Analytical and Spectroscopic Characteristics of Refuse Compost-Derived Humic Substances

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Abstract: Animal manure composts contain a substantial amount of organic matter, subsequently containing a significant amount of humic substances. In this study, we investigated the yields of refuse compost-derived humic substances and their analytical and spectroscopic characteristics. The humic acid (HA) and fulvic acids (FAs) of humic substances were extracted from refuse compost and purified. The composition of humic substances were determined by elemental analysis and that of functional groups by Fourier transform infrared spectroscopy (FTIR) and solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS ^{13}C NMR). Elemental analysis indicates that C and N contents of HA surpass those of FAs. However, total functional group acidities of FAs were much greater than that of HA. Both FTIR and ^{13}C NMR spectra indicate that carboxyl contents of FAs surpass that of HA. The characteristics of compost-derived humic substances affects their reactions with metals especially heavy metals, which in turn affects the mobility and biotoxicities of heavy metals in soil and the associated environments.

1. Introduction

The extension of organic farming and sustainable agriculture has led to increasing applications of organic fertilizers. Composts of organic fertilizers contain a substantial amount of organic matter, with a significant amount of humic substances [1]. The contents of elements and functional groups of a variety of humic acids (HAs), FAs and humins of humic substances from soil, lake sediment, coal and water have been determined by Schnitzer and Khan [2]. Humic substances contain a variety of functional groups, including COOH, phenolic OH, enolic OH, alcoholic OH, quinone, hydroxyquinone, lactone, and ether [3]. Numerous laboratory studies on the reaction between metals and humic substances have been reported in the literature. Several review articles have also been devoted to this subject [3-4]. On the study of the rate of sorption-desorption of volatile organic compounds (VOCs) by humic substances, Chang et al. [5] reported that the sorption of toluene to humic acid, an integral member of a soil humic substance, is found to be reversible and diffusion controlled. In addition, Shih and Wu [6] con-

cluded that partitioning is believed to be the major mechanism of the sorption of toluene to humin. In soil and the associated environments, naturally occurring humic substances exert strong binding strength with metals (including heavy metals). It is generally attributable to the interaction of metal ions with two important acidic binding sites of carboxylic and phenolic hydroxyl groups of humic substances [7-10]. Organic ligands derived from humic substances react with heavy metals to form complexes, which influence the concentrations of free and labile heavy metal ions and their subsequent mobilities and biotoxicities in soil and the associated environments [11-12]. Analytical and spectroscopic characterization of humic acid (HA) extracted from one kind of worm compost have already been studied by Deiana et al. [1]. However, those of refuse compost-derived humic substances including HA and FAs were seldom reported.

2. Materials and methods

2.1. Materials

The refuse compost was purchased from former Feng-Fu Organic Fertilizer Company (Changhua County, Taiwan) to perform the experiment. According to the method recommended by Aiken [13], Hayes [14], Leenheer [15], and Swift [16], HA (MW>1000) and fulvic acid (FA) were extracted from the compost and purified. Based on the method proposed by Wang and Huang [17], the purified H^+-saturated FA solution was concentrated by using a rotary evaporator to an adequate volume. The concentrated FA solution was transferred to dialysis tubes with molecular-weight cutoff of 1000 to separate the fraction of FA (MW>1000) from that of FA (MW<1000). The equilibrated solution outside the dialysis tubes was then concentrated by using a rotary evaporator to collect the fraction of FA (MW<1000). All HA (MW>1000), FA (MW>1000) and FA (MW<1000) were freeze-dried and their yields were determined. The extractions and subsequent purification of the humic substances was triplicate.

2.2. Characterization of humic substances

The C, H, N, O and S contents of the freeze-dried HA (MW>1000), FA (MW>1000) and FA (MW<1000) samples were obtained with a Heraeus CHN-O-rapid elemental analyzer with Tacussel coulomax 78 automatic coulometric titrator. Fourier transform infrared (FTIR) spectra of the freeze-dried HA (MW>1000), FA (MW>1000) and FA (MW<1000) samples were obtained from KBr discs (each disc contained 1 mg sample and 300 mg KBr). The spectra were recorded in the 400 to 4000 cm^{-1} range on a Bio-Rad FTS-7 Fourier transform infrared spectrophotometer. The freeze-dried HA (MW>1000), FA (MW>1000) and FA (MW<1000) samples were subjected to solid state ^{13}C NMR analysis on a Bruker MSL-200 spectrometer. It was set with cross polarization and magic-angle spinning (CPMAS), proton decoupling, spectrometer frequency of 50.33 MHz, acquisition time (delay time) of 25.6 ms, contact time of 1 ms, recycle time of 1 s and magic angle spinning rate of around 3.5 kHz. According to the method described by Schnitzer and Khan [2], the freeze-dried HA (MW>1000), FA (MW>1000) and FA (MW<1000) samples were subjected to the determination of total acidity values by titration. The obtained elemental analyses as well as atomic ratios, functional group acidity, and distribution percentages of the characteristic carbons of the humic substances were subjected to statistical analysis using SAS procedures [18] and Duncan's multiple range test at $p < 0.05$ [19].

3. Results and discussion

3.1. Yields and elemental analyses of humic substances

Yields of HA (MW>1000), FA (MW>1000) and FA (MW<1000) extracted from the refuse compost were 23.4, 0.294 and 0.610 g kg^{-1}, respectively. The C, H, N, O and S contents of the extracted humic substances (Table 1) were close to those of natural humic substances derived from soils and waters [2,20]. The sequence of the C contents of the extracted humic substances was HA (MW>1000) (552 g kg^{-1}) > FA (MW>1000) (479 g kg^{-1}) > FA (MW<1000) (381 g kg^{-1}) and that of N contents was HA (MW>1000) (65.3 g kg^{-1}) > FA (MW>1000) (34.3 g kg^{-1}) and FA (MW<1000) (31.7 g kg^{-1}), and their differences were significant. However, the sequence of the O content was in a reverse trend, and their differences were also significant (Table 1). Accordingly, as reported by Schoenau and Bettany [21], HA (MW>1000) extracted from the refuse compost may act as an organic nutrient supplier, while the extracted FA (MW>1000) and FA (MW<1000) are nutrient such as metal transporters in soil and the related environment. The H and S contents of the HA (MW>1000) and/or FA (MW>1000) were significantly lower than that of FA (MW<1000) (Table 1). The elemental compositions of the extracted humic substances reflected their atomic ratios. Due to the lowest C content and the highest O content of the FA (MW<1000), it had the highest H/C and O/C ratios. The sequence of the O/C ratio of the humic substances was FA (MW<1000) (1.00) > FA (MW>1000) (0.68) > HA (MW>1000) (0.44), and their differences were significant. Moreover, the sequence of the total acidity of the humic substances coincided with that of the O/C ratio (Table 1). This indicates that O-containing functional groups of the humic substances contributed to their total acidity. Further, the highest O/C ratio and total acidity of the FA (MW<1000) among the humic substances extracted (Table 1) implicate its weighted importance of O content. Steelink [22] used the O/C ratios of humic substances to suggest their origins. He found that O/C ratio of both soil HA and lake sediment HA were around 0.50, and those of soil FA and lake sediment FA were around 0.70 and 0.80, respectively. The O/C ratio of the FA (MW>1000) in this study was close to those reported for natural FA, while that of the FA (MW<1000) was higher (Table 1).

Table 1. Elemental analyses as well as atomic ratios and total functional group acidity of humic substances extracted from the compost

Humic substances	Elemental content[†]					Atomic ratio			Total acidity
	C	H	N	O	S	H/C	C/N	O/C	
			g kg^{-1}						meq g^{-1}
HA (MW>1000)	552a[‡]	47.9ab	65.3a	324.0c	10.7b	1.04b	9.86b	0.44c	8.28c
FA (MW>1000)	479b	43.6b	34.3b	433.0b	10.1b	1.09b	16.3a	0.68b	12.08b
FA (MW<1000)	381c	52.3a	31.7b	508.5a	26.4a	1.65a	14.0a	1.00a	14.42a

[†] Expressed on ash-free weight basis.
[‡] Different lower case letters in columns indicate significant differences in means at p < 0.05 using Duncan's multiple range test.

In addition, Steelink [22] reported that humic substances have higher degree of polymerization and higher content of aromatic compound if their H/C and O/C ratios were lower. On the contrary, humic substances had higher content of carbohydrate if their H/C and O/C ratios were higher. In this study, H/C and O/C ratios of the HA (MW>1000) extracted were significantly lower than those of FA (MW<1000) (Table 1), indicating higher degree of polymerization and higher content of aromatic compound of the former than that of the latter. Furthermore, H/C and O/C ratios of FA (MW<1000) were significantly higher than those of FA (MW>1000) (Table 1), showing higher content of carbohydrate of FA (MW<1000) than that of FA (MW>1000).

3.2. FTIR spectra of humic substances

The relative intensities of the FTIR absorption bands for HA (MW>1000), FA (MW>1000) and FA (MW<1000) extracted from the compost were different to some extent (Figure 1). However, the FTIR spectra of the humic substances extracted in this study resembled those of soil HAs and FAs [23-26]. The absorption bands between 3400 and 3450 cm^{-1} is due to the stretching vibrations of OH or N-H groups with varying degrees of H bonding. The absorption bands in the 2830 to 2965 cm^{-1} region are assigned to the stretching vibrations of aliphatic CH, CH_2 and CH_3 side chain groups of the aromatic nuclei. The occurrence of the shoulder and/or band at 2615 cm^{-1} is attributed to the OH vibration of the carboxyl group. The spectra showed the peaks and/or bands at 1701-1719 cm^{-1} (carboxyl as well as aldehydic and ketonic carbonyl). The spectra also showed the peaks and/or bands at 1641-1682 cm^{-1} (C=C stretching vibrations in olefinic and aromatic compounds), 1381-1387 cm^{-1} (C-H deformation of CH_2 and CH_3, salts of carboxylic acid and/or aliphatic CH), and 1222-1288 cm^{-1} (C-O stretching vibrations of esters, ethers and

phenols). The band at around 1082 cm^{-1} was attributed to alcohols and carbohydrates. The FTIR spectra of FA (MW<1000) and FA (MW>1000) showed a strong absorption band or peak at 738 cm^{-1} (Figures 1b and 1c), which is attributed to the H-bonded OH stretching vibration of carboxylic groups. This is in good agreement with the higher total acidity of FA (MW<1000) and FA (MW>1000) among the humic substances (Table 1). In addition, the spectrum of FA (MW<1000) showed a strong absorption band at 513 cm^{-1} (Figure 1c), which is attributed to the deformation of COOH. The FTIR spectra of HA (MW>1000) and FA (MW>1000) showed a strong absorption band at 1641 cm^{-1} and 1682 cm^{-1} (C=C bond) (Figures 1a and 1b), respectively, while the spectrum of FA (MW<1000) showed weak absorption band at 1642 cm^{-1} (Figure 1c). This indicates greater weighted percentages of C=C bond in HA (MW>1000) and FA (MW>1000) than in FA (MW<1000). The FTIR spectrum of FA (MW<1000) showed greater absorption band at around 1719 cm^{-1} (C=O bond) (Figure 1c), while the spectrum of HA showed a weak absorption band at 1701 cm^{-1} (Figure 1a). This clearly indicates that C=O bond content is higher in FA (MW<1000) than in HA (MW>1000). This C=O bonding site is the main reaction site with metals (including heavy metals) [9-10,27]. The characteristics of the FTIR spectra (Figure 1) of the humic substances extracted from the compost coincides the results from elemental analyses (Table 1).

3.3. CPMAS ^{13}C NMR spectra of humic substances

Since the signals obtained by solid-state NMR spectroscopy are much broader than those by liquid-state, the resolution of the former is not as high as the latter. In liquid-state NMR, however, the samples of humic substances need to be dissolved by an alkali solvent, such as NaOH solution and hence

result in the alteration of their forms to some extent [28-29]. Due to the recent development of the applied technique for solid-state NMR, commercially available instruments are now incorporated dipolar decoupling, magic angle spinning and cross polarization, which thus make possible acquisition of high-resolution ^{13}C spectra from solids [29-32].
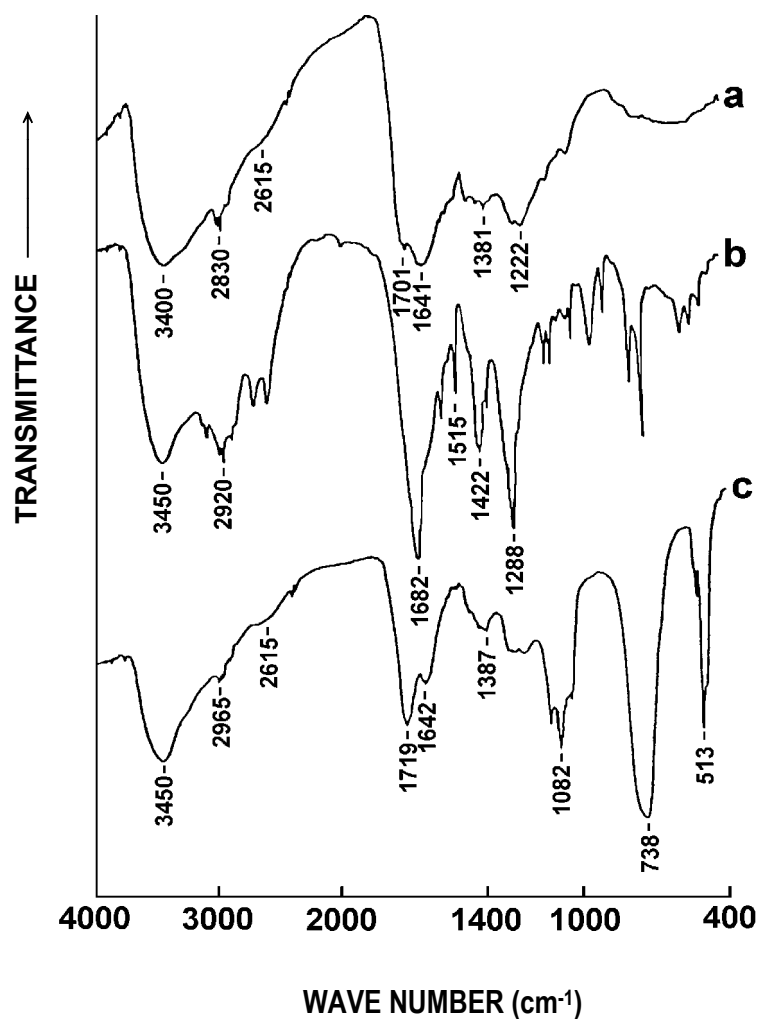

Figure 1. Fourier transform infrared spectra of humic substances extracted from the compost. Spectra a, b, and c are of HA (MW>1000), FA (MW>1000), and FA (MW<1000), respectively.

In this study, although it was in the same instrumental settings, the ^{13}C CPMAS NMR spectra of FA (MW>1000) and FA (MW<1000) (Figures 2b and 2c) showed a little weaker resonance absorption bands than that of HA (MW>1000) (Figure 2a). They were very similar, except for the relative intensities of resonance bands at some or in a narrow range of chemical shifts. In addition, they were very similar to those of humic substances extracted from soils [30,33-40]. The ^{13}C CPMAS NMR spectra were analyzed according to the chemical shift assignments made by Perminova [41] and Chefetz et al. [42]. The spectra clearly showed the presence of carbons in aldehydes and ketones (201 and

200 ppm), carbonyl carbon in carboxyl and ester groups (174 and 173 ppm), aromatic carbons (152, 130, 129, and 102 ppm), CO carbons-alcohols, esters, ethers, carbohydrates (73, 65, 63, and 56 ppm) and alkyl carbons (50, 35, 25, 23, and 10 ppm). According to the models proposed by Wilson [29] and Malcolm [31], the CPMAS ^{13}C NMR spectra of HA (MW>1000), FA (MW>1000) and FA (MW<1000) were divided into six ranges of chemical shifts in 0-50, 50-90, 90-110, 110-160, 160-190 and 190-210 ppm. The areas covered by the six sections of a spectrum were used to compute the distribution percentages of the characteristic carbons of HA (MW>1000), FA (MW>1000) or FA (MW<1000). The aliphatic carbon content of HA (MW>1000) was greater than those of FA (MW<1000) and FA (MW>1000). Their differences were significant (Table 2). The sequence of the carboxylic carbon content and that of the total functional group acidity were

FA (MW<1000) > FA (MW>1000) > HA (MW>1000) (Table 2 and Table 1), showing the main contribution of carboxylic carbon to total functional group acidity. Total amounts of functional group acidity of the humic substances derived from soils are mainly contributed by carboxyls and acidic hydroxyls, not associated with carbonyl groups [2,43]. The characteristic relation of carboxylic carbon contents to total acidity of the humic substances extracted from the refuse compost is thus the same as that of the humic substances derived from natural soils. Moreover, the acidic hydroxyls not associated with carbonyl groups and especially the carboxyls of humic substances mainly govern cation exchange and complexation reactions [3,7-8,10,20]. The sequence of the reactivities of the humic substances extracted from the refuse compost was thus FA (MW<1000) > FA (MW>1000) > HA (MW>1000).
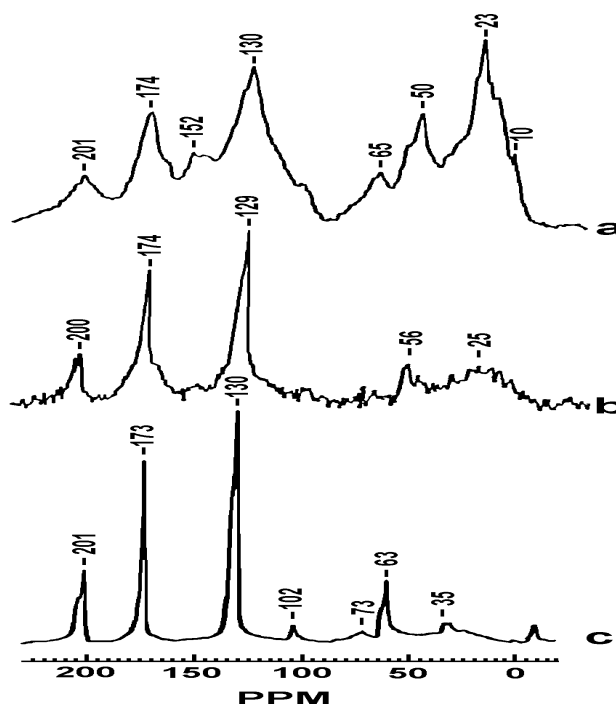

Figure 2. Solid-state ^{13}C nuclear magnetic resonance spectra with cross-polarization and magic-angle spinning (CPMAS ^{13}C NMR) of humic substances extracted from the compost. Spectra a, b, and c are of HA (MW>1000), FA (MW>1000), and FA (MW<1000), respectively.

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Table 2. Distribution percentages of the characteristic carbons in ^{13}C nuclear magnetic resonance spectra with cross-polarization and magic-angle spinning (CPMAS ^{13}C NMR) of humic substances extracted from the compost

Chemical shift and characteristic group C ppm	HA (MW>1000)	FA (MW>1000)	FA (MW<1000)
		%	
0-50	30.5a[†]	14.5c	20.8b
50-90	14.5a	16.0a	12.4b
90-110	3.40b	4.31a	4.36a
110-160[‡]	31.6a	36.0a	34.5ab
160-190[§]	14.1c	19.1b	21.8a
190-210	5.86b	10.1a	6.20b
Aliphatic C[¶]	48.4a	34.8b	37.6b

[†] Different lower case letters in rows indicate significant differences in means at $p < 0.05$ using Duncan's multiple range test.
[‡] Characteristic carbons in this range of chemical shift are assigned as aromatic C.
[§] Characteristic carbons in this range of chemical shift are assigned as carboxylic C.
[¶] Expressed by the sum of the percentages of the characteristic carbons in the ranges of chemical shifts of 0-50, 50-90 and 90-110 ppm.

4. Conclusions

The elemental compositions, atomic ratios, and FTIR and ^{13}C CPMAS NMR spectra of the HA (MW>1000), FA (MW>1000), and FA (MW<1000) extracted from refuse compost were close to those reported for soil, sediment, and water HA and FA. However, the FA (MW<1000) had exceptional high O and carboxylic C contents, O/C ratio, and total acidity. The reactivity of humic ligands plays role in governing the reaction of humic substances with heavy metals. Consequently, the effect of compost-derived humic substances in agriculture practice on the mobility and subsequent biotoxicity of heavy metals in soil and associated environments merits close attention.

Acknowledgments

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