

Effects of Pig Slurry Application on Soils and Soil Humic Acids

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The effect of three annually consecutive additions of pig slurry at two rates (90 and 150 m³ ha⁻¹ year⁻¹) on soils and soil humic acids (HAs) was investigated in a field experiment under semiarid conditions. Soils and pig slurries were analyzed by standard methods. The HAs were isolated from soils and pig slurry by a conventional procedure based on alkaline extraction, acidic precipitation to pH 1, purification by repeated alkaline dissolutions and acidic precipitations, water washing, dialysis, and final freeze-drying. The HAs obtained were analyzed for elemental (C, H, N, S, and O) and acidic functional group (carboxylic and phenolic) composition, and by UV–vis, FT-IR, fluorescence, and ESR spectroscopies. With respect to the control soil, the pig slurry amended soils had greater pH and electrical conductivity, slightly larger total N content, and smaller values of C/N ratio. A decrease of total organic C was observed only in soils amended for 2 and 3 years at the higher slurry rate. With respect to control soil HA, pig slurry HA was characterized by larger contents of S- and N-containing groups, smaller acidic functional group and organic free radical contents, a prevalent aliphatic character, extended molecular heterogeneity, and smaller aromatic polycondensation and humification degrees. Amendment with pig slurry HA determines a number of modifications in soil HAs, including increase of C, S, and COOH contents, C/N ratios, and aliphaticity and decrease of extraction yields and N, O, phenolic OH, and organic free radical contents. These effects are generally more evident after the first year of slurry application and tend to disappear with increasing number of treatments. Most probably, over the years the slightly humified slurry HA is mineralized through extended microbial oxidation, whereas only the most recalcitrant components, such as S-containing, phenolic, and aliphatic structures, are partially accumulated by incorporation into soil HA.

KEYWORDS: Pig slurry; soil amendment; humic acids; elemental analysis; functional groups; FT-IR spectroscopy; fluorescence spectroscopy; ESR spectroscopy

INTRODUCTION

The use of animal manures as fertilizers has been practiced since the beginning of agriculture in order to improve soil fertility and productivity, especially in semiarid agroecosystems where the low organic matter content may favor soil degradation and erosion (1, 2). This practice has raised, however, serious environmental concern in recent years, due to the frequent excessive application rates of animal wastes of various origins and natures.

Pig breeding is characterized by confined growth facilities where slurries are collected in great amounts; thus, it may occur that surrounding soils receive large amounts of slurry every year and for several consecutive years. Uncontrolled application of

these slurries to soil can generate, among other effects, an excess of nitrates, salts, undesirable microorganisms, pathogens, and greenhouse gas emissions (3, 4). Furthermore, pig manure may represent a potential source of pollution also for the atmosphere and water if the amounts, methods, and times of disposal are not properly monitored.

Although pig slurry (PS) is not rich in organic matter compared to other manures (5), it has frequently produced favorable effects in improving soil quality and fertility, with respect to inorganic fertilizers (6). The decomposition of PS in soils has been evaluated in several laboratory and field studies. Large variations have been measured in decomposition rates of different slurries in laboratory incubation studies (7, 8). These differences have been mainly ascribed to the variable composition of pig slurry, which may result from various factors including animal type and storage and handling conditions. For example, Bernal and Kirchmann (9) found that ~75% of C added to soil with PS was mineralized after 70 days in a

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laboratory incubation, whereas Saviozzi et al. (10) reported a value of 44% mineralization after 230 days under similar conditions. Results of a long-term field study showed a rapid increase in soil microbial biomass, which paralleled PS decomposition and was independent of the dose employed (11). The same experiment also showed that half of the total CO₂ emissions occurred during the first week after PS addition and that soil C content was not significantly increased after 19 consecutive years of PS application.

Furthermore, the nature of organic matter contained in PS, in comparison to native soil organic matter, has been investigated in several chemical and physicochemical studies. Giusti et al. (12) reported that the molecular structure of the acid-insoluble and acid-soluble fractions of total organic carbon dissolved in PS was similar, respectively, to that of humic acids (HAs) and fulvic acids isolated from soils and other organic wastes. These authors concluded that organic matter dissolved in PS could easily enter into the natural soil C cycle. The PS HAs were characterized by extended molecular heterogeneity, small degree of aromaticity and structural polycondensation, prevalent aliphatic character, small acidic functional group content, and a marked presence of proteinaceous and polysaccharidic components (13, 14). All of these properties are typical of a small humification degree and are very different from those of native soil HAs.

Although the ascertained chemical, compositional, structural, and functional differences between PS HA and native soil HA may be reasonably expected to induce more or less extended modifications of HAs in amended soils, as a function of amendment rate and number, considerably less experimental work has been done on this subject. Knowledge of the type and extent of soil HA changes possibly caused by any organic amendment is of great importance for a better evaluation of this practice. The use of spectroscopic methods combined with chemical approaches can provide valuable information in this respect (15).

The objectives of this study were to investigate the extent to which the rate and number of PS applications can affect general soil properties and the compositional, structural, and functional properties of soil HA, by use of chemical methods and spectroscopic techniques including elemental and functional group analyses, E₄/E₆ ratio, and FT-IR, fluorescence, and ESR spectroscopies.

MATERIALS AND METHODS

Pig Slurries, Soils, and Field Experiment. Samples of PS were collected from a pig-breeding farm located in the Toledo province, Spain, which employs a closed-cycle production system. The field experiment was conducted on a sandy loam soil (sand, 59%; silt, 22%; clay, 19%) classified as a Typic Haploxeralf (16) on the experimental farm "La Higuera" located in Santa Olalla, very close to the pig farm. The site is characterized by a continental semiarid climate with an average annual rainfall of ~487 mm and an average annual temperature of 14 °C.

The experimental design included plots (10 × 8 m²) cropped to barley (*Hordeum vulgare* L.) and amended with PS at rates of 90 (PS90) and 150 (PS150) m³ ha⁻¹ year⁻¹ over a 3-year period. One plot was not amended and represented the control soil (C). The PS was applied once per year, prior to barley planting (mid-October of 1997, 1998, and 1999), by spreading from a tank and immediate incorporation by disking to a 0–15-cm depth of soil. Three replicates were performed for each treatment. Soon after barley harvest (late June of 1998, 1999, and 2000), surface soil subsamples (Ap horizon, 0–15-cm depth) were collected randomly from each plot. A composite soil sample was then obtained for each treatment by mixing equal amounts of the three corresponding soil subsamples.

Soil and Pig Slurry Analysis. Prior to analyses, soil samples were air-dried, crushed, and passed through a 2-mm sieve after removal of plant residues and stones.

The principal chemical properties of soils and slurries were determined according to standard methods in triplicate analysis of each sample. In the case of soils, pH was measured on mixtures of soil/water = 1:2.5, electrical conductivity (EC) on water extracts obtained at a soil/water ratio of 1:5, and total organic carbon (TOC) by dichromate oxidation and subsequent titration with ferrous ammonium sulfate. In the case of slurries, pH and EC were measured directly on a fresh homogenized sample, and TOC was determined by combustion at 550 °C. Total N content in soils and slurries was obtained by using the Kjeldahl method and spectrophotometric determination by a Technicon Autoanalyzer AAII (Buffalo Grove, IL).

Isolation of Humic Acids. The three PS samples collected each year were freeze-dried, and a composite sample was obtained by mixing equal weights of the freeze-dried preparations. HAs were isolated from the untreated and PS-treated soils and from the composite, freeze-dried PS sample by using a conventional procedure that is briefly described below.

Soil carbonates were removed by mechanical stirring of the sample for 30 min with 2 M H₃PO₄. The treatment was repeated three times. Then soil samples were washed with distilled water until the suspension reached a pH of 7. Free-carbonate soils and freeze-dried PS sample were first extracted with 0.1 M Na₄P₂O₇ (pH 9.8) and then by 0.1 M NaOH, at room temperature (RT; ~293 K), and using a sample/extractant ratio of 1:10. Each extraction was repeated three times. For each extraction step, the mixture was shaken mechanically for 3 h and then centrifuged at 15300g for 15 min, and the supernatant was filtered through a Whatman no. 31 filter paper. The combined alkaline extracts were then acidified with HCl to pH 1, left standing for 24 h in a refrigerator to allow the complete precipitation of HA, and then centrifuged at 30100g for 15 min. The HA precipitates were purified by dissolution in 0.1 M NaOH, centrifugation at 30100g, elimination of the residue, and acidification of the alkaline supernatant with HCl to pH 1. The suspension was left standing for 12 h at RT and then centrifuged at 30100g for 15 min. This procedure was repeated three times. The precipitated HAs were then recovered with distilled water, dialyzed until free of Cl⁻ ions, and finally freeze-dried.

Humic Acid Analyses. Moisture and ash contents were measured by heating the HAs for one night at 105 and 550 °C, respectively. The elemental composition was determined by a C, H, N, S analyzer (Fisons Instruments, Crawley, U.K.) model EA 1108 in triplicate analysis of each HA sample. Oxygen content was calculated by difference: O% = 100 - (C + H + N + S)%. Total acidity was determined according to the Ba(OH)₂ method and carboxyl group content according to the Ca(CH₃COO)₂ method; and phenolic hydroxyl group content was calculated by difference (17).

The ratio of absorbances at 465 and 665 nm (E₄/E₆ ratio) was determined on solutions of 3.0 mg of each HA in 10 mL of 0.05 M NaHCO₃, with the pH adjusted to 8.3 with 0.02 M NaOH. The absorbances at 465 and 665 nm were measured using a Perkin-Elmer (Norwalk, CT) Lambda 15 UV-vis spectrophotometer (18).

The Fourier transform infrared (FT-IR) spectra of HAs were recorded over the range of 4000 to 400 cm⁻¹ on pellets obtained by pressing under reduced pressure a mixture of 1 mg of HA and 400 mg of dried KBr, spectrometry grade. A Nicolet (Madison, WI) 5PC FT-IR spectrophotometer operating with a peak resolution of 2 cm⁻¹ and Omnic 1.2 software were used to obtain the spectra.

Fluorescence spectra in the emission, excitation, and synchronous scan modes were obtained on aqueous, slightly alkaline solutions of HA at a concentration of 100 mg L⁻¹ after overnight equilibration at RT and adjustment to pH 8 with 0.05 M NaOH. Spectra were recorded using a Perkin-Elmer (PE; Norwalk, CT) LS-5 luminescence spectrophotometer equipped with a PE Data Station 3600 for data generation and processing by PE Computerized Luminescence Spectroscopy (PECLS) software. Emission and excitation slits were set at a 5-nm bandwidth, and a scan speed of 120 nm min⁻¹ was selected for both monochromators. Emission spectra were recorded over the range of 380–550 nm at a constant excitation wavelength of 360 nm. The overall relative fluorescence intensity (RFI) was expressed in arbitrary units

Table 1. Main Characteristics (\pm Standard Errors) of Pig Slurries

property	pig slurry		
	1997	1998	1999
dry matter (g L ⁻¹)	20 \pm 0.3	16 \pm 0.3	27 \pm 0.3
ash (g L ⁻¹)	7.5 \pm 0.1	5.9 \pm 0.1	7.4 \pm 0.1
pH	7.5 \pm 0.0	7.6 \pm 0.0	7.2 \pm 0.0
EC (dS m ⁻¹)	16.4 \pm 0.1	16.9 \pm 0.0	14.5 \pm 0.0
TOC (g L ⁻¹)	7.3 \pm 0.2	5.7 \pm 0.1	11.2 \pm 0.2
total N (g L ⁻¹)	2.8 \pm 0.1	2.4 \pm 0.1	2.7 \pm 0.0

Table 2. Some Chemical Properties (\pm Standard Errors) of the Control Soil (C) and Soils Amended with Pig Slurry

soil	pH (H ₂ O)	EC (dS m ⁻¹)	TOC (g kg ⁻¹)	total N (g kg ⁻¹)	C/N ratio
C	5.8 \pm 0.3	0.06 \pm 0.01	13.1 \pm 0.1	1.2 \pm 0.1	10.6 \pm 0.1
P90-1 ^a	6.7 \pm 0.4	0.11 \pm 0.01	13.2 \pm 0.2	1.3 \pm 0.1	10.2 \pm 0.1
P90-2 ^a	7.0 \pm 0.5	0.13 \pm 0.00	13.2 \pm 0.1	1.3 \pm 0.0	9.9 \pm 0.1
P90-3 ^a	6.8 \pm 0.4	0.11 \pm 0.01	13.2 \pm 0.1	1.4 \pm 0.0	9.6 \pm 0.1
P150-1 ^b	7.3 \pm 0.4	0.11 \pm 0.01	13.0 \pm 0.3	1.3 \pm 0.0	9.9 \pm 0.1
P150-2 ^b	8.0 \pm 0.1	0.17 \pm 0.01	12.4 \pm 0.1	1.4 \pm 0.0	9.0 \pm 0.1
P150-3 ^b	7.8 \pm 0.2	0.13 \pm 0.02	12.2 \pm 0.2	1.3 \pm 0.0	9.0 \pm 0.1

^a Soils amended with pig slurry at a rate of 90 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively. ^b Soils amended with pig slurry at a rate of 150 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively.

as the unitless reciprocal to the gain used to normalize each emission spectrum (19). Excitation spectra were obtained over a scan range of 300–500 nm by measuring the emission radiation at a fixed wavelength of 520 nm. Synchronous scan excitation spectra were measured by scanning simultaneously both the excitation (varied from 300 to 550 nm) and the emission wavelengths while maintaining a constant, optimized wavelength difference $\Delta\lambda = \lambda_{\text{exc}} - \lambda_{\text{em}} = 18$ nm (19).

The electron spin resonance (ESR) spectra were recorded at RT on solid HA samples packed in quartz ESR tubes (4 mm o.d., 3 mm i.d.) by a Bruker ER-200D SRC ESR spectrophotometer operating at X-band frequency with 100 kHz magnetic field modulation. The magnetic field range scanned was 10 mT and centered at about the resonance field of the free electron. A modulation amplitude of 0.63 mT, a microwave frequency of 9.52 GHz, and a microwave attenuation of 13 dB (corresponding to a microwave power of \sim 10 mW) were used. The absolute free radical concentration of organic free radicals was calculated using standard procedures and equations and expressed in spins per gram (20).

RESULTS AND DISCUSSION

Soils and Pig Slurries. In comparison to other types of manures and organic wastes (5), the PS samples are characterized by a very small dry matter content, a slightly alkaline pH, a large EC value and total N concentration, and a relatively small amount of TOC (Table 1). Although PS properties are often influenced by several factors such as animal type and age, time of sampling, and storage and handling conditions, the properties of the three PS samples used in this study are quite similar to one to another—probably due to the same source and collection at the same time of the year—and fall into ranges commonly reported for these materials (21).

The control soil has a slightly acidic pH, a small EC, a level of N adequate for barley growth, and a small content of TOC (Table 2). With respect to the control soil, the PS-amended soils have greater pH (around neutrality or slightly alkaline) and EC (still low), similar or smaller (samples PS150-2 and PS150-3) TOC contents, similar or slightly larger total N content, and smaller C/N ratios (Table 2).

The effect of PS addition on pH, which is more evident at large amendment rate, is similar to that reported previously by other authors (22). Changes in pH of soils amended with cattle manure are attributed to the action exerted by the large content of CaCO₃ in the manure, which originates from the cattle diet that is rich in CaCO₃ (23).

Despite the large amount of N added with PS, only a small increase of N content is measured in manured soils, with respect to that of control soil. This smaller than expected effect on N content in manured soils may be mostly attributed to loss of ammonia N by volatilization as a result of the increased pH of manured soils and, secondarily, to N uptake by crops and nitrate leaching.

The similar or smaller TOC content measured in PS-amended soils, that is, the “priming effect” (5, 24) here observed, may be ascribed to the small, mostly easily decomposable amount of organic C and the relatively large N content of PS applied to soil. In these conditions, soil microorganisms have an increased amount of fresh N available for their protein metabolism but not enough fresh C as the energy source; thus, microbial oxidation of soil native organic C must occur. Previous long-term field studies that have used PS as fertilizer also did not show enhancement of organic C in amended soils. For example, Rochette et al. (11) measured no significant increase of soil C content after 19 consecutive years of pig slurry application at rates of 60 and 120 m³ ha⁻¹ year⁻¹. However, Hountin et al. (25) observed an increase of soil C content with increasing rates (from 30 to 120 m³ ha⁻¹ year⁻¹) of amendment with an organic-rich (TOC, 29 g L⁻¹) liquid pig manure after 14 years of application to a poorly drained, silty loam soil in a rainy and cold area of the province of Quebec (Canada). Thus, different effects may be observed on soil TOC content as a function of pig slurry and soil characteristics and climatic conditions in which experiments are conducted.

Humic Acids: Yield, Ash, Elemental and Functional Group Composition, and E₄/E₆ Ratio. Although the yield of PS-HA is larger than that of C-HA, the yields of PS-amended soil HAs are slightly smaller than that of C-HA and slightly decrease with increasing amendment rate and number (Table 3). These results support the previously discussed trend for TOC in amended soils, suggesting that enhanced microbial activity may lead to partial mineralization of native soil HA through microbial respiration.

As expected, the elemental composition of PS-HA is markedly different from that of both C-HA and PS-amended soil HAs. In particular, C, H, N, and S contents of PS-HA are larger or much larger, and O content and C/N, C/H, and O/C ratios are much smaller or smaller than the corresponding values of any soil HA. These results are in general agreement with previous findings on HAs from various organic amendments in comparison to HAs from the corresponding unamended and amended soils (15).

The elemental composition of amended soil HAs is generally slightly different from that of C-HA and varies as a function of amendment rate and number. In particular, with respect to C-HA, HAs from PS-amended soils have similar or slightly larger C and S contents, similar or slightly smaller H and O contents, smaller N content, larger C/N ratio, and similar C/H and O/C ratios.

It is important to note that, with the exception of S content and despite the increasing rate and number of amendment, the most pronounced differences between amended soil HAs and C-HA are measured after the first year of PS application and

Table 3. Yield, Ash, Elemental Composition, and Atomic Ratios (\pm Standard Errors) of Humic Acids (HAs) Isolated from the Control Soil (C), Pig Slurry (PS), and PS-Amended Soils

origin of HA sample	yield ^b (g kg ⁻¹)	ash ^b (%)	% ^a					atomic ratios		
			C	H ^c	N ^c	S ^c	O	C/N ^c	C/H ^c	O/C ^c
C	1.6	1.6	56.6 \pm 0.9	4.9	4.8	0.3	33.4 \pm 0.9	13.8	1.0	0.4
PS	2.5	1.5	62.7 \pm 0.3	9.4	5.2	1.3	21.3 \pm 0.3	14.1	0.6	0.3
PS90-1 ^d	1.6	0.1	61.4 \pm 0.3	4.5	4.0	0.3	29.9 \pm 0.4	18.1	1.1	0.4
PS90-2 ^d	1.5	0.4	58.6 \pm 0.4	4.4	4.1	0.4	32.6 \pm 0.5	16.9	1.1	0.4
PS90-3 ^d	1.5	0.4	56.9 \pm 0.4	4.6	4.3	0.5	33.8 \pm 0.5	15.6	1.0	0.4
PS150-1 ^e	1.5	0.4	59.5 \pm 0.0	5.0	4.4	0.3	30.7 \pm 0.0	15.7	1.0	0.4
PS150-2 ^e	1.3	0.8	57.4 \pm 0.0	5.0	4.5	0.5	32.5 \pm 0.0	14.7	0.9	0.4
PS150-3 ^e	1.3	0.1	56.7 \pm 0.3	4.8	4.5	0.5	33.5 \pm 0.3	14.8	1.0	0.4

^a On moisture- and ash-free basis. ^b On moisture-free basis. ^c Standard errors of three laboratory replicates for H, N, and S % and C/N, C/H, and O/C ratios are in any case <0.1. ^d Soils amended with pig slurry at a rate of 90 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively. ^e Soils amended with pig slurry at a rate of 150 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively.

Table 4. Acidic Functional Group Contents, E₄/E₆ Ratios, Relative Fluorescence Intensity (RFI), and Concentration of Organic Free Radicals (OFR) of Humic Acids (HAs) Extracted from the Control Soil (C), Pig Slurry (PS), and PS-Amended Soils

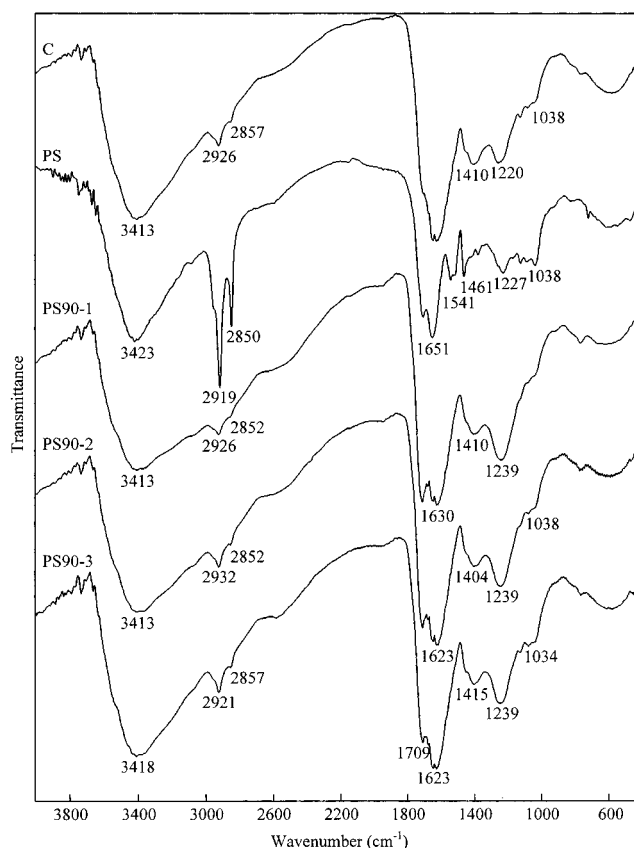
origin of HA sample	mmol g ⁻¹			E ₄ /E ₆ ratio	RFI ^b	OFR ^a spins (g ⁻¹ \times 10 ¹⁷)
	total acidity ^a	COOH ^a	phenolic OH ^a			
C	7.7	2.9	4.8	4.9	6.6	3.73
PS	4.7	2.5	2.2	4.0	7.3	0.55
PS90-1 ^c	8.2	4.4	3.8	4.9	2.7	3.45
PS90-2 ^c	7.8	3.8	4.0	4.6	1.9	3.30
PS90-3 ^c	7.3	3.3	4.0	4.7	1.9	3.26
PS150-1 ^d	7.6	3.7	3.9	4.7	3.2	3.17
PS150-2 ^d	7.1	3.6	3.5	4.4	5.1	3.38
PS150-3 ^d	6.4	3.6	2.8	4.5	2.7	3.57

^a On moisture- and ash-free basis. ^b Arbitrary units. ^c Soils amended with pig slurry at a rate of 90 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively. ^d Soils amended with pig slurry at a rate of 150 m³ ha⁻¹ year⁻¹ for 1, 2, and 3 years, respectively.

especially at low rate, that is, for sample PS90-1-HA. At both rates, with increasing amendment number, that is, passing from 1 to 2 and to 3 years of amendment, the differences in elemental composition between amended soil HAs and C-HA tend to decrease and C and O contents tend to reach the values of C-HA. The only exception is S content, which slightly increases with amendment number at both amendment rates.

Total acidity and carboxyl and phenolic OH group contents of PS-HA are markedly smaller than those of any soil HA (Table 4), in agreement with previous findings on similar systems (15). The HAs extracted from PS-amended soils show a greater COOH group content, a much smaller phenolic OH content, and a similar or smaller total acidity, as compared to C-HA (Table 4). With the exception of phenolic OH content in HAs from soils amended at small rate, which remain similar, the acidic functional group contents of the remaining soil HAs tend to diminish with increasing amendment number. However, they reach values that are still larger for COOH groups and smaller for phenolic OH and total acidity than the corresponding values of C-HA.

The E₄/E₆ ratio of PS-HA is smaller than that of any soil HA (Table 4). The E₄/E₆ values of amended soil HAs are generally slightly smaller than that of C-HA and tend to slightly diminish after the first slurry application at both rates. According to Chen et al. (18), the E₄/E₆ ratio of HAs is mainly governed by particle size and molecular weight and is correlated positively with total acidity. Smaller E₄/E₆ ratios in PS-amended soil HAs,

**Figure 1.** FT-IR spectra of humic acids isolated from the control soil (C), pig slurry (PS), and soils amended with 90 m³ ha⁻¹ year⁻¹ of pig slurry for 1, 2, and 3 years (PS90-1, PS90-2, and PS90-3, respectively).

as compared to C-HA, might thus suggest a greater contribution of high molecular weight particles in the former.

Humic Acids: FT-IR Spectra. The FT-IR spectrum of PS-HA differs markedly from those of soil HAs (Figures 1 and 2). The main common and different IR features of PS-HA, as compared to soil HAs, and their corresponding assignments, according to Bellamy (26), MacCarthy and Rice (27), and Stevenson (28), are (a) a common, intense broad band at \sim 3400 cm⁻¹ usually attributed to O-H stretching and, secondarily, to N-H stretching of various functional groups; (b) two sharp bands at about 2930–2920 and 2860–2850 cm⁻¹ due to aliphatic C-H group stretching, the relative intensities of which are much higher for PS-HA than for soil HAs; (c) an absorption at about 1715 to 1710 cm⁻¹ due to C=O stretching of COOH

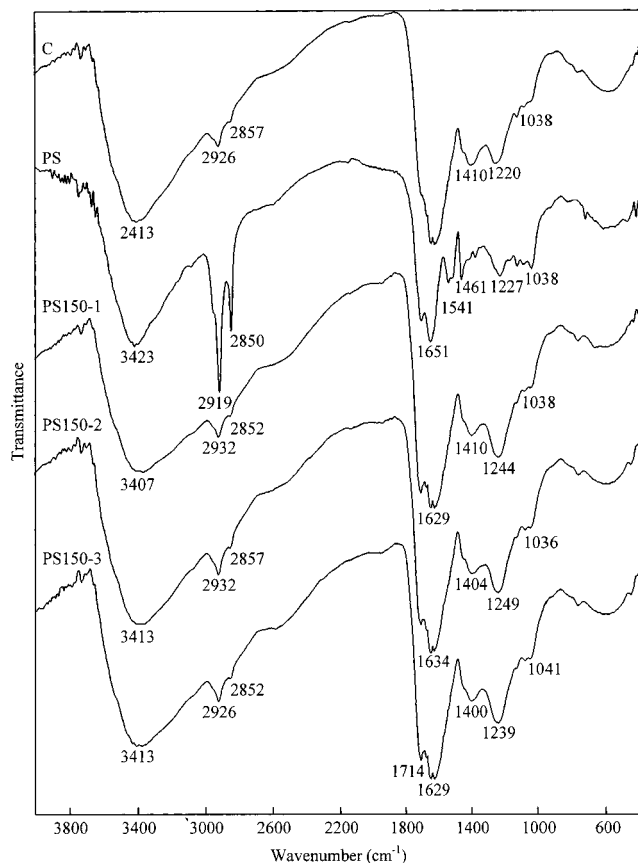


Figure 2. FT-IR spectra of humic acids isolated from the control soil (C), pig slurry (PS), and soils amended with $150 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ of pig slurry for 1, 2, and 3 years (PS150-1, PS150-2, and PS150-3, respectively).

and other carbonyl groups, which is evident in PS-HA and all amended-soil HAs but is only a shoulder in C-HA; (d) a broad band in the region between 1660 and 1600 cm^{-1} , which is generally considered an envelope of unresolved absorptions mainly due to aromatic C=C, C=O stretching of amide groups (amide I band), quinonic C=O, and/or C=O of H-bonded conjugated ketones, which is less intense in PS-HA than in soil HAs; (e) a band of medium intensity at $\sim 1540 \text{ cm}^{-1}$ preferentially ascribed to N-H deformation and C=N stretching of amides (amide II band), which is evident only for PS-HA; (f) a medium-intensity band at $\sim 1460 \text{ cm}^{-1}$ attributed to aliphatic C-H, which is very sharp in PS-HA but only a weak shoulder in soil HAs; (g) a broad band of medium intensity centered between 1415 and 1400 cm^{-1} and preferentially assigned to O-H deformation and C-O stretching of phenolic OH, which is apparent for all soil HAs and is a shoulder for PS-HA; (h) a faint absorption at $\sim 1380 \text{ cm}^{-1}$ possibly due to C-H deformation of CH_2 and CH_3 groups and/or to antisymmetric stretching of COO^- groups, which appears only in PS-HA; (i) a common, broad band of medium-strong intensity centered between 1240 and 1220 cm^{-1} and generally ascribed to C-O stretching and O-H deformation of carboxyls and C-O stretching of aryl ethers; and (j) an absorption at $\sim 1040 \text{ cm}^{-1}$, generally attributed to C-O stretching of polysaccharides or polysaccharide-like substances and Si-O of silicate impurities, which is of weak intensity in PS-HA and a shoulder in all soil HAs.

The FT-IR spectra of HAs isolated from PS-amended soils are very similar one to another and more similar to the spectrum of C-HA than to that of PS-HA (Figures 1 and 2). However, the spectra of PS-amended soil HAs differ from that of C-HA for (a) the stronger relative intensity of the bands at ~ 1710

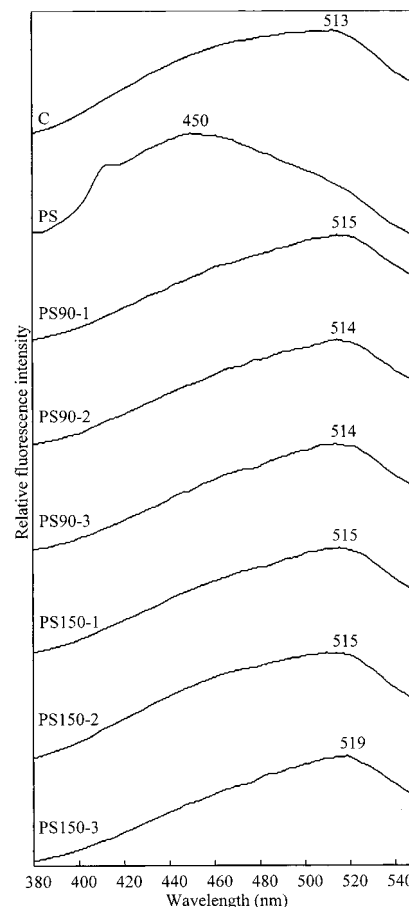


Figure 3. Fluorescence emission spectra of humic acids isolated from the control soil (C), pig slurry (PS), and soils amended with either 90 or $150 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ of pig slurry for 1, 2, and 3 years (PS90-1, PS90-2, and PS90-3 and PS150-1, PS150-2, and PS150-3, respectively).

cm^{-1} and in the 1250 to 1240 cm^{-1} region, which tend to slightly decrease with the number of PS applications; and (b) the slight increase of the relative intensities of the bands at about 2930 to 2920 and 2860 to 2850 cm^{-1} and of the shoulder at $\sim 1040 \text{ cm}^{-1}$, which tend to slightly increase with the number of PS applications.

A comparison of FT-IR spectra described above with typical FT-IR spectra of humic substances from soils and other sources, as classified by Stevenson and Goh (29), shows that the FT-IR spectra of soil HAs are generally similar to IR spectra of Type I, typical of HAs from common aerated soils. In contrast, the FT-IR spectrum of PS-HA more closely resembles IR spectra of Type III, typical of HAs formed in poorly aerated systems. The latter result suggests that the PS-HA has developed during anaerobic digestion, which also explains the low amounts of carboxyl and phenolic OH groups measured in this HA. These results agree with those obtained on similar systems by other authors (30, 31).

Results provided by FT-IR spectroscopy support and complement those of elemental and functional group analyses discussed previously in confirming the prevalent aliphatic character and the large presence of N-containing groups, most probably polypeptidic chains, of PS-HA. Furthermore, FT-IR results confirm the similarity of C-HA and HAs from PS-amended soils and the larger presence of carboxyl groups and slightly greater aliphaticity of the latter HAs with respect to C-HA.

Humic Acids: Fluorescence Spectra. The RFI value of PS-HA is larger than that of C-HA and much larger than those of

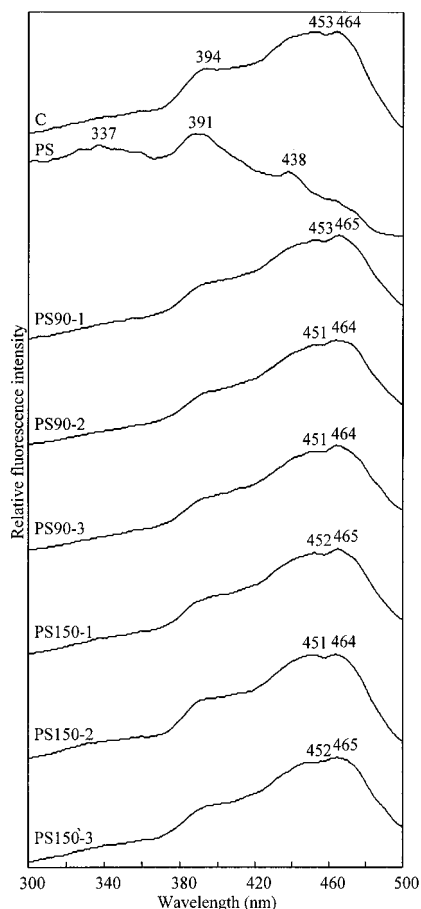


Figure 4. Fluorescence excitation spectra of humic acids isolated from the control soil (C), pig slurry (PS), and soils amended with either 90 or 150 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ of pig slurry for 1, 2, and 3 years (PS90-1, PS90-2, and PS90-3, and PS150-1, PS150-2, and PS150-3, respectively).

PS-amended soil HAs, which are all much smaller than that of C-HA (**Table 4**).

The main feature of the emission spectra is a unique, typical, broad band with the maximum centered at a wavelength that is much shorter (450 nm) for PS-HA than for any soil HA (513–519 nm) (**Figure 3**). The PS-amended soil HAs feature the emission maximum at a slightly longer wavelength than that of C-HA and a broad shoulder that extends to shorter wavelengths.

The fluorescence excitation spectrum of PS-HA is very different from those of soil HAs and is characterized by a prominent band in the intermediate-wavelength region (391 nm) and some small bands and shoulders at short and long wavelengths (337 and 438 nm) (**Figure 4**). The excitation spectrum of C-HA features two prominent bands of almost equal relative intensity at long wavelength (453 and 464 nm) and an intense shoulder at 394 nm. The excitation spectra of PS-amended soil HAs are similar to one to another and to that of C-HA (**Figure 4**). However, with respect to C-HA, PS-amended soil HAs exhibit a less intense shoulder at intermediate wavelength and the band at 464–465 nm is slightly more intense than that at 451–453 nm.

Also, the fluorescence synchronous scan spectrum of PS-HA is very different from those of soil HAs (**Figure 5**). The PS-HA features a most intense band at intermediate wavelength (391 nm) and several less intense peaks and shoulders at shorter (327 nm) and longer (between 429 and 465 nm) wavelengths. The C-HA shows a unique peak at long wavelength (472 nm) accompanied by shoulders at longer wavelengths and a slight

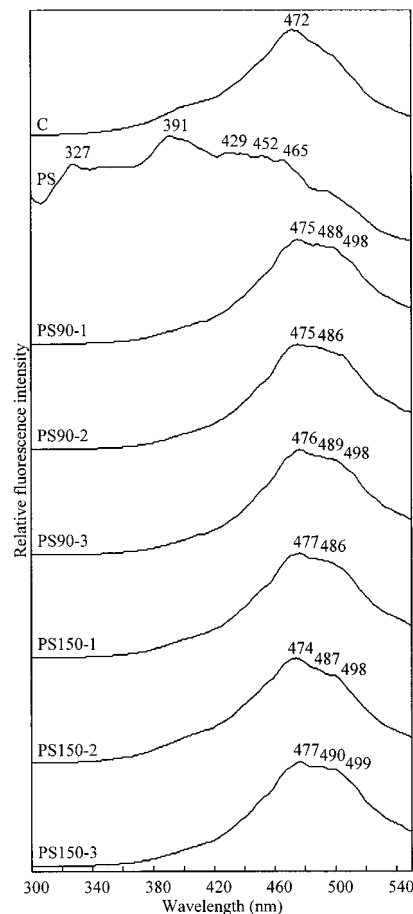


Figure 5. Fluorescence synchronous scan spectra of humic acids isolated from the control soil (C), pig slurry (PS), and soils amended with either 90 or 150 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ of pig slurry for 1, 2, and 3 years (PS90-1, PS90-2, and PS90-3 and PS150-1, PS150-2, and PS150-3, respectively).

shoulder around 390 nm (**Figure 5**). The PS-amended soil HAs show a unique, broad band at long wavelength (474–479 nm), in which a number of bands and shoulders merge together, with a maximum at 474–477 nm (**Figure 5**).

In general, fluorescence spectra of PS-HA are similar to those of HAs isolated from other types of organic amendments, for example, sewage sludge HAs, and the spectra of soil HAs closely resemble those of typical soil HAs (15, 19, 32). The large overall fluorescence intensity and the short wavelengths measured for the main fluorescence peaks of PS-HA suggest the presence of simple structural components of wide molecular heterogeneity and low molecular weight, degree of aromatic polycondensation, level of conjugated chromophores, and humification degree. On the contrary, the small fluorescence intensities and long wavelengths of major peaks of soil HAs may be ascribed to the presence of an extended, linearly condensed aromatic ring network and other unsaturated bond systems capable of a great degree of conjugation in large molecular weight units of great humification degree.

Humic Acids: ESR Analysis. A sharp and narrow single-line resonance centered at about the resonance field of the free electron appears in ESR spectra (not shown) of all HA samples. This signal is typical of HA of any nature and origin and is attributed to indigenous organic free radicals of semiquinone nature conjugated with an extended aromatic network (33). The concentration of organic free radicals that can be calculated from the intensity of this signal is generally related positively with

the aromatic polycondensation, polymerization, and humification degrees of HA (33).

The data in **Table 4** indicate that the concentration of organic free radicals in PS-HA is ~ 1 order of magnitude smaller than that of any soil HA. This result confirms the much smaller polycondensation, polymerization, and humification degrees of this HA with respect to soil HAs. The slight decrease of organic free radical concentration measured in PS-amended soil HAs, with respect to that of the control soil HA, provides evidence of the limited effect of PS amendment on this property.

The results obtained would suggest pig slurry should be subjected to appropriate treatments prior to its application to soil, to transform its HA fraction into more stabilized forms by increasing its humification degree and thus enhance its potential as a soil organic fertilizer.

ABBREVIATIONS USED

HA, humic acid; RT, room temperature; PS, pig slurry; C, control soil; PS90-1, PS90-2, and PS90-3 and PS150-1, PS150-2, and PS150-3, soils amended with either 90 or 150 $\text{m}^3 \text{ha}^{-1}$ of PS for 1, 2, and 3 years, respectively; EC, electrical conductivity; TOC, total organic carbon; FT-IR, Fourier transform infrared; RFI, relative fluorescence intensity; ESR, electron spin resonance.

LITERATURE CITED

- García, C.; Hernández, T.; Costa, F. Biochemical parameters in soils regenerated by the addition of organic wastes. *Waste Manage. Res.* **1994**, *12*, 457–466.
- García-Gil, J. C.; Plaza, C.; Soler-Rovira, P.; Polo, A. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem.* **2000**, *32*, 1907–1913.
- Amberger, A. Use of organic wastes as fertilizers and its environmental implications. In *Fertilization and the Environment*; Merckx, R., Vereecken, H., Vlassak, K., Eds.; Kluwer Academic Publishers: London, U.K., 1990; pp 314–329.
- Cabral, F.; Vasconcelos, E.; Pinto, F. C.; Santos, J. Q. Agricultural use of pollutant organic wastes. In *Treatment and Use of Sewage Sludge and Liquid Agricultural Wastes*; L'Hermite, P., Ed.; Elsevier Applied Science: London, U.K., 1991; pp 425–428.
- Ndayegamiye, A.; Côté, D. Effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. *Can. J. Soil Sci.* **1989**, *69*, 39–47.
- Bernal, M. P.; Roig, A.; Lax, A.; Navarro, A. F. Effects of the application of pig slurry on some physico-chemical and physical properties of calcareous soils. *Bioresour. Technol.* **1992**, *42*, 233–239.
- Kirchmann, H.; Lunvall, A. Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry. *Biol. Fertil. Soils* **1993**, *15*, 161–164.
- Dendooven, L.; Bonhomme, E.; Merckx, R.; Vlassac, K. Injection of pig slurry and its effects on dynamics of nitrogen and carbon in a loamy soil under laboratory conditions. *Biol. Fertil. Soils* **1998**, *27*, 5–8.
- Bernal, M. P.; Kirchmann, H. Carbon and nitrogen mineralization and ammonia volatilization from fresh, aerobically and anaerobically treated pig manure during incubation with soil. *Biol. Fertil. Soils* **1992**, *13*, 135–141.
- Saviozzi, A.; Levi-Minzi, R.; Riffaldi, R.; Vanni, G. Role of chemical constituents of wheat straw and pig slurry on their decomposition in soil. *Biol. Fertil. Soils* **1997**, *25*, 401–406.
- Rochette, P.; Angers, D. A.; Côté, D. Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year: I. Carbon dioxide fluxes and microbial biomass carbon. *Soil Sci. Soc. Am. J.* **2000**, *64*, 1389–1395.
- Giusquiani, P. L.; Concezzi, L.; Businelli, M.; Macchioni, A. Fate of pig sludge liquid fraction in calcareous soil: agricultural and environmental implications. *J. Environ. Qual.* **1998**, *27*, 364–371.
- Loffredo, E.; D'Orazio, V.; Senesi, N. Adsorption of triallate onto soils and pig slurry. *J. Environ. Sci. Health* **1997**, *32*, 25–36.
- D'Orazio, V.; Loffredo, E.; Brunetti, G.; Senesi, N. Triallate adsorption onto humic acids of different origin and nature. *Chemosphere* **1999**, *39*, 183–198.
- Senesi, N.; Miano, T. M.; Brunetti, G. Humic-like substances in organic amendments and effects on native soil humic substances. In *Humic Substances in Terrestrial Ecosystems*; Piccolo, A., Ed.; Elsevier: New York, 1996; pp 531–593.
- Soil Survey Staff. *Keys to Soil Taxonomy*, 8th ed.; Natural Resources Conservation Service, USDA: Washington, DC, 1998.
- Perdue, E. M. Acidic functional groups of humic substances. In *Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation, and Characterization*; Aiken, G. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P., Eds.; Wiley-Interscience: New York, 1985; pp 493–536.
- Chen, Y.; Senesi, N.; Schnitzer, M. Information provided on humic substances by E_4/E_6 ratios. *Soil Sci. Soc. Am. J.* **1977**, *41*, 352–358.
- Senesi, N.; Miano, T. M.; Provenzano, M. R.; Brunetti, G. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. *Soil Sci.* **1991**, *152*, 259–271.
- Senesi, N. Electron spin (or paramagnetic) resonance spectroscopy. In *Methods of Soil Analysis: Chemical Methods*; Sparks, D. L., Ed.; ASA, CSSA, SSSA Publication: Madison, WI, 1996; pp 323–356.
- Scottford, I. M.; Cumby, T. R.; White, R. P.; Carton, O. T.; Lorenz, F.; Hatterman, U.; Provolò, G. Estimation of the nutrient value of agricultural slurries by measurement of physical and chemical properties. *J. Agric. Eng. Res.* **1998**, *71*, 291–305.
- Whalen, J. K.; Chang, C.; Clayton, G. W.; Carefoot, J. P. Cattle manure amendments can increase the pH of acid soils. *Soil Sci. Soc. Am. J.* **2000**, *64*, 962–966.
- Eghball, B. Liming effects of beef cattle feedlot manure or compost. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 2563–2570.
- Kuzyakov, Y.; Friedel, J. K.; Stahr, K. Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* **2000**, *32*, 1485–1498.
- Hountin, J. A.; Couillard, D.; Karam, A. Soil carbon, nitrogen and phosphorus contents in maize plots after 14 years of pig slurry applications. *J. Agric. Sci.* **1997**, *129*, 187–191.
- Bellamy, L. J. *The Infrared Spectra of Complex Molecules*; Chapman and Hall: London, U.K., 1975.
- MacCarthy, P.; Rice, J. A. Spectroscopic methods (other than NMR) for determining functionality in humic substances. In *Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation, and Characterization*; Aiken, J. R., McKnight, D. M., Wershaw, R. L., MacCarthy, P., Eds.; Wiley-Interscience: New York, 1985; pp 527–559.
- Stevenson, F. J. *Humus Chemistry: Genesis, Composition, Reactions*; Wiley-Interscience: New York, 1994; 496 pp.
- Stevenson, F. J.; Goh, K. M. Infrared spectra of humic acids and related substances. *Geochim. Cosmochim. Acta* **1971**, *35*, 471–483.
- Boyd, S. A.; Sommers, L. E.; Nelson, D. W. Changes in the humic acid fraction of soil resulting from sludge application. *Soil Sci. Soc. Am. J.* **1980**, *44*, 1179–1186.
- Piccolo, A.; Zaccaro, P.; Genevini, P. G. Chemical characterization of humic substances extracted from organic-waste-amended soils. *Bioresour. Technol.* **1992**, *40*, 275–282.

- (32) Senesi, N.; Miano, T. M.; Provenzano, M. R.; Brunetti, G. Spectroscopic and compositional comparative characterization of I.H.S.S. reference and standard fulvic and humic acids of various origin. *Sci. Total Environ.* **1989**, *81/82*, 143–156.
- (33) Senesi, N. Application of electron spin resonance (ESR) spectroscopy in soil chemistry. In *Advances in Soil Science*; Stewart, B. A., Ed.; Springer-Verlag: New York, 1990; Vol. 14, pp 77–130.

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