolysis-gas chromatography-mass spectrometry. *Org. Geochem.*, **24**: 859-873.
- Simjouw J P, Minor E C, Mopper K. 2005. Isolation and characterization of estuarine dissolved organic matter: comparison of ultrafiltration and C<sub>18</sub> solid-phase extraction techniques. *Mar. Chem.*, **96**: 219-235.
- Stevenson F J. 1994. Humus Chemistry: Genesis, Composition, Reactions. John Wiley and Sons, New York. 443p.
- Stuermer D H, Payne J R. 1976. Investigation of seawater and terrestrial humic substances with carbon-13 and proton nuclear magnetic resonance. *Geochim. Cosmochim. Acta*, **40**: 1 109-1 114.
- Tian L, Wu Y, Lin J, Zhu Z, Zhang J. 2010. Distribution and composition of organic carbon in the northeastern Hainan rivers and coasts during winter. *Journal of Tropical Oceanography*, **29**(5): 136-141. (in Chinese with English abstract)
- Tipping E. 2002. Cation Binding by Humic Substances. Cambridge University Press, Cambridge. 444p.
- Thurman E M. 1985. Organic Geochemistry of Natural Waters. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht. 516p.

- Tremblay L, Alaoui G. 2011. Characterization of aquatic particles by direct FTIR analysis of filters and quantification of elemental and molecular compositions. *Environ. Sci. Technol.*, **45**: 9 671-9 679.
- Tremblay L B, Dittmar T, Marshall A G, Cooper W J, Cooper W T. 2007. Molecular characterization of dissolved organic matter in a North Brazilian mangrove porewater and mangrove-fringed estuaries by ultrahigh resolution Fourier Transform-Ion Cyclotron Resonance mass spectrometry and excitation/emission spectroscopy. *Mar. Chem.*, **105**: 15-29.
- Wang B, Chen S, Gong W, Ling W, Xu Y. 2006. Development and Evolution of Estuarine Coast in Hainan Island, Ocean Press, Beijing. 215p. (in Chinese)
- Wang X, Lou T, Xie H. 2009. Photochemical production of dissolved inorganic carbon from Suwannee River humic acid. *Chin. J. Oceanol. Limnol.*, **27**(3): 570-573.
- Wilson M A, Gillam A H, Collin P J. 1983. Analysis of the structure of dissolved marine humic substances and their phytoplanktonic precursors by <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance. *Chem. Geol.*, **40**: 187-201.
- Xu Y, Mead R N, Jaffé R. 2006. A molecular marker-based assessment of sedimentary organic matter sources and distributions in Florida Bay. *Hydrobiologia*, **569**: 179-192.
- Zhang Y, Du J, Zhang F, Yu Y, Zhang J. 2011. Chemical characterization of humic substances isolated from mangrove swamp sediments: the Qinglan area of Hainan Island, China. *Estuar. Coast. Shelf Sci.*, **93**: 220-227.
- Zhang Y, Yang G, Chen Y. 2009. Chemical characterization and composition of dissolved organic matter in Jiaozhou Bay. *Chin. J. Oceanol. Limnol.*, **27**(4): 851-858.

Certain units appeared in this paper, such as 'ppm', 'cm<sup>-1</sup>', '%', are used upon authors' request, though not recommended by CJOL editorial office.