

Chemical characterization of humic substances isolated from mangrove swamp sediments: The Qinglan area of Hainan Island, China

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ABSTRACT

In order to improve the understanding of structural and reactive features of sediment organic matter from mangrove swamp as well as evaluate the relationship between such features and the impact from different sources (marine and terrestrial), humic and fulvic acids were isolated from two mangrove swamp sediments located in the Qinglan harbors on Hainan Island, China. One is a forest surface sediment site (WWM2), and the other is an estuary subaqueous sediment site (BMW). The humic and fulvic acids were characterized and compared using chemical and spectroscopic methods, including elemental analysis, thermogravimetric analysis (TGA), Fourier Transformed Infrared Spectroscopy (FTIR), ¹³C nuclear magnetic resonance (¹³C NMR) and potentiometric titrations. The results indicated that there were less aliphatic compounds but more aromatic compounds and oxygen-containing functional groups in fulvic acids. Humic acids contained more long-chain hydrocarbons and nitrogen compounds. Comparison of the C/N ratios and $\delta^{13}\text{C}$ values for the humic substances at both sites indicated a larger marine and/or microbial contribution to the BMW site. Humic substances at the WWM2 site have more acidic functional groups than those of the BMW site. Compared to the literature, more phenolic groups existed in the samples of both sites, which may be due to the autochthonous contribution of mangrove plants.

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1. Introduction

Mangroves forests are transitional coastal ecosystems between terrestrial and marine environments. They are highly productive, biodiverse ecosystems that form the fringe areas of 60–75% of all tropical coasts (Blasco et al., 1996; Clough, 1998). Up to half of the net primary production of mangroves is the exportation of leaves or detritus. Although mangroves cover only 0.1% of the continents' surface, they can account more than 10% of the terrestrial-derived refractory dissolved organic matter transported to the oceans (Jennerjahn and Ittekkot, 2002; Dittmar et al., 2006). This export mechanism and its waste greatly affect the ecosystems of coastal waters. Therefore, much research focusses on the dissolved organic matter from the mangroves swamp (Dittmar and Lara, 2001a,b; Dittmar et al., 2006). However, mangroves act as not only a carbon source (carbon out-welling) but also a carbon sink (burial of mangrove assimilated carbon in sediments) (Eong, 1993). Mangroves cover an estimated area of ca.15.71 million ha worldwide, with an overall average carbon sequestration rate of 210 g CO₂ m⁻² yr⁻¹

per unit area. The average sediment carbon density of mangroves swamps is 0.05 ± 0.004 g cm⁻³ (Chmura et al., 2003; Ferreira et al., 2009), which constitutes a large carbon pool and plays a crucial role in the global carbon biogeochemical cycle in coastal system. However, there are only a few studies detailing the soil organic matter in mangrove sediments (Sierra et al., 2005; Ferreira et al., 2009; Ranjan et al., 2010). This limits our understanding of the biogeochemistry processes involved in sediment organic matter in mangrove area.

Humic substances (HSs), the dominant component of soil organic matter, are formed by the decomposition of plant, animal, and microbial materials; they are complex, heterogeneous organic compounds with different structures and a wide range of molecular weights but they are still poorly understood (Stevenson, 1994; Lead et al., 1994, 1999; Maurice and Namiesnik-Deianovic, 1999). They are potentially important in the binding and bioavailability of trace metals and organic pollutants because of their large surface areas and strong structural binding sites (Dwane and Tipping, 1998; Tipping, 2002). In order to investigate the structural features of HSs, various analytical methods have been employed, such as direct and indirect potentiometric titrations (Perdue, 1985; Du et al., 1994), radiometric determination (Pompe et al., 1996) and infrared spectroscopy (MacCarthy and Rice, 1985; Stevenson, 1994). In addition to these methods, carbon-13 nuclear magnetic resonance spectroscopy

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(^{13}C NMR) is a valuable tool for investigating the functionality of HSs both qualitatively and quantitatively and for distinguishing between different functional groups (Wershaw, 1985; Leenheer et al., 1987; Stevenson, 1994; Bortiatynski et al., 1996).

In order to improve the understanding of structural and reactive features of sediment organic matter from mangrove swamp as well as evaluate the relationship between such features and the impact from different sources (marine and terrestrial), two humic substances samples were isolated from different sites (one is forest surface sediment and another is estuary subaqueous sediments) in Qinglan Harbor ($110^{\circ}43'–110^{\circ}49'E$, $19^{\circ}30'–19^{\circ}40'N$), China, where natural reserves of mangrove swamps were established in the 1980s. The elemental composition, thermogravimetric analysis (TGA), Fourier Transform Infrared Spectroscopy (FTIR), ^{13}C NMR, and potentiometric titrations were used to characterize the humic substances.

2. Materials and methods

2.1. Site description

Sampling was carried out in Qinglan, in the coastal Hainan province of China (Fig. 1). The mangrove reserves occur in the estuary formed by the confluence of the Wenchang River and the South China Sea. The selected sites are WWM2 ($110^{\circ}48'00''E$, $19^{\circ}36'11''N$) and BMW ($110^{\circ}49'06''E$, $19^{\circ}35'13''N$) within in these mangrove reserves. The dominant mangrove species present are *Rhizophora*, *Avicennia*, *Lumnitzera*, *Ceriops*, *Kandelia*, *Bruguiera* and *Sonneratia* (Mao et al., 2006). The estuary is meso-tidal with tidal amplitudes >1.5 m. At low tides, the creek water is dominated by pore water, which flows from the mangrove sediments into the creek, producing high concentrations of nutrients and organic matters. We collected forest surface

sediments at WWM2 and estuary subtidal sediments at BMW, where the water depth is *ca.* 3 m. The light silt and sand sediments were collected at 0–20 cm. The basic properties of the two sediments (Table 1) indicate BMW has less organic carbon but more nitrogen than those of WWM2. As expected, the carbon in humic substance constituted 66.9–79.7% of total sediment organic carbon. Among the HSs fractions, humin (HU) carbon occupied the largest proportion, then humic acid (HA) and fulvic acid (FA) carbon in decrease. These were consistent with the results measured by Tremblay and Gagné (2007) in the St. Lawrence Estuary and the Saguenay Fjord. The particle size of WWM2 is slightly coarser than BMW.

2.2. Extraction and purification of humic substances

The extraction of FA and HA fractions follows the International Humic Substances Society (Swift, 1996). Each 2-mm sieved sediment sample was air dried and pre-extracted in 0.1 M HCl for 1 h under constant agitation. The HCl was then decanted and the residues were neutralized with 1 M NaOH. A 0.1 M NaOH solution was then added to the residues using an extractant to sample ratio of 10:1 under nitrogen gas. The extracted humic substances were then separated by centrifugation into FA and HA fractions by acidifying the extract to pH 1 with 6 M HCl. The HA precipitates were re-dissolved with a minimal volume of 0.1 M KOH under nitrogen gas, and KCl was added to obtain a concentration of 0.3 M K^+ . After the removal of suspended solids by centrifugation, the HA fraction was again precipitated by adjusting the pH to 1 using 6 M HCl. This mixture was centrifuged, and the supernatant was discarded. The HA precipitate was treated with 0.1 M HCl and 0.3 M HF several times and dialyzed against distilled water until the dialysis water gave a negative Cl^- test with AgNO_3 . The FA fraction

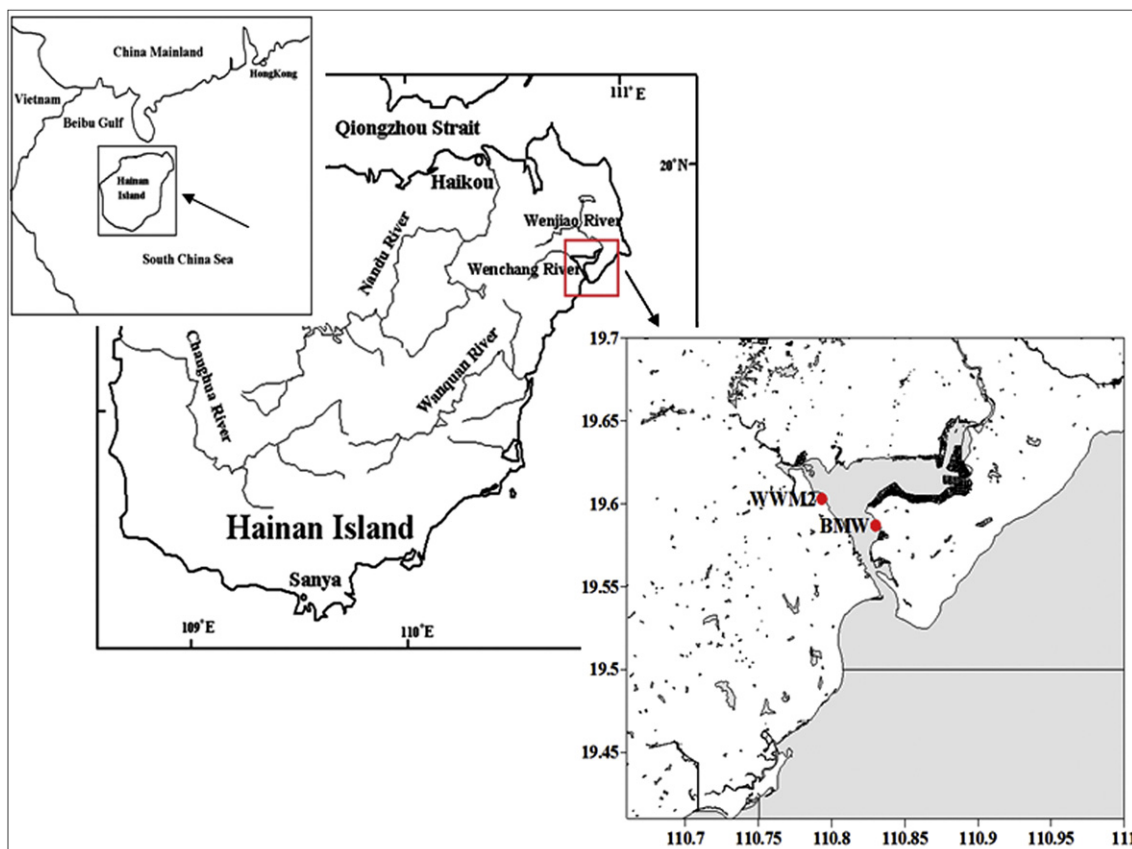


Fig. 1. The sampling locations of BMW and WWM2.

Table 1
Basic properties of the sediments used for this study.

	BMW Site	WWM2 Site
Location	110°49'06"E, 19°35'13"N	110°48'00"E, 19°36'11"N
Collection depth (cm)	0–20	0–20
Sediment color	Gray	Gray
%TC	0.96	1.04
%TOC	0.64	0.86
%IC	0.32	0.18
%TN	0.10	0.07
%C _{FA} /C _{org}	2.66	2.07
%C _{HA} /C _{org}	10.91	10.15
%C _{HU} /C _{org}	53.33	67.51
%Sand	71.602	85.870
%Silt	16.545	9.576
%Clay	11.853	4.554
δ ¹³ C (‰)	−22.624	−25.704

was concentrated and purified by flowing FA through an XAD-8 adsorption resin column, and the 0.1 M NaOH eluate was passed through a H⁺-saturated cation exchange resin column. Subsamples of both fractions were freeze-dried for further analysis.

After using the exhausted extraction method described by Xing et al. (2005), the residue solid containing HU were collected and freeze-dried. The TOC content of the HU fractions was determined by combustion at 700 °C for 16 h.

2.3. Chemical characterization of HA and FA

2.3.1. Elemental analysis and stable carbon isotopic composition

Elemental compositions (C, H, and N) of HA and FA were determined using a Vario EL 3 Elemental Analyzer (Elementary, Germany). Oxygen content was calculated by subtracting the percentage of C, H, and N from 100%. C-stable isotopic analysis was measured on a Delta plus XP isotope ratio mass spectrometer (Thermo Finnigan, USA). Isotopic ratios were reported in the standard delta notation using Peedee Belemite (PDB) as a standard:

$$\delta^{13}\text{C value (‰)} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (1)$$

where R is the ratio of ¹³C/¹²C.

2.3.2. Thermogravimetric analysis

Thermogravimetric analysis was carried out using a TGA/SDTA 851 (Mettler Toledo, Switzerland). HA and FA samples ranging from 5 mg to 10 mg were heated at 10 °C per minute from 25 °C to 100 °C and held at 100 °C for 60 min for drying. Then the samples were heated from 100 °C to 750 °C at 10 °C per minute. The gases given off by the samples were carried away by air at a flow rate of 20 ml/min.

2.3.3. Infrared spectroscopy

Fourier Transform Infrared (FTIR) Spectra of the samples were obtained using a Nicolet Nexus 670 spectrometer (Thermo Electron Corporation, USA). Spectra were acquired from 4000 to 650 cm^{−1} at 4 cm^{−1} resolution.

2.3.4. Solid-state ¹³C nuclear magnetic resonance

Solid-state ¹³C NMR spectra were acquired using a Bruker Advance DSX 300 MHz spectrometer equipped with a 4-mm magic angle spinning (MAS) probe. Each HA or FA sample was packed into a zirconia rotor and spun at 8 kHz during data acquisition. Spectra were acquired using a cross-polarization (CP) pulse sequence with composite pulse TPPM15 proton decoupling and magic angle spinning. The contact time was 1.5 ms, and 20,480 scans were

averaged for each sample. Chemical shifts were externally referenced to the glycine resonance at 176 ppm.

2.3.5. Potentiometric titrations

The content of carboxylic and phenolic functional groups was measured by potentiometric acid–base titrations. The experiments were conducted in a 100-ml Teflon vessel with a polyethylene lid. The vessel was surrounded with a glass jacket to maintain a temperature of 25 ± 0.5 °C. All titrations were conducted using a computer-controlled PC-titration system (DL50 Automatic Titrator, Mettler Toledo) with a pH electrode (Delta 320). A Teflon bar was used for stirring and argon was bubbled continuously to exclude CO₂ gas.

Solutions containing 500 mg L^{−1} of either FA or HA were titrated at ionic strength 0.1 M with NaNO₃ as the inert electrolyte. To dissolve the samples, the stock solution of HA also contained NaOH before each titration, and sufficient HNO₃ was added to neutralize the NaOH. Sample volumes of 40 ml were placed in the thermostated cell and titrated by adding small aliquots of 0.3577 M CO₂-free NaOH solution. More than 100 points were included in each titration for a range of pHs from 3 or 4 to approximately 11. Each step was allowed to stabilize until the pH drift was less than 0.005 pH units per minute (Chen et al., 2008).

The potentiometric titration gave a direct relationship between the amount of NaOH added and the resulting pH of the mixture. This result can be reorganized to show the total organic charge of dissociated functional groups as a function of pH, and the carboxylic and phenolic content were estimated directly from those titration curves (Ritchie and Perdue, 2003).

The organic charge at any point in a titration can be calculated using the electroneutrality equation:

$$\sum_i [\text{Org}_i^-] = [\text{Na}^+] + [\text{H}^+] - [\text{NO}_3^-] - [\text{OH}^-] \quad (2)$$

where [Na⁺] and [NO₃[−]] are dilution-corrected concentrations of added NaOH and HNO₃, and [Org_{*i*}[−]] is the dilution-corrected concentration of the *i*th organic charge in the sample. The [Org_{*i*}[−]] calculated from Eq. (2) was normalized to the dilution-corrected concentration of dissolved organic carbon to yield Q_{tot} with units of meq gC^{−1}. Q_{tot} was plotted against pH, and the concentrations of carboxylic groups were estimated to be the value of Q_{tot} at pH 8, while those of phenolic groups were estimated as two times the change in Q_{tot} between pH 8 and pH 10 (Ritchie and Perdue, 2003).

3. Results and discussion

3.1. Elemental analysis and stable carbon isotopic composition

The elemental composition and the δ¹³C values of the purified FA and HA as well as the calculated ratios of the C/N, H/C, and O/C (Table 2) show that both HA samples were richer in carbon, nitrogen and hydrogen than the FA samples, but the oxygen content was higher in the FA than in the HA samples. The difference in oxygen content showed that more oxygen-containing functional groups such as carboxylic acids, phenols, alcoholic hydroxyls, carbonyls, and methoxyls existed in the FA fractions (Schnitzer and Khan, 1972). The C/N ratio of the FA samples was larger than that of the HA samples, which indicated that resistant forms of nitrogen tended to be retained in the HA samples, whereas they were depleted in the FA samples during the humification process (Almendros et al., 1996; Gondar et al., 2005; Chai et al., 2007). The H/C ratio of the FA was lower than that of HA, suggesting that HA had more aliphatic and fewer aromatic organic compounds at both sites. These findings agree with Giovanela et al. (2004) and Sierra et al. (2005), who also found that the FAs have higher C/N and lower H/C ratios than those

Table 2
Elemental composition and acidic functional group contents of HAs and FAs isolated from two mangrove sediments.

Sample	C%	N%	H%	O%	C/N (atomic)	H/C (atomic)	O/C (atomic)	$\delta^{13}\text{C}$	Carboxylic (meq gC ⁻¹)	Phenolic (meq gC ⁻¹)
BMW-FA	51.45	3.15	4.13	41.27	19.06	0.96	0.60	-23.117	10.93	4.17
BMW-HA	55.32	6.13	5.42	33.13	10.52	1.17	0.45	-22.436	4.93	2.52
WWM2-FA	50.75	1.16	3.89	44.20	51.08	0.91	0.65	-26.408	11.56	5.09
WWM2-HA	58.74	5.23	5.24	30.79	13.09	1.06	0.39	-25.543	5.84	4.32

of the HAs in all their mangrove sediments HSS, and these trends also were shown by the HSs isolated from the marine environment sediments in their research. However, the higher H/C ratio which means the less aromatic carbon exist in HA differed with many findings from the soil HSs (Gondar et al., 2005; Santín et al., 2008). This question will be discussed in detail in the NMR data analysis.

A typical value of $\delta^{13}\text{C}$ in mangroves is about -27‰ (Maie et al., 2006). The $\delta^{13}\text{C}$ values from the total sediments for BMW and WWM2 sites are -22.624 and -25.704‰ , respectively, which indicates that both sites subjected to the influence from the marine contribution, but BMW encountered more than WWM2 (Table 1). Hedges et al. (1997) showed the typical marine $\delta^{13}\text{C}$ value is about -22‰ , with a C/N ratio of 10 although natural organic matter of terrestrial samples has a wide range of C/N ratios (15–30), with a $\delta^{13}\text{C}$ value of about -28‰ . Combining the higher content of N of the whole sediment of BMW reinforces the fact that this site encountered more marine and/or microbial influence (Santín et al., 2009). Table 2 shows that FAs had slightly lower $\delta^{13}\text{C}$ values than their HA counterparts at both sites, which are in line with the results of Tremblay and Gagné (2007). This finding suggested a higher vascular plant composition and/or a more degraded state for FA.

3.2. Thermogravimetric analysis

The ash contents within FA and HA from both sites were less than 1% according to the weight loss percentage data. Thermogravimetric

(TG) and the corresponding differential thermogravimetric (DTG) curves for each HA and FA sample are shown in Fig. 2. The DTG curves for both of the FA samples show three peaks with the weight loss: the first occurs between 30 and 107 °C with a mass loss of 10.8–13%; the second is between 101–418 °C shows a mass loss of 33.9–40.5%; the third lies between 371–600 °C with a mass loss of 46.5–55.3%. The DTG curves for the two HA samples both display four weight loss zones: they are between 30–103 °C, 100–430 °C, 419–474 °C and 467–633 °C corresponding to the mass loss of 7.0–10.4%, 34.9–35.9%, 10.0–10.1% and 43.6–48.1%, respectively. Many studies (Tao et al., 1997; Esteves and Duarte, 1999; Peuravuori et al., 1999; Calace et al., 2006) show the primary features of the DTG curves can be interpreted as follows: (a) in all samples, the progressive loss of weight up to 100 °C was mainly due to the loss of adsorbed water; (b) the second peak in the 100–430 °C region for all HA and FA samples was generally attributed to the degradation of carbohydrates, carboxylic, phenolic, carbonyl, alcoholic groups, and dehydration of aliphatic compounds; (c) the third peak that showed a shoulder in the 419–474 °C region for the two HA samples, which also was found by Giovanella et al. (2004) in their HA samples isolated from mangrove sediments, could be attributed to the degradation of long-chain hydrocarbons and nitrogen compound (e.g. proteinaceous material). As stated from previous H/C atomic ratio and following FTIR and NMR data, HAs in both sites have higher aliphatic compounds than those of FAs. Upon heating, these long aliphatic chains can be condensed to cyclic structure and further to aromatic

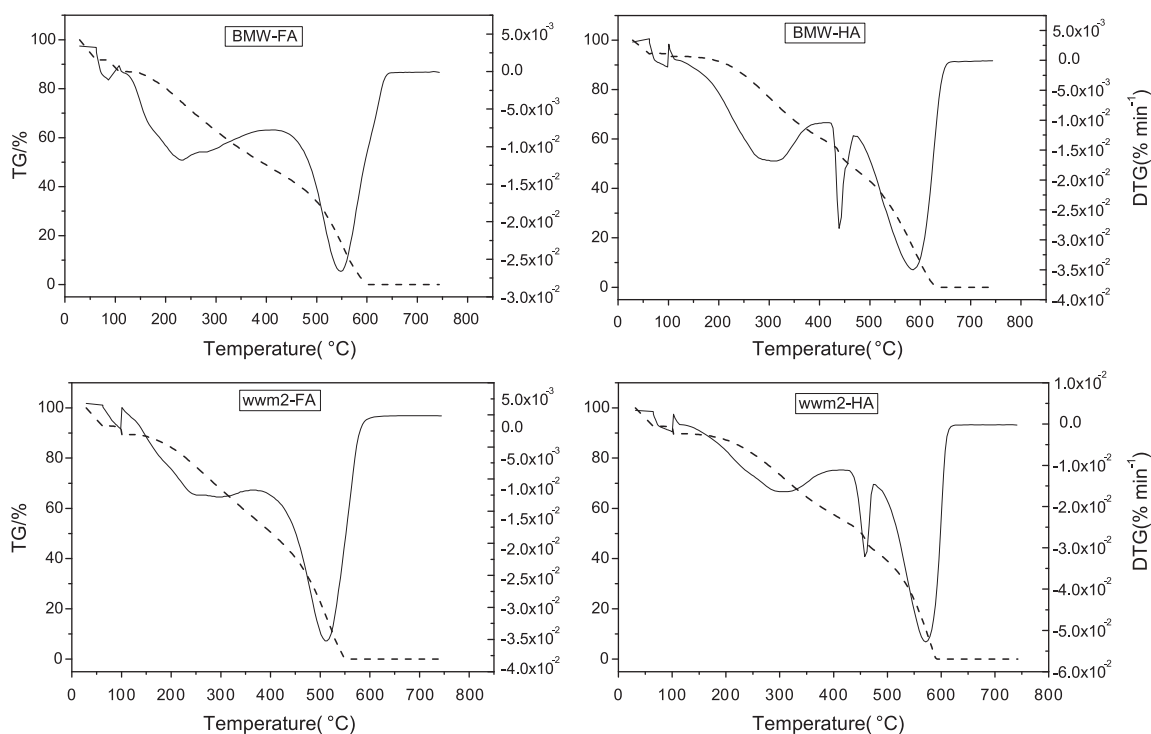


Fig. 2. The TG and DTG curves of HAs and FAs isolated from two mangrove swamp sediments.

structures. These newly formed aromatic compounds added to those already existing in the sample enhancing the observed amount of the structures in the DTG profiles (Giovanela et al., 2004). This peak could also indicate the degradation of nitrogen-containing compounds as there was a higher nitrogen content in HAs than in FAs and these compounds can be thermally degraded around 450 °C (Francioso et al., 2005); and (d) the third peak of the FA samples in the 371–600 °C region and the fourth peak of the HA samples in the 467–633 °C region were related to the combustion of aromatic structures and the cleavage of C–C bonds.

The TG and DTG profiles of the FA and HA samples at both sites were very similar, regardless of the influence from different source (Fig. 2). The corresponding temperature ranges and amounts of weight loss were similar to other recently reported values in sediment and soil humic substances (Giovanela et al., 2004; Fernández et al., 2008). Generally, the HSs in both sites are highly thermo-resistant retaining most of the weight even after being heated to 400 °C. At both sites, FAs have higher percentage of components that degrade at temperatures below 430 °C, which indicated FAs possess more polar functional groups than those of HAs. Again, there were more long-chain hydrocarbons and nitrogen-containing compound in HA fractions as confirmed by TG analysis.

3.3. Fourier Transform Infrared Spectroscopy

The Fourier Transform Infrared Spectra (Fig. 3) in general shows that the two HA and FA samples from the 2 sites exhibited similar spectroscopic features. Identification of the main absorption bands was based mainly on the data published by Stevenson (1994). The broad band around 3400 cm^{-1} was due to O–H stretching and trace N–H stretching. The absorption peaks around 2920 cm^{-1} , 2850 cm^{-1} , and 1460–1450 cm^{-1} were ascribed to aliphatic C–H stretching. The intensities of those peaks (2920, 2850, and 1460–1450 cm^{-1}) were higher for HA than FA at both sites, thus illustrating that the HA contained more aliphatic compounds than the FA.

The peak around 1720 cm^{-1} , attributed to C=O stretching of COOH and trace ketones, was obvious for FA but only appeared as a shoulder in the HA, perhaps because most carboxyl groups were in the salt form in the HA (El-Sayed et al., 1996). The strong absorption at 1650 cm^{-1} in the HA, which is attributed to aromatic C=C vibrations and the stretching of COO⁻ groups (Chai et al., 2007), seemed to support this hypothesis. The peak at 1400–1390 cm^{-1} (COO⁻ antisymmetric stretching) and the peak around 1220 cm^{-1}

(C–O stretching and OH deformation of COOH) also indicated the presence of a large amount of carboxylic acids in both the FA and HA. From the intensities of these peaks at 1720 and 1220 cm^{-1} , it can be easily recognized that the FA contained more carboxylic acids than the HA, which agrees well with the elemental composition data (Table 2).

The presence of protein-like fractions was represented by two peaks, one at 1660–1630 cm^{-1} (C=O stretching of amide groups) and another at 1560–1510 cm^{-1} (N–H deformation and C=N stretching of amide groups). The two peaks were all comparatively stronger for the HA than the FA, indicating that more nitrogen-containing compounds existed in the HA as also shown by the elemental and thermogravimetric analysis.

Finally, the peak around 1160 cm^{-1} was ascribed to the –C–OH stretch of aliphatic OH (Jung et al., 2005). The band between 1080 and 1030 cm^{-1} was assigned to C–O stretching of polysaccharides and to Si–O vibration of silicate impurities (Santín et al., 2008).

3.4. ¹³C CP/MAS NMR spectroscopy

Solid-state CPMAS ¹³C NMR spectra for the FA and HA samples in the two sites (Fig. 4) shows that all major spectral bands agree with those observed for soil and aquatic HSs, although the relative intensities of the peaks may vary among samples (Bortiatynski et al., 1996; Ussiri and Johnson, 2003; Templier et al., 2005). In general, all spectra could be divided into five chemical-shift regions, 0–45 ppm, 45–110 ppm, 110–160 ppm, 160–185 ppm and 185–220 ppm corresponding approximately to aliphatic C, O-alkyl C, aromatic C, carboxylic acid C and carbonyl C, respectively.

For the alkyl region, the peak at 22 ppm was attributed to aliphatic carbon of branched and short-chain compounds in alkyl chains, while the signal at 30 ppm was due to long-chain alkyl compounds (Santín et al., 2008). The peak centered at 30 ppm is better defined and more prominent in HAs than in FA spectra, suggesting that HAs contain more long-chain alkyl than that of FAs, which confirmed the findings from the TG analysis (Fig. 2).

The O-alkyl C region can be subdivided into methoxyl groups (45–65 ppm) and carbohydrate (65–110 ppm) regions. The signal at 55 ppm was assigned to methoxyl groups associated with lignin and lignin-like products (Kögel-Knabner et al., 1994), the peak at 74 ppm to O-alkyl C containing the ring carbons of carbohydrate and the side-chains of lignin, and the peak at 104 ppm to di-O-alkyl C, including anomeric-C of carbohydrate (Preston and Newman, 1995). For the WWM2, the O-substituted alkyl C is the largest percentage of carbon in FA (34.9%) and the second largest in HA (34.1%, the first being the aliphatic carbons) among the regions considered, suggesting that this fraction constitute a major structural component of this site. Comparatively, BMW site had less carbohydrate-like matter in FA and HA (26.55% and 31.23%, respectively).

For the aromatic region, there were two peaks around 130 and 150 ppm that could be due to aromatic structures contained in lignin (Ussiri and Johnson, 2003). Fig. 4 shows that the WWM2 HA had higher peaks than that of the BMW HA, which may indicate that there was a larger proportion of plant-derived material at WWM2. The integration of aromatic carbon decreased from FA to HA at both sites, which is in line with the H/C ratios from elemental analysis but differs from other results for soil samples (Gondar et al., 2005; Santín et al., 2008). However, this agrees with Sierra et al. (2005) for their mangrove sediments HSs. This distinct difference may be due to the specific environment in mangrove area. Some labile compounds in FA, such as short-chain aliphatic matter, may be removed by tidal action because FA is soluble in sea water (pH 8.2), but HA is not (Stevenson, 1994).

Generally, BMW has a slightly higher aromatic carbon than WWM2 for both FA and HA (28.91% and 21.67% VS. 26.26% and

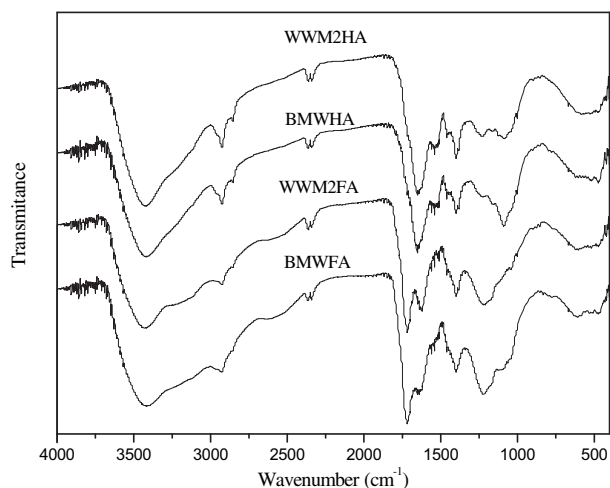


Fig. 3. Fourier Transform Infrared Spectra of FAs and HAs isolated from the BMW and WWM2 sites.

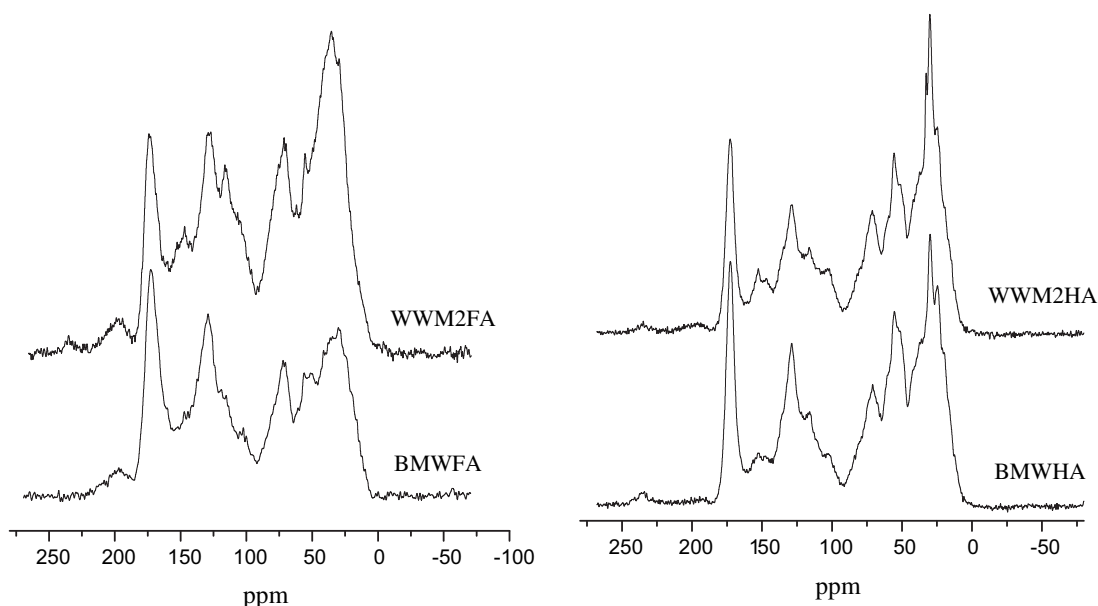


Fig. 4. ^{13}C NMR spectra of FAs and HAs isolated from the BMW and WWM2 sites.

20.43%, respectively). Combining the results of the integration from O-alkyl C region, it is implied that the HSs from BMW has a higher degree of humification. Hur et al. (2009) pointed out the enrichment of aromatic moieties and the reduction of O-alkyl carbon structure in HSs is related to the higher degree of humification process. The humification process can be influenced by many factors such as type and amount of organic matter input, grain size composition, redox conditions, bioturbation, etc (Calace et al., 2006). Based on the nature of the BMW and WWM2 sites, we would expect a larger degree of humification at WWM2 due to the larger particle size and increase in oxidation during low tides, however the constant input of fresh leaves and detritus from the mangroves forest at WWM2 provide an explanation for the lower humification degree that we estimated from the NMR data.

In the carboxylic acid and carbonyl C region, the peaks at 174 ppm can be assigned to carboxyl, amide and ester C (Preston and Newman, 1995). The higher integration values of carboxylic and carbonyl C for FAs in both sites indicated that the FA was highly oxidized and had more acidic functional groups and higher

reactivity. These findings reflect the elemental composition, FTIR and following potentiometric titrations results.

3.5. Potentiometric titrations

The Q_{tot} –pH curves and the content of acidic functional groups (Fig. 5 and Table 2, respectively) generally shows that HSs at WWM2 contained more acidic functional groups than those of BMW, thus indicating that the WWM2 HSs had higher chemical reactivity and more binding sites than the BMW HSs. At both sites, the charge on the FA was higher than that on the HA for the whole pH range, consistent with many authors' results (e.g. Milne et al., 2001; Ritchie and Perdue, 2003; Gondar et al., 2005). The carboxyl contents of the samples ranged from 4.93 to 11.56 meq gC^{-1} , accounting for 6–14% of the carbon in each sample. The average ratio of carboxylic groups in the HA compared to the carboxylic groups in the FA was 0.48 in our samples, which was lower than the previously reported values of 0.66, 0.67 and 0.59 (Milne et al., 2001; Ritchie and Perdue, 2003; Gondar et al., 2005). This is likely due to the slightly lower content of carboxylic groups in our HA samples. The content of carboxylic groups constituted 69%–72% of the total acidity for fulvic acids and 57%–66% for humic acids.

Overall, the phenolic content in our samples range from 2.52 to 5.09 meq gC^{-1} , and the average ratio of phenolic to carboxyl groups in the four samples was 0.52, which is similar to the ratio used in Model V (Tipping and Hurley, 1992) but higher than the 21:79 ratio published by Ritchie and Perdue (2003). The higher percentage of phenolic hydroxyl groups may be due to the presence of lignin phenolic groups derived from autochthonous mangrove plants (Rasyid et al., 1992).

4. Conclusions

The chemical and spectroscopic characterization from two humic substances in mangrove forest sediment (WWM2) and estuary sediment (BMW, water depth 3 m) show that:

More long-chain hydrocarbons and nitrogen compounds existed in humic acids than fulvic acids at both sites. Humic acids had more aliphatic and fewer aromatic organic compounds in both sites. The content of carboxylic groups accounted for most of the

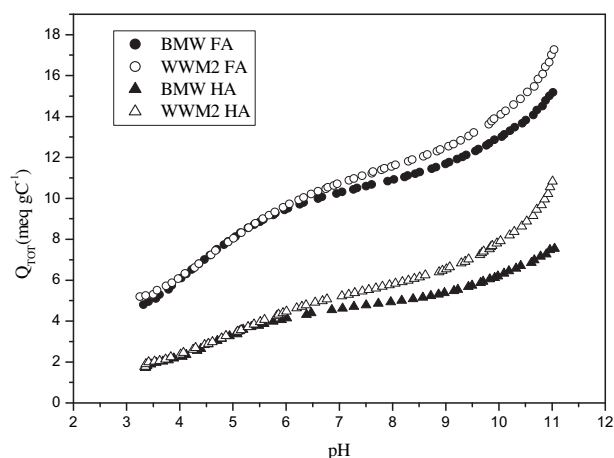


Fig. 5. Potentiometric titration curves for the FAs and HAs isolated from the BMW and WWM2 sites.

total acidity and contributed more to the fulvic acids than the humic acids. The higher aromatic carbon in the fulvic acids than in humic acids could be due to the presence of some labile compounds in the fulvic acids, such as short-chain aliphatic compounds, taken away by tide or sea rush.

HSs extracted from both sites were influenced by the marine and/or microbial contribution, but this was great at the BMW site than that of WWM2. HSs in WWM2 site had a lower degree of humification due to the constant input of fresh leaves and detritus from the mangroves forest. In addition, HSs in WWM2 had larger proportion of plant-derived material and more density acidic functional groups.

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