

Characterization of Humic Substances Derived from Swine Manure-Based Compost and Correlation of Their Characteristics with Reactivities with Heavy Metals

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Various composts contain a significant amount of humic substances including humic acid (HA) and fulvic acids (FAs). The FA fraction in soils is considered to be sensitive to agronomic and environmental factors. In this study, three fractions of humic substances, HA (MW > 1000 Da), FA (MW > 1000 Da), and FA (MW < 1000 Da) were extracted from swine manure-based compost and characterized, and then, their reactivities were correlated with heavy metals. Compositions of the three fractions of humic substances were characterized by elemental and total acidity analyses and electron spin resonance (ESR), Fourier transform infrared (FTIR), and ¹³C nuclear magnetic resonance with cross-polarization and magic-angle spinning spectroscopic techniques. Elemental analyses indicated that HA has higher contents of C, H, N, and S than those of FAs. However, FA (MW > 1000) and especially FA (MW < 1000) have higher contents of O than that of HA (MW > 1000). The *g* values of the ESR spectra of the three fractions showed that the organic free radical characteristics and the widths of the spectra and free radical concentrations of the three fractions are significantly different. The FTIR spectra indicated that HA (MW > 1000) is abundant in C=C bonds while FA (MW > 1000), especially FA (MW < 1000), are abundant in C=O bonds. In addition, ¹³C NMR spectra indicate that carboxylic contents of FA (MW > 1000), especially FA (MW < 1000), are higher than that of HA (MW > 1000). The sequence of the reactivity in terms of acidic functional groups was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000). Elemental and functional group compositions of the three fractions significantly correlated with reported reactivities with heavy metals. The application of swine manure-based compost containing HA and FAs fractions to soil and associated environments may thus significantly affect the concerned reactions with organic and inorganic compounds including pollutants.

KEYWORDS: Swine manure-based compost; humic acid; fulvic acid; reactivity; total acidity

INTRODUCTION

The disposal of large quantities of organic wastes produced by municipal, agricultural, and agroindustrial activities causes energetic, economic, and environmental problems (1, 2), but agroindustrial waste such as swine manure waste, known for its high content in organic matter and mineral nutrients, could be used to restore soil fertility. In subtropical Taiwan, the extension of organic farming and sustainable agriculture has led to increasing applications of organic fertilizers including various composts derived mostly from agricultural and agroindustrial wastes. For manufacturing compost, composting is an absolutely necessary process and is defined as a process of aerobic thermophilic microbial degradation or an exothermic biological oxidation of various organic wastes by many popula-

tions of indigenous microorganisms, leading to the formation of a stabilized, mature, deodorized, hygienic product, free of pathogens and weed seeds, rich in humic substances, easy to store, and marketable as an organic amendment or fertilizer (3–5). Microorganisms involved in the biological degradation are bacteria, actinomycetes, and fungi, but in particular, microbial associations are highly effective to form the organic matter and speed up the humification processes (6). Hence, various composts of organic fertilizers contain substantial amounts of organic matter, with a significant amount of humic substances (7, 8).

Castaldi et al. (7) studied the composting process of municipal solid wastes and found that during the process of composting there was slight increase in the humic acid (HA) and fulvic acid (FA) contents and a higher rate of structural changes. Grube et al. (8) evaluated the composting process of sewage sludge-based compost by Fourier transform infrared (FTIR) spectroscopy and reported that the appearance of a well-pronounced intense band

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Table 1. Selected Physical and Chemical Properties of the Swine Manure-Based Compost^a

sample	pH	EC (mS cm ⁻¹)	g kg ⁻¹						mg kg ⁻¹					
			organic C	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	S	B
compost	5.76	3.18	493	20.3	6.80	2.18	16.1	5.50	1899	119	74	106	2058	273

^a All data are means of triplicates. The standard deviation of pH value is ± 0.04 , while those of the remaining data are less than 5% of their respective means.

of nitrate at 1384 cm⁻¹ was evidence for the maturity of sewage sludge-based compost.

Humic substances contain a variety of functional groups, including COOH, phenolic OH, enolic OH, alcoholic OH, quinone, hydroxyquinone, lactone, and ether (9). Many laboratory studies and review articles on the reaction between metals and humic substances have been reported in the literature (9–11). In soil and the associated environments, naturally occurring humic substances exert a 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 (12–15). HAs may exist in either dissolved or suspended form in natural aqueous systems. In the dissolved form, HAs have the ability to form complexes with metals, and when present as an organic solid phase, HAs provide a surface for metal adsorption from the aqueous system. Lin et al. (16) investigated the effect of water extract of swine manure-based compost (WEC) on the adsorption of arsenate by two calcareous soils. They reported that organic anions of dissolved organic carbon of WEC and arsenate oxyanions competed for binding sites on soils. Using the same batch of swine manure-based compost, Lin et al. (17) studied the complexation of arsenate with humic substances in WEC. They suggested that cations, such as Ca and Mg, and especially Fe, Al, and Mn, act in cation bridging in the complexation of arsenate with humic substances (verified as FA).

In all of these studies reported on the characterization and related reactivities of humic substances, only HAs and FAs were fractionated without any emphasis on further separation and subsequent characterization of FA (MW > 1000) from FA (MW < 1000). Swine manure-based compost is being extensively applied to agricultural soils to improve crop production and quality in Taiwan. Organic ligands derived from humic substances of swine manure-based compost react with heavy metals to form complexes and influence the concentrations of free and labile heavy metal ions (11). The complexation with organic ligands may affect the subsequent mobilities and biotoxicities of heavy metals in soil and the associated environments. Hence, the application of swine manure-based compost to the soils affects the agronomical and environmental factors of soils substantially. In the studies reported by Aiken (18), Hayes (19), Leenheer (20), and Swift (21), both fractions of FAs [FA (MW < 1000) and FA (MW > 1000)] operationally defined as the FA fraction were conventionally extracted and grossly purified and then subjected to characterization without the isolation of FA (MW < 1000) and FA (MW > 1000), although Wang and Chang (22) carried out the extraction of humic substances from a Taiwanese soil and then separated the fractions of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000). Therefore, the characterization of elemental and functional group compositions of FA (MW < 1000) and FA (MW > 1000) extracted from the organic matter of compost related materials such as swine manure-based compost has not so far been achieved. The aim of this study was the extraction and subsequent purification of HA and FAs with specific

emphasis on the separation of FA (MW > 1000) and FA (MW < 1000) fractions of swine manure-based compost, characterization by analytical and spectroscopic techniques, and correlation of the elemental and functional group compositions of the three fractions of humic substances with reported reactivities with heavy metals. Thus, this study emphasized the importance of the lower molecular weight FAs for the agronomic value of compost and its effects on supplying plant nutrients and governing the reactions with heavy metals in soil.

MATERIALS AND METHODS

Materials. The swine manure-based compost was purchased from the former Talin Sugarcane Mill, Taiwan Sugar Co. (Talin Township, Chia-I County, Taiwan), to perform the experiment. For the treatment of swine manure from the plant in a swine farm, the suspension of swine manure combined with flushed water from the floor of the swine house was mechanically filtered to separate the solid waste from the liquid waste. The liquid waste portion was subjected to further treatment to combine it with water used for sugarcane farm irrigation. The solid waste was collected for further composting to manufacture swine manure-based compost. Although the maturity of the bagged swine manure-based compost was guaranteed by the manufacturing company, for certainty of sufficient maturity of the compost during the experimental process, the purchased compost was examined for maturity before it was used as experimental material in the laboratory. Several bags of purchased swine manure-based compost were poured out together on a clean plastic cloth (washed with detergent solution and then rinsed with tap water and deionized water) and then thoroughly mixed. Using the quadruple division method, an adequate amount of representative compost sample was taken and ground using a Warning blender to pass through a 0.84 mm sieve and then put into a clean plastic dish (washed with detergent solution and then rinsed with tap water and deionized water) with a size of around 50 cm in length, 30 cm in width, and 15 cm in depth. A total of 10 plastic dishes were evenly filled with ground swine manure-based compost until around 10 cm depth and then placed on the shelves in a ventilation cabinet. An adequate and equal amount of ground compost was taken from each of the 10 plastic dishes and mixed as a composite sample once in a week. Each time interval of composite sample of the swine manure-based compost was subjected to the determinations of organic carbon (C), total nitrogen (N), and then the calculation of C/N ratio for testing the maturity of purchased swine manure-based compost. Another portion of the air-dried composite swine manure-based compost was subjected to the analyses of physical and chemical characteristics. The pH of the compost in deionized water (1:5, w/v) was measured using a pH meter model 682 Titroprocessor (Metrohm, Swiss) (23). The filtrate of the suspension for measuring compost pH was also used to measure the electrical conductivity (EC) of the compost (24). After the swine manure-based compost was digested with 0.01 M HCl (25), its organic C and total N contents were determined by a Heraeus CHN-O-rapid elemental analyzer. After the swine manure-based composts were digested with concentrated HNO₃ and 30% H₂O₂, they were analyzed for contents of P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B by an inductively coupled plasma atomic emission spectrometer (26). All of the measurements and determinations were of triplicates of the composite compost sample. Selected physical and chemical compositions of the swine manure-based compost are presented in **Table 1**.

Extraction and Purification of Compost Humic Substances. Five hundred grams of the matured and ground swine manure-based compost was taken from each of the 10 plastic dishes in the cabinet and then

Table 2. Elemental Analyses (Expressed on Ash-Free Weight Basis) as Well as Atomic Ratios and Total Functional Group Acidity of Humic Substances Extracted from the Swine Manure-Based Compost^a

humic substance	elemental content (g kg ⁻¹)					atomic ratios			total acidity (mequiv g ⁻¹)
	C	H	N	O	S	H/C	C/N	O/C	
HA (MW > 1000)	539 a	55.9 a	66.0 a	325 c	14.2 a	1.24 b	9.52 b	0.45 c	7.98 c
FA (MW > 1000)	450 b	50.4 b	52.4 b	435 b	12.2 a	1.33 b	10.0 b	0.73 b	11.1 b
FA (MW < 1000)	417 c	57.6 a	23.1 c	503 a	0.2 b	1.65 a	21.1 a	0.91 a	14.9 a

^a Means of the data followed by different lower case letters in columns indicate significant differences in means at $P < 0.05$ using Duncan's multiple range test.

thoroughly mixed as a composite sample for the extraction of humic substances. HA and FA were extracted from the composite swine manure-based compost and purified by following the methods developed by the International Humic Substances Society (18–21). A brief description of the extraction and purification procedure is as follows. Compost samples were treated with 0.1 M NaOH solution under N₂ atmosphere several times at a 1:10 (w/v) ratio to extract the humic substances until the extracts were clear. All of the extracts were combined and acidified to pH 1.0 with 6 M HCl. The acidified solution was equilibrated at room temperature for 24 h and then centrifuged at 2000g to separate the HA fraction from the FA fraction (supernatant). The precipitated HA fraction was suspended in a 0.1 M HCl:0.3 M HF solution in plastic containers and shaken overnight at room temperature. The suspension was centrifuged at 2000g to precipitate the HA fraction, suspended in deionized distilled water, and then transferred to dialysis tubes to separate the fraction of HA (MW > 1000). The FA solution was purified by using an adsorption resin XAD-8, and the 0.1 M NaOH alkaline eluate was passed through H⁺-saturated cation exchange resin. The purified H⁺-saturated FA solution was concentrated using a rotary evaporator to an adequate volume by the method proposed by Wang and Huang (27). The concentrated FA solution was transferred to dialysis tubes with a molecular weight cutoff of 1000 to separate the fraction 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 FA (MW < 1000). All HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) fractions were freeze-dried, and their yields were determined. The extractions and subsequent purifications of the humic substances were carried out in triplicate.

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 a Tacussel coulmax 78 automatic coulometric titrator. The elementary composition was calculated and referred to on an ash-free basis. The three replicated HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000) samples were mixed thoroughly and made three composite samples based on the yield ratios. The electron spin resonance (ESR), FTIR, and ¹³C nuclear magnetic resonance (¹³C NMR) spectra of the three composite HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) samples were recorded for further qualitative and/or semiquantitative comparison with ESR, FTIR, and ¹³C NMR spectra obtained individually for three of each of the HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000) samples derived from the swine manure-based compost. The ESR spectra of the three fractions of humic substances were obtained with a Bruker ER EMX-10 spectrometer operating at 100 kHz and a frequency of 9620 MHz at room temperature. The FTIR spectra of the freeze-dried samples of the three fractions of humic substances were obtained using KBr discs (each disc contained 1 mg of sample and 300 mg of KBr). The spectra were recorded in the 400–4000 cm⁻¹ range on a Bio-Rad FTS-7 FTIR spectrophotometer with a spectral resolution of 3 cm⁻¹. The freeze-dried samples of the three fractions of humic substances were subjected to solid-state ¹³C NMR analysis using a Bruker MSL-200 spectrometer. It was set with cross-polarization and magic-angle spinning (CPMAS), proton decoupling, a spectrometer frequency of 50.33 MHz, an acquisition time (delay time) of 25.6 ms, a contact time of 1 ms, a recycle time of 1 s, and a magic angle spinning rate of around 3.5 kHz. The total acidity values of the freeze-dried samples of the three fractions of humic substances were determined by the

titration method as described by Schnitzer and Khan (28). 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 (29) and Duncan's multiple range test at $P < 0.05$ (30).

RESULTS AND DISCUSSION

Yields and Elemental Analyses of Humic Substances.

Yields of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) extracted from the swine manure-based compost were 98.1, 0.4, and 0.6 g kg⁻¹, respectively. This indicates that the yield of HA (MW > 1000) was substantially higher than those of FA (MW > 1000) and FA (MW < 1000), becoming the major component of organic matter of the swine manure-based compost. The C, H, N, O, and S contents of the extracted humic substances are presented in **Table 2**, and these results indicate that C, H, N, O, and S contents of the extracted humic substances extracted from the swine manure-based compost are close to those of natural humic substances derived from soils and waters (22, 28, 31). Chang Chien et al. (32) studied the fertilizer effect (including swine manure-based compost and this compost complemented with various chemical fertilizer nitrogen rates) applied to a paddy-upland rotation on characteristics of HAs (MW > 1000). They found that the average contents of C, H, N, O, and S of HAs extracted from the surface soils of the four replicated plots of the seven treatments were in the ranges of 50.0–50.8, 5.19–5.56, 3.88–4.74, 33.8–37.5, and 0.40–0.67%, respectively. As compared with the contents of C, H, N, O, and S of HA (MW > 1000) extracted from the swine manure-based compost (**Table 2**) to those of HAs (MW > 1000) extracted from the surface soils amended with swine manure-based compost (32), the data showed that after long-term application of swine manure-based compost to soil, the contents of C, H, N, O, and S of HA (MW > 1000) of the compost were changed due to the interactions mediated by physical, chemical, and biological processes occurring in the soil environment involving soil–crop root systems. Furthermore, the observed changes are significant in the cases of N and S, which serve as plant nutrients, as compared to C, O, and H (**Table 2**; 32). This emphasizes how various elements of HAs/organic matter are utilized in soil–crop root systems. The sequence of the C and N contents of the humic substances extracted from the swine manure-based compost was HA (MW > 1000) > FA (MW > 1000) > FA (MW < 1000), and their differences were significant. However, the sequence of the O content was in a reverse trend with significant differences (**Table 2**). With higher C and N contents, the HA (MW > 1000) serves as an organic nutrient supplier in soils. It shows significant correlation at $P < 0.01$ with a correlation coefficient of 0.979** between the O content and the total acidity of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) (**Table 2**). In addition to the water-soluble characteristics of FAs under all pH conditions (33), higher total acidities of FA (MW > 1000) as well as FA (MW < 1000) have higher capacities to complex

or chelate with metals, including heavy metals (11); hence, FAs serve as nutrient transporters in soil environments. The H content of the FA (MW > 1000) was significantly lower than those of HA (MW > 1000) and FA (MW < 1000), while the S content of the FA (MW < 1000) was significantly lower than those of HA (MW > 1000) and FA (MW > 1000) (Table 2). The lowest C and N contents and the highest O content of the FA (MW < 1000) result in the highest H/C, C/N, and O/C atomic ratios. The sequence of the O/C atomic ratio of the humic substances derived from the swine manure-based compost was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000) with significant differences. This sequence was also found in the humic substances extracted from a Taiwan soil reported by Wang and Chang (22), showing the close relation between humic substances derived from the organic matter of compost and that of the soil environment. Moreover, the sequence of total acidity of the humic substances derived from the swine manure-based compost coincided with that of the O/C atomic ratio (Table 2). This indicates that O-containing functional groups of the humic substances derived from the swine manure-based compost contributed to their total acidity. Furthermore, the highest O/C atomic ratio and total acidity of the FA (MW < 1000) among the humic substances extracted (Table 2) implicate its significant importance of O content. Steelink (34) used the O/C atomic ratios of humic substances to predict their origins. He found that O/C atomic ratios 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 atomic ratios of the HA (MW > 1000) and FA (MW > 1000) in this study were close to those reported for natural HA and FA, respectively, while that of the FA (MW < 1000) was higher (Table 2). In addition, Steelink (34) and Ussiri and Johnson (35) reported that humic substances had a higher degree of polymerization and a higher content of aromatic compound if their H/C and O/C atomic ratios were lower and had a higher content of carbohydrate if their H/C and O/C atomic ratios were higher. In this study, H/C and O/C atomic ratios of the HA (MW > 1000) extracted were significantly lower than those of FA (MW < 1000) (Table 2), indicating a higher degree of polymerization and a higher content of aromatic compounds in HA (MW > 1000). Furthermore, H/C and O/C atomic ratios of FA (MW < 1000) derived from the swine manure-based compost were significantly higher than those of FA (MW > 1000) (Table 2), suggesting a higher carbohydrate content of FA (MW < 1000) than that of FA (MW > 1000). Moreover, results from the separation indicated that the yield of FA (MW < 1000) was 1.5 times greater than that of FA (MW > 1000). This study further demonstrated the distinct properties of FA (MW < 1000) derived from swine manure-based compost, which was significantly different from those of FA (MW > 1000) as reported in Table 2. The separation of FA (MW < 1000) from FA (MW > 1000) of extraction and purification and their subsequent characterization of compost and its related materials thus merit close attention.

ESR Spectra of Humic Substances. There were not many differences among the three replicated ESR spectra of HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000) extracted from the swine manure-based compost and the spectra of their respective composite humic substances (not shown). Moreover, there were no apparent differences among the ESR spectra of the composite HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) of the compost except in spectral width. Therefore, to label the specific organic free radical characteristics and spectral widths, the ESR spectrum of the composite HA

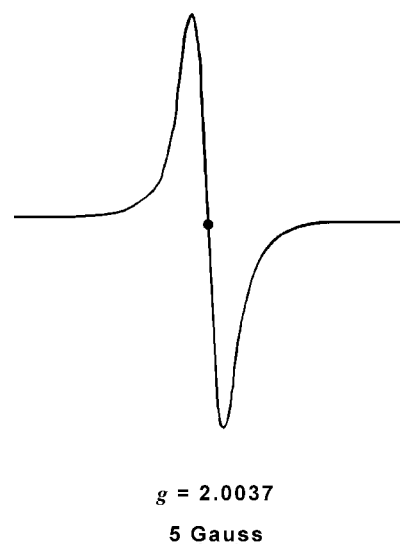


Figure 1. ESR spectra of HA (MW > 1000).

Table 3. Characteristic Parameters of ESR Spectra of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) Derived from Swine Manure-Based Compost^a

humic substance	g value	width (G)	free radical concentration (spins g ⁻¹)
HA (MW > 1000)	2.0037 a	4.15 c	3.6 × 10 ¹⁷ a
FA (MW > 1000)	2.0036 a	4.47 b	5.3 × 10 ¹⁵ b
FA (MW < 1000)	2.0037 a	4.92 a	9.3 × 10 ¹⁴ c

^a Explained in Table 2.

(MW > 1000) derived from the swine manure-based compost is shown as an example (Figure 1). All nine ESR spectra of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) of the compost were single lines, devoid of hyperfine splitting. The means of g values, widths between two extreme peaks on the derivative curves, and the free radical concentrations of the three fractions of humic substances of the compost are summarized in Table 3. The means of g values of the HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) derived from the swine manure-based compost were 2.0037, 2.0036, and 2.0037, respectively, indicating that the main free radicals of the three humic substances were semiquinone or substituted semiquinone (36). Schnitzer (37) reported the g values in the range of 2.0032–2.0050 and the spectral widths from 2.5 to 4.0 G for ESR spectra of HAs and FAs extracted from soils of widely differing climatic zones. He also reported that the free radical concentrations of HA and FA solutions were of the order of 10¹⁷–10¹⁸ spins g⁻¹. Wang and Chang (22) reported that the g values ranged from 2.0036 to 2.0037 and the widths ranged from 3.76 to 5.70 G for ESR spectra of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) extracted from a Taiwanese soil. They also reported that the free radical concentrations of the three humic substances were in the order of 10¹⁴–10¹⁷ spins g⁻¹. Chang Chien et al. (32) reported that the g values ranged from 2.0035 to 2.0036 and the widths ranged from 3.55 to 3.72 G for ESR spectra of HA (MW > 1000) extracted from the surface soils of the treatments applied with swine manure-based compost and the compost supplemented with various chemical fertilizer nitrogen rates. They also reported that the free radical concentrations of the HA (MW > 1000) extracted from the surface soils of the treatments were of the order of 10¹⁸ spins g⁻¹. In our study also, the means of the g values, spectral widths, and free radical concentrations

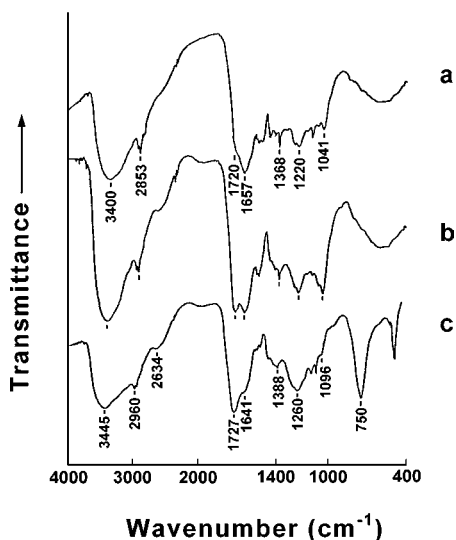


Figure 2. FTIR spectra of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000). Spectra a, b, and c are of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000), respectively.

obtained by ESR spectra of the three fractions of humic substances derived from swine manure-based compost were in the reported ranges (Table 3), showing the close relevance of the characteristic parameters of ESR spectra of humic substances derived from compost to those of humic substances extracted from soils with the amendment of compost. However, there were significant differences in the means of widths and of free radical concentrations of the ESR spectra of the three fractions of humic substances derived from the swine manure-based compost (Table 3). The sequence of the spectral width was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000), while that of the free radical concentration was in a reverse trend. This shows that the parameters, ESR spectral width, and free radical concentration serve as the indicator characteristics for degree of humification of humic substances derived from the organic matter of swine manure-based compost.

FTIR Spectra of Humic Substances. Examination of FTIR spectra of the three replicated HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000) samples derived from swine manure-based compost and the spectrum of their respective composite humic substance (not shown) revealed that there were no significant differences in absorption band positions. However, as seen from Figure 2, the relative intensities of the FTIR absorption bands for composite HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) have some differences. The FTIR spectra of the humic substances extracted from swine manure-based compost resembled those of soil and water HAs and FAs (22, 31, 37, 38). Moreover, the spectrum of composite HA (MW > 1000) sample (Figure 2a) resembled that of HA (MW > 1000) extracted from surface soils of the plots with long-term application of this swine manure-based compost (32) in spectral peaks, bands, and/or shoulders positions but not in their relative intensities, showing that the inherited transformations of compost-derived HA due to physical, chemical, and biological processes occurred in the soil environment.

The qualitative and semiquantitative information of the functional groups of humic substances derived from swine manure-based compost were elucidated by assigning the peaks, bands, and/or shoulders of FTIR spectra as proposed by Stevenson (9) and Silverstein and Webster (39). The absorption bands between 3400 and 3445 cm^{-1} are due to the stretching vibrations of OH or N–H groups with varying degrees of H

bonding. The absorption bands in the 2853–2960 cm^{-1} region are assigned to the stretching vibrations of aliphatic CH, CH₂, and CH₃ side chain groups of the aromatic nuclei. The occurrence of the shoulder at 2634 cm^{-1} is attributed to the OH vibration of the carboxyl group. The spectra showed the peaks and/or bands at 1720–1727 cm^{-1} (carboxyl as well as aldehydic and ketonic carbonyl). There are peaks and/or bands at 1641–1657 cm^{-1} (C=C stretching vibrations in olefinic and aromatic compounds), 1368–1388 cm^{-1} (C–H deformation of CH₂ and CH₃, salts of carboxylic acid and/or aliphatic CH), and 1220–1260 cm^{-1} (C–O stretching vibrations of esters, ethers, and phenols) in the spectra. The bands at 1041–1096 cm^{-1} are attributed to alcohols and carbohydrates. The FTIR spectrum of composite FA (MW < 1000) showed a strong absorption band at around 750 cm^{-1} (Figure 2c), which is attributed to the H-bonded OH stretching vibration of carboxylic groups. This is in good agreement with the highest total acidity of FA (MW < 1000) among the humic substances derived from swine manure-based compost (Table 2). The FTIR spectrum of composite HA (MW > 1000) showed a strong absorption band at 1657 cm^{-1} (C=C bond) (Figure 2a), while the spectra of both composite FA (MW > 1000) and composite FA (MW < 1000) showed a relatively weak absorption band and shoulder, respectively (Figure 2b,c). This indicates more C=C bonds in HA (MW > 1000) than those in FA (MW > 1000) and FA (MW < 1000). The FTIR spectra of composite FA (MW > 1000), especially composite FA (MW < 1000), showed a stronger absorption band at around 1720–1727 cm^{-1} (C=O bond) (Figure 2b,c), while the spectrum of composite HA (MW > 1000) showed a weak absorption shoulder (Figure 2a). This clearly indicates that C=O bond contents are higher in both FA (MW > 1000) and FA (MW < 1000) than in HA (MW > 1000). Moreover, the absorption band at 1720–1727 cm^{-1} of the FTIR spectrum of composite FA (MW < 1000) was much stronger than that of composite FA (MW > 1000) (Figure 2b,c), indicating more C=O bonds in FA (MW < 1000) than in FA (MW > 1000). This C=O bonding site is the main reaction site with metals (including heavy metals) (12–15, 40). The characteristics of the FTIR spectra (Figure 2) of humic substances derived from the swine manure-based compost are consistent with the results from both elemental and total acidity analyses (Table 2). Furthermore, the FTIR spectral studies confirmed that FA (MW < 1000) has more content of reactive functional groups such as C=O and carboxylic groups, which may be correlated to the higher reactivity of FA (MW < 1000) than FA (MW > 1000) and HA (MW > 1000) as revealed by the study of Chang Chien et al. (11).

CPMAS ¹³C NMR Spectra of Humic Substances. The CPMAS ¹³C NMR spectra of the three replicated HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000) samples derived from the swine manure-based compost and that of their composite humic substance were found to be very similar (not shown). In this study, with the same instrumental settings, the CPMAS ¹³C NMR spectra of composite FA (MW < 1000) (Figure 3c) showed a little weaker resonance absorption peaks than those of composite HA (MW > 1000) and composite FA (MW > 1000) (Figure 3a,b). They were similar, except for the relative intensities of resonance absorption bands at some or in a narrow range of chemical shifts. In addition, they were similar to those of humic substances extracted from soils (22, 41–43). The CPMAS ¹³C NMR spectra were analyzed according to the assignment of chemical shifts by Silverstein and Webster (39), Perminova et al. (44), and Chefetz et al. (45). The spectra clearly showed the presence of carbons in aldehydes

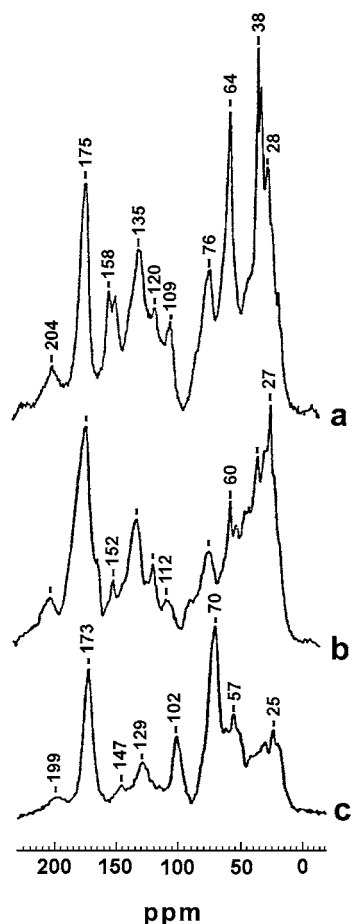


Figure 3. Solid-state CPMAS ^{13}C NMR spectra of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000). Spectra a, b, and c are of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000), respectively.

and ketones (204 and 199 ppm), carbonyl carbon in carboxyl and ester groups (175 and 173 ppm), aromatic carbons (158, 152, 147, 135, 129, 120, 112, 109, and 102 ppm), CO carbons in alcohols, esters, ethers, carbohydrates (76, 70, 64, 60, and 57 ppm), and alkyl carbons (38, 28, 27, and 25 ppm). According to the models proposed by Malcolm (46) and Wilson (47), the CPMAS ^{13}C NMR spectra of composite HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) were divided into six ranges of chemical shifts, 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 composite HA (MW > 1000), FA (MW > 1000), or FA (MW < 1000). The sequence of the aliphatic carbon content of the humic substances extracted was FA (MW > 1000) > HA (MW > 1000) > FA (MW < 1000), while that of the aromatic carbon content was HA (MW > 1000) > FA (MW < 1000) > FA (MW > 1000) and their differences were significant (Table 4). These data were in good agreement with the results revealed by the FTIR spectra of the same humic substances (Figure 2). The sequence of the carboxylic carbon content and that of the total functional group acidity was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000) (Tables 4 and 2), 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 (28, 48). The characteristic relation of carboxylic carbon contents to total acidity of the humic substances derived from the swine manure-

Table 4. Distribution Percentages of the Characteristic Carbons in CPMAS ^{13}C NMR Spectra of Humic Substances Extracted from the Swine Manure-Based Compost^a

chemical shift (ppm) and characteristic group C assignment	HA (MW > 1000) (%)	FA (MW > 1000) (%)	FA (MW < 1000) (%)
0–50	30.7 b	22.7 c	35.1 a
50–90	22.9 b	33.9 a	16.7 c
90–110	4.93 b	7.94 a	3.07 c
110–160 ^b	24.2 a	14.6 c	17.5 b
160–190 ^c	13.1 c	16.4 b	22.1 a
190–210	4.03 c	4.65 b	5.57 a
aliphatic C ^d	58.5 b	64.7 a	54.9 c

^a Means of the data followed by different lower case letters in rows indicate significant differences in means at $P < 0.05$ using Duncan's multiple range test.

^b Characteristic carbons in this range of chemical shift are assigned as aromatic C.

^c Characteristic carbons in this range of chemical shift are assigned as carboxylic C.

^d 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.

based 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 (10, 13).

Correlation of Reactivities with Characteristics of Humic Substances and Their Significance.

Chang Chien et al. (11) investigated the average conditional concentration quotients of the complexes formed from the reaction of metals with humic substances including HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) extracted from the same batch of swine manure-based compost that is used in this study. They found that for one kind of metal the sequence of the reactivity expressed as average conditional concentration quotients of the humic substances was FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000). In this study, on the basis of the summarized results from elemental and total acidity functional groups and FTIR and CPMAS ^{13}C NMR spectra analyses of three fractions of humic substances derived from the swine manure-based compost, the sequence of the reactivity in terms of acidic functional groups that are responsible for reactivities of humic substances was also FA (MW < 1000) > FA (MW > 1000) > HA (MW > 1000) (Tables 2 and 4 and Figures 2 and 3). Among the humic substances, the fractionated FA (MW < 1000) has the highest O and carboxylic C contents, O/C atomic ratio, and total acidity, which were in good agreement with the relative higher reactivity of FA (MW < 1000) than those of HA (MW > 1000) and FA (MW > 1000). The correlations of carboxylic C contents (Table 4) with total acidities and O contents (Table 2) of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) give correlation coefficients 0.995** and 0.959**, respectively, indicating significant correlations at the $P < 0.01$ probability level by the analysis of variance. In addition, the correlations of carboxylic C contents of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) (Table 4) with the average conditional concentration quotients of these three fractions of humic substances reacting with Pb, Cu, Cd, and Zn at initial pH 4.00 and 6.50 (11) were also analyzed. The data in Table 5 show the significant correlations at $P < 0.05$ and mostly at $P < 0.01$ probability levels except for the humic substances reacting with Zn carried out at 1:0.05 in molar (1:0.1 in equivalent) ligand: metal stoichiometry at pH 6.50. This clearly indicates that carboxylic C contents, total acidities, and O contents of the three fractions of humic substances derived from swine manure-based

Table 5. Correlations of Carboxylic C Contents of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) with the Average Conditional Concentration Quotients of the Three Fractions Obtained by Reactivities with Pb, Cu, Cd, or Zn at Initial pH 4.00 and 6.50

initial pH	correlation coefficient and its significant level ^a			
	Pb	Cu	Cd	Zn
4.00	0.987**	0.950**	0.976**	0.670*
	0.989**	0.886**	0.973**	0.963**
	0.989**	0.951**	0.920**	0.973**
6.50	0.997**	0.990**	0.977**	NS ^b
	0.993**	0.995**	0.954**	0.960**
	0.998**	0.919**	0.868**	0.672*

^a Each data set for each heavy metal at pH 4.00 or 6.50 indicates that the correlation coefficients for the reactions of HA (MW > 1000), FA (MW > 1000), and FA (MW < 1000) with Pb, Cu, Cd, or Zn were carried out at 1:0.05, 1:0.25, and 1:0.5 in molar (1:0.1, 1:0.5, and 1:1 in equivalent) ligand:metal stoichiometry, in which all of the experiments were carried out in triplicate. ^b Not significant. ** and * indicate significant correlations at 1 and 5% probability levels, respectively, by the analysis of variance.

compost are in good correlation with the reactivities of the three fractions of humic substances with heavy metals. According to the operational definition in the scope of studying humic substances, HA is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2) but becomes soluble at higher pH conditions; FA is the fraction of humic substances that is soluble under all pH conditions (33). Therefore, on the basis of the consideration of characteristic solubility of FA and its related reactivity in terrestrial and aquatic ecosystems, the extraction, subsequent purification, and characterization of FA (MW > 1000) and especially FA (MW < 1000) from environmental and associated materials deserve significance. Furthermore, large amounts of manure-based compost incorporated with or without chemical fertilizers have been frequently applied to agricultural soils. The roles of humic substances including HA (MW > 1000), FA (MW > 1000), and especially FA (MW < 1000) derived from manure-based compost in soil and associated environments thus deserve attention.

To corroborate the field study with long-term amendment of swine manure-based compost to agricultural soil, the characteristics of HA obtained in the study showed the inherited transformations of humic substances in soil environment. Furthermore, because of frequent application of manure-based compost to agricultural soils, the roles of humic substances including HA (MW > 1000), FA (MW > 1000), and especially FA (MW < 1000) derived from manure-based compost in supplying plant nutrients and governing the reactions with organic and inorganic compounds including pollutants in soil and associated environments deserve further studies.

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