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Chemical and structural evolution of humic acids during organic waste composting

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Abstract

The effects of the composting process on the chemical and structural properties of humic acids have been studied in seven different organic waste mixtures from different origin. Only slight changes in elemental composition have been found in the humic acids after the composting process pointing to a more aromatic structure with higher proportions of oxygen and nitrogen. Functional groups were the most sensitive to the changes caused by the composting process showing a marked increase in the total acidity and phenolic, carboxyl and carbonyl groups. Gel permeation chromatography showed a slight increase in the average molecular weight of the humic acids. Infrared spectroscopy did not show important differences in humic acid structure but a clear decrease in the intensity of the bands in the region 3000–2850 cm^{-1} corresponding to the aliphatic fractions. As a general result, the composting process yields humic acids in which the elemental and functional composition are closer to that of the more humified soil humic acids.

Introduction

Composting is considered a useful way for organic waste stabilisation involving the partial humification of the organic matter. Traditionally, the study of the humification processes taking place during composting has been one important research topic in the area. The interest in these humification processes is twofold. First, the organic matter humification during composting has been considered as a suitable index for assessing compost stability and second, the agricultural value of the final compost increases when the organic matter reaches a high level of humification, especially when composts are used as a source of soil organic matter.

Organic matter humification is usually studied in order to understand the transformations undergone by both the whole organic matter (González-Vila et al. 1999; Chefetz et al. 1996; Hsu & Lo 1999), and the humic fractions extracted from the composts (Chefetz et al. 1998; Miikki et al. 1997; Veeken et al. 2000). The changes in the constituents of the organic matter caused by the humification process during composting are used to propose stabilization indices as quality criteria for the end product. These indices are usually based on the ratios between different alkali extractable fractions of the organic matter such as humic acids, fulvic acids, total extractable carbon, etc (Senesi 1989; Iglesias & Pérez 1989, 1992). Nevertheless, the humic substances formed during composting process are called humic-like substances as they are not expected to have the same properties that those extracted from soils or from more humified materials, like coals or peats, where the humification process takes place over a considerably longer time scale than in the composting pile.

One of the main agricultural uses of compost is as a source of organic matter for the soil and the beneficial effects of the soil organic matter on soil fertility are well known (Stevenson 1994). The main effects are the improvement of the soil structure, the improvement of soil aeration, water-holding capacity, buffer capacity and cation exchange capacity. The addition of compost to the soil with a more humified organic matter leads to longer lasting effects of this organic matter in the soil, increasing the agricultural value of the composts. Hence, a better understanding of the humification process during composting would help to achieve a final product with a high degree of stabilisation of the organic matter and with an increased agricultural value.

The aim of the present work is to compare the chemical composition and the spectroscopic properties of humic acids extracted from seven different composts at the beginning and at the end of the composting process in order to show the main changes that the composting process produces on the humic acids.

Materials and methods

Description of materials

Seven compost samples of different origin were used for the extraction of the humic acids and Table 1 shows some general characteristics of the mature composts used. The composts were prepared from a wide number of organic wastes produced in agricultural and industrial activities and sewage treatment plants in different municipalities from the south-east of Spain. The compost samples had the following initial organic waste mixtures (dry weight):

Compost I:	28% poultry manure + 5% pig slurry + 67% sweet sorghum bagasse
Compost II:	44% sewage sludge + 56% barley straw
Compost III:	35% sewage sludge + 65% maize straw
Compost IV:	46% sewage sludge + 54% cotton waste
Compost V:	13% poultry manure + 87% cotton waste + 2.3 L/kg olive mill wastewater
Compost VI:	9% sewage sludge + 91% cotton waste + 0.7 L/kg olive mill wastewater
Compost VII:	18% sewage sludge + 82% maize straw + 3.2 L/kg olive mill wastewater.

Approximately 1500–2000 kg of initial organic waste mixtures were composted in a pilot plant by the Rutgers static-pile composting system (Finstein et al. 1985). This system maintains a temperature ceiling in the pile, through the on-demand removal of heat by ventilation. This encourages a high decomposition rate by avoiding high temperatures, which inhibit and slow down decomposition by reducing microbial activity. Air was blown into the base of the pile using three perforated PVC tubes, 3 m in length and 12 cm in diameter. The timer was set for 30 s ventilation every 15 min. The ceiling temperature for continuous air blowing was 55 °C.

The biooxidative phase of composting (active phase) was considered to be finished when the temperature of the pile was near to that of the atmosphere. Air blowing was stopped and the mixtures were then allowed to mature over a period of two months (maturation phase). This stage was reached after 112 (compost I), 119 (compost II), 112 (compost III), 105 (compost IV), 105 (compost V), 140 (compost VI) and 119 (compost VII) days in the different mixtures. The moisture levels of the piles were controlled during the biooxidative phase of composting and adjusted by weekly additions of the necessary amount of water to obtain values between 45–65% of total weight. Two representative samples were taken, one after the first week of the composting process and other at the end of the maturation phase. The samples were taken by mixing six subsamples from six sites along the length of the pile and from the whole profile (from the top to bottom of the pile). These were then air dried and ground to 0.5 mm for further humic acid extraction.

Extraction and purification of humic acids

The humic acids were extracted and purified according to Sánchez-Monedero et al. 2002. The samples were treated with 0.25 M NaOH (1 : 20 w/V) by constant shaking under N_2 gas atmosphere in sealed bottles for 12 h. After centrifugation for 15 min at 23,500 g, the supernatants were carefully removed and adjusted to pH 2.0 by adding concentrated HCl. The precipitates formed at pH 2, conventionally known as humic acids (HA), were allowed to coagulate for 24 h at 4 °C and were then separated from the soluble fulvic acids by centrifugation for 15 min at 23,500 g. These were then washed twice with 0.1 N HCl and once with desionized water. The HA were then purified with a 0.5% HCl–HF (5 ml HCl and 5 ml HF per l) solution for 36 h. The suspension were centrifuged for 15 min at 23,500 g and the solid washed twice with

Table 1. General characteristics of the composts

	pH	OM (g kg^{-1})	TOC (g kg^{-1})	N_T (g kg^{-1})	C/N	N–NH_4^+ (mg kg^{-1})	CEC/TOC (mmol$_c$ g^{-1})	GI (%)
Initial								
I	7.7	798	404	16.8	24.1	1778	1.17	46.8
II	6.3	811	419	17.5	24.0	–	–	–
III	5.6	810	413	37.5	11.0	16982	0.94	18.4
IV	7.6	815	439	20.8	21.1	1424	1.22	77.8
V	7.5	784	407	27.1	15.0	3385	2.28	13.2
VI	7.1	807	405	19.2	21.1	1365	1.88	38.6
VII	6.1	896	472	15.2	31.1	1608	0.73	19.6
Mature								
I	8.6	598	303	27.6	11.0	95	1.83	59.7
II	6.8	650	348	27.3	12.7	–	–	–
III	7.1	535	273	31.6	8.6	431	2.76	71.5
IV	7.3	648	356	37.9	9.4	437	3.50	69.4
V	7.4	629	337	34.7	9.7	286	3.15	76.9
VI	7.8	564	294	31.1	9.4	314	4.54	94.4
VII	7.5	748	394	33.3	11.8	661	2.28	91.3

OM: organic matter; TOC: total organic carbon; N_T: total nitrogen; CEC/TOC: cation exchange capacity to total organic carbon ratio; GI: germination index.

Table 2. Elemental composition (data expressed as ash free basis) of the humic acids extracted before and after the composting process

	C (g kg^{-1})	N (g kg^{-1})	H (g kg^{-1})	O (g kg^{-1})	O/C[1]	H/C[1]	N/C[2]
Initial							
I	524^{c}	25^{h}	58^{c}	393^{cd}	0.54	1.59	0.42
II	509^{ef}	54^{c}	68^{ab}	369^{g}	0.53	1.65	0.90
III	513^{de}	57^{b}	70^{a}	360^{hi}	0.48	1.50	0.95
IV	533^{b}	54^{c}	70^{a}	343^{j}	0.48	1.58	0.86
V	527^{c}	49e	68^{ab}	356^{i}	0.51	1.55	0.80
VI	488^{h}	53^{c}	57^{c}	402^{i}	0.62	1.41	0.94
VII	552^{a}	27^{h}	69^{a}	352^{i}	0.56	1.32	0.42
Mature							
I	466^{j}	37^{f}	54^{c}	443^{a}	0.57	1.52	0.68
II	503^{g}	54^{c}	64^{b}	379^{ef}	0.54	1.58	0.92
III	504^{fg}	63^{a}	66^{ab}	367^{gh}	0.56	1.27	1.07
IV	518^{d}	52^{cd}	57^{c}	373^{fg}	0.54	1.32	0.86
V	506^{fg}	53^{cd}	54^{c}	387^{d}	0.57	1.29	0.89
VI	478^{i}	51^{de}	55^{c}	416^{b}	0.65	1.38	0.91
VII	524^{cf}	33^{g}	55^{c}	388^{de}	0.71	1.38	0.54
Significance	***	***	***	***	–	–	–
Soil HA[3]	538–587	8–43	32–62	328–383	–	–	–

[1] Atomic ratio.

[2] Atomic ratio ×10.

[3] Usual range for soil humic acids (Stevenson, 1994).

***Significant at probability level $P < 0.001$. Figures in the same column followed by the same letter does not show significant statistical differences according to Duncan Test at the probability level of $P < 0.05$.

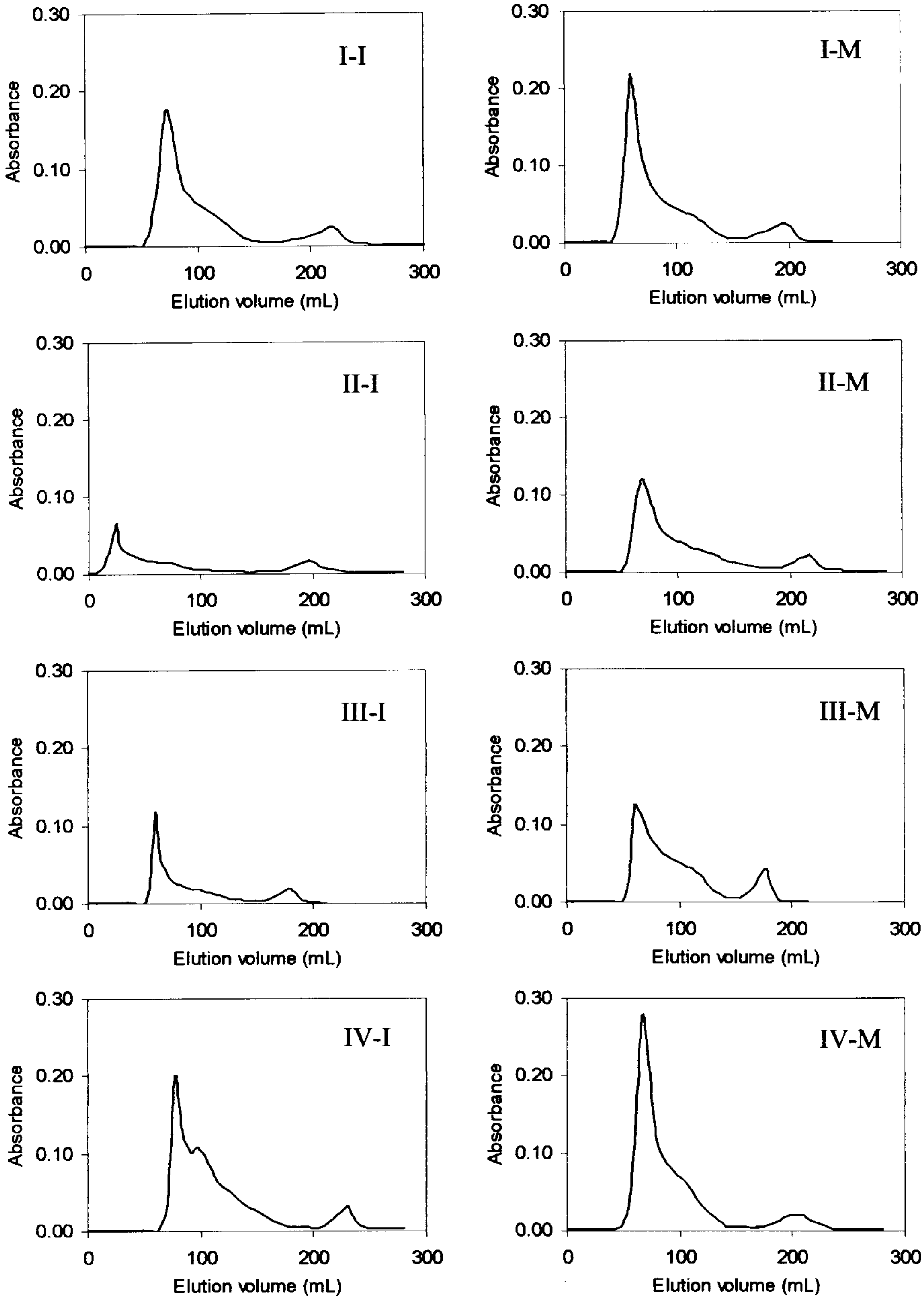

Figure 1. Gel permeation chromatograms of the humic acids I–IV (I: initial; M: mature compost).

Table 3. Functional group composition (acidity, meq g^{-1})

	Total acidity	Carboxylic acidity	Phenolic acidity	Carbonyl acidity	Of/O[1]
Initial					
I	3.08^{g}	1.83^{j}	1.25^{e}	0.76^{f}	23.1
II	2.72^{h}	1.95^{i}	0.77^{f}	1.36^{cd}	26.2
III	2.66^{h}	2.11^{h}	0.55^{f}	1.12^{e}	26.2
IV	3.51^{ef}	2.25^{g}	1.26^{e}	1.59^{bc}	34.3
V	3.77^{e}	2.41^{f}	1.36^{e}	1.28^{de}	33.5
VI	4.46^{d}	2.55^{e}	1.91^{d}	1.94^{a}	35.7
VII	3.31^{fg}	1.82^{j}	1.49^{e}	1.12^{e}	28.3
Mature					
I	6.66^{a}	3.96^{a}	2.70^{a}	1.32^{de}	43.1
II	4.59^{d}	2.71^{d}	1.88^{d}	1.89^{bc}	37.3
III	4.81^{d}	2.76^{d}	2.05^{cd}	1.12^{e}	38.0
IV	5.16^{c}	2.93^{c}	2.23^{bc}	1.96^{a}	43.1
V	5.53^{b}	3.18^{b}	2.35^{ab}	1.71^{b}	43.2
VI	5.34^{bc}	2.91^{c}	2.43^{ab}	1.57^{bc}	37.9
VII	3.89^{ef}	2.60^{e}	1.29^{e}	1.20^{de}	29.8
Significance	***	***	***	***	–
Soil HA[2]	5.6–8.9	1.5–5.7	2.1–3.6	0.1–5.6	–

[1] Of/O = Functional O (carboxylic + phenolic + carbonyl) as % of total O.

[2] Usual range for soil humic acids (Stevenson, 1994).

*** Significant at probability level $P < 0.001$. Figures in the same column followed by the same letter does not show significant statistical differences according to Duncan Test at the probability level of $P < 0.05$.

0.1 N HCl and once with desionized water and finally freeze-dried for storage.

Characterisation of humic acids

The elemental composition of the humic acids (C, H, N) was determined by an ANA 1500 Carlo Erba microanalyser and the O was calculated by the difference from 100. Total acidity and carboxyl groups were measured using the barium hydroxide and the calcium acetate methods, respectively (Perdue 1985). Phenolic groups were calculated as the difference between total acidity and carboxyl groups, and the carbonyl groups were determined by the oximation method (Schnitzer & Khan 1972). Chemical analysis was carried out in triplicate. Analysis of variance and Duncan's multiple range tests were used to determine differences caused by purification on elemental and functional composition.

Gel permeation chromatography was performed using Sephadex G-150 and a 45 × 2.8 cm Pharmacia column. Elution pressure was maintained by a peristaltic pump at a constant flow rate of 24 ml h^{-1}. A modification of the method described by Swift & Posner (1971) was used in order to adjust the ionic strength (Tsutsuki & Kuwatsuka 1984). The gel packing solution and the eluent was 0.025 M tris-hydroxymethil-aminomethane (Tris) adjusted to pH 7.5 with phosphoric acid. Samples for chromatography were prepared as follows: 25 mg portions of HAs were dissolved in 25 ml of 0.1 M NaOH, precipitated with 3 ml of 1 M HCl, stored for 24 h and centrifuged at 23,500 g for 15 min. The precipitate was redissolved in the 0.025 M tris-buffer solution up to a volume of 10 ml. Column eluates were passed through a continuous flow Uvikon 940 spectrophotometer and the absorbance was measured at 465 nm. The column void volume V_0 was measured with blue dextran 2000, and the total volume V_t was obtained by eluting with glucose. The distribution coefficient of a determined fraction (K_{av}) is calculated by the following expression:

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}, \tag{1}$$

where V_e is the elution volume of the fraction.

Infrared spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrophotometer using the pellet tech-

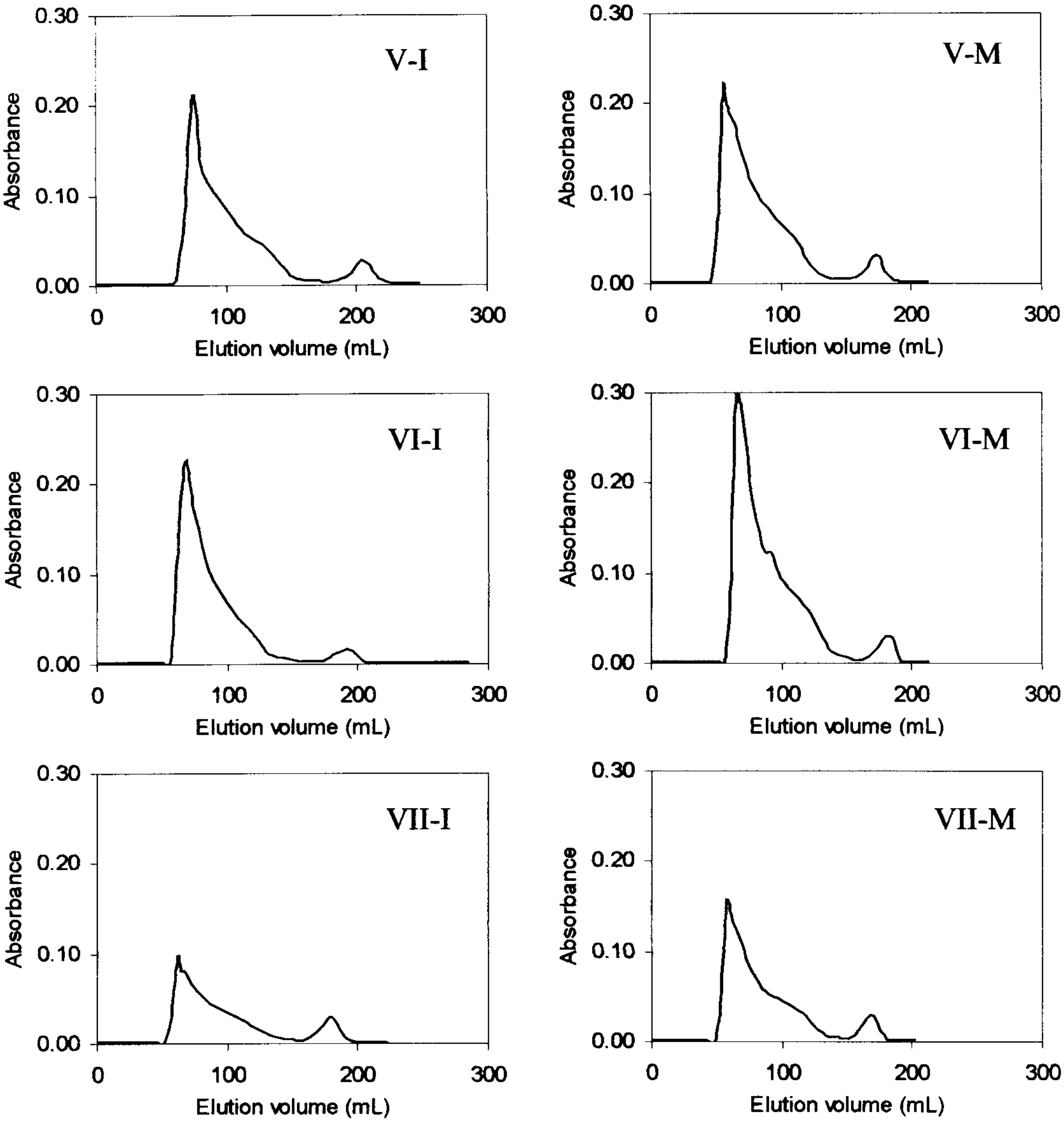

Figure 2. Gel permeation chromatograms of the humic acids V–VII (I: initial; M: mature compost).

nique, by mixing 1 mg of dried humic acid with 300 mg of predried and pulverized spectroscopic grade KBr.

Results and discussion

Elemental composition analysis

Table 2 shows the elemental composition (C, N, H and O concentrations) of the humic acids extracted at the beginning and at the end of the composting process. The elemental composition of all the materials are very close to the usual levels found in humic acids extracted from soils (Stevenson 1994). C concentration is in the lower limit of the range, whereas N, H and O are close to the upper limit, as has also been found by other authors, especially in the case of N (Deiana et al. 1990; He et al. 1992; Unsal & Sozudogru Ok 2001). As a consequence, the atomic ratios O/C, H/C and N/C showed more clear differences with respect to soil humic acids. As a general pattern, the humic acids extracted from the organic materials had higher atomic ratios than the normal values for soil humic acids. The biggest difference occurs for H/C atomic ratio which varied in the range 1.27–1.65, whereas it is expected

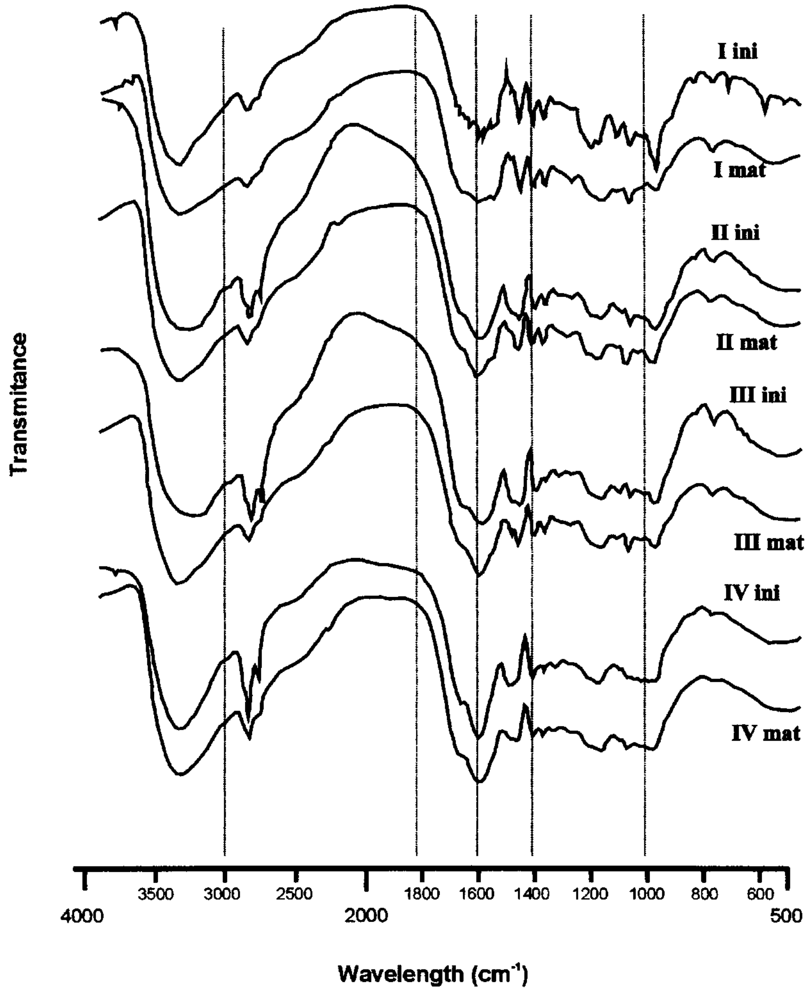

Figure 3. Infrared sprecta of the humic acids I–IV (ini: initial; mat: mature compost).

to be around 1 in soil humic acids, indicating the less aromatic structure of the humic acids extracted from the organic materials compared to the soil humic acids.

The composting process produced slight but statistical differences in the elemental composition of the humic acids. The effects on the elemental composition during the composting process were a decrease of 4 and 12% in the C and H concentrations, respectively, and an increase of 7% for both N and O concentrations. As a consequence, there was an slight increase in O/C and N/C ratios and a decrease of the H/C ratio that suggest that the composting process produced an increase in aromaticity and oxygenation of the humic substances as well as an enrichment in nitrogen compounds. These slight differences in elemental composition caused by composting are in agreement with the findings of others authors, such as Kakezawa et al. (1992) and Miikki et al. (1997). However, other authors such as Inbar et al. 1990, did not find any significant difference on chemical composition in the humic acids extracted during composting of the solid fraction of cattle manure.

The changes in chemical composition during the composting process could be due to the degradation of substances with a low degree of humification and more aliphatic structure (with high H/C ratio) such as carbohydrates, polysaccharides or fatty acids that might be originally linked to the humic "core" of the humic substances (Chefetz et al. 1998; Veeken et al. 2000). Chefetz et al. (1996) and Chefetz et al. (1998), based on ^{13}C-NMR spectroscopy, suggested that the carbohydrates, polysaccharides, alcohols, lipids and proteins initially linked to humic acids, were lost

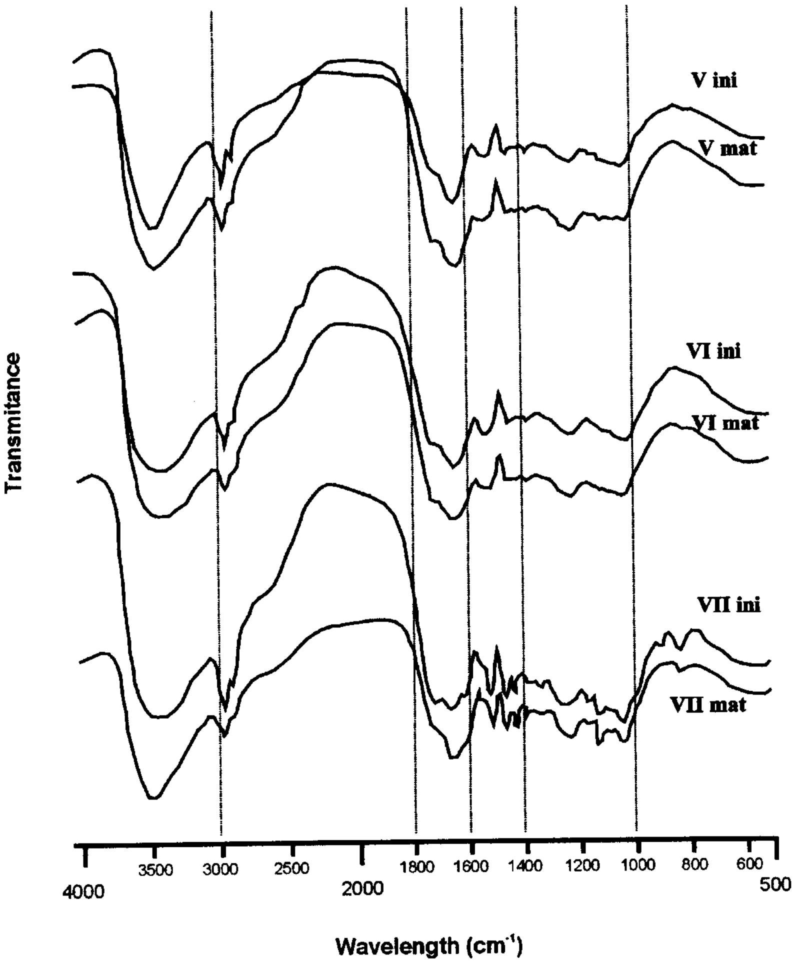

Figure 4. Infrared sprecta of the humic acids V–VII (ini: initial; mat: mature compost).

by degradation during composting, while the residual humic acids were enriched with aromatic structures.

The increase in the nitrogen concentration in the humic acids extracted after the composting process could be due to the greater resistance of the complex between nitrogen and the humic "core" (Kakezawa et al. 1992; Miikii et al. 1997), that produce a relative enrichment of these N-containing compounds. Moreover, it is also important to consider the contribution of the nitrogen compounds in the different humification pathways (Veeken et al. 2000). Nitrogen compounds are incorporated into the humic "core" through condensation of proteins and modified lignin, reactions between N-containing compounds and quinones arising from lignin, or sugar-amine condensation (Stevenson 1994). Schnitzer et al. (1993) suggested that the decrease of the concentration of heterocyclic-N compounds measured in the water extract during composting, might be due to the incorporation of these compounds to the humic structures.

Functional group analysis

Table 3 shows the functional group composition of the humic substances studied. The functional groups include total acidity, phenolic, carboxylic and carbonyl groups. As a general pattern the composting process produced an increase in all the functional groups in most of the humic acids studied. This effect has been more noticeable in the functional groups than in elemental composition. The functional groups increased for the range 2.66–4.46 to 3.89–6.66 for total acidity, being closer to the normal range of soil humic acids (between 5.6 and 8.9 meq g^{-1}) (Stevenson 1994). This increase was produced in both phenolic and carboxylic groups, with a significant statistical increment of 29 and 42%, respectively, in the average concentrations of these functional groups before and after the composting process. The increase of the carbonyl group was lower than that for the others groups and accounted for 18% after the composting process.

The effects of the composting process on the functional group concentration of the humic acids are consistent with the main pathways of humus synthesis in soil (Stevenson 1994). Hence, the increase in functional groups might be due either to the oxidation of methoxyl and alcoholic groups of side chains of lignin, or to the microbial degradation of carbohydrates to phenolic, quinone, ketone and carboxyl groups, causing the noticeable increment of the total acidity of the humic acids. Kakezawa et al. (1992) and Chefetz et al. (1998) suggested that one main transformation suffered by the humic substances extracted from organic materials takes place in the coating materials (as carbohydrates) of the humic "core" rather than in the "core" itself which remained almost unaltered. The results are also consistent with the hypothesis of the incorporation of simple compounds such as quinones and phenolic-degradation products (Schnitzer et al. 1993) to the humic structure through condensation reactions.

The composting process caused an important increase in the ratio between the oxygen involved in the functional groups and the total oxygen (Of/O) (Table 2). Both the oxidation of methoxyl and alcoholic groups already present in the original humic acids and the incorporation of new chemical fractions containing a high proportion of functional groups, would cause an increment in the proportion of functional oxygen (Of/O).

Gel permeation chromatography

Humic acids extracted from both fresh and composted organic matter showed similar gel permeation chromatographic patterns. In all cases the gel permeation chromatography separated the humic substances into two different fractions according to the molecular size distribution (Figures 1 and 2). Table 4 shows the percentage of the area for each fraction with respect to the total chromatographic area. The excluded fraction (A_1) corresponds to the higher molecular weight fraction whereas the included fraction (A_2) corresponds to the smallest one. The excluded fraction represents the biggest area and accounts for between 75 and 95% of the total area in all the humic acids studied.

As a general trend the composting process produced a slight enrichment of the excluded fraction and the included fractions run to earliest elution times. The increase of the excluded fraction could be due to condensation reactions of different fractions to the humic "core" producing molecules of higher molecular weight, or to the degradation of the smallest fractions, that produce an enrichment of the biggest ones. Prudent et al. (1995) and Ciavatta et al. (1993) also suggested that the composting process increased the complexity of the humic substances, based on gel permeation chromatography and electrofocusing, respectively.

Infrared spectroscopy

Figure 3 and 4 show the infrared spectra of all the humic acids studied. The humic acids extracted from the 7 composts showed very similar infrared spectra. In all of them the main absorbance bands were: a broad band around 3400 cm^{-1} due to H-bond and OH groups, two bands in the region 3000–2850 cm^{-1} due to aliphatic C–H stretching, a shoulder in the region 1740–1720 cm^{-1} due to C=O groups, a strong band at 1650 cm^{-1} due to C=C in aromatic structures and hydrogen bonded C=O, a shoulder at 1600 cm^{-1} due to aromatic C=C, a shoulder around 1540 cm^{-1} probably due to C=O from peptide groups, a peak around 1510 cm^{-1} due to aromatic C=C, a peak at 1460 cm^{-1} due to aliphatic C–H groups, a broad band in the region 1290–1220 cm^{-1} due to C–O stretch and OH deformation, a peak around 1125 cm^{-1} due to aliphatic CH_2, OH or C–O groups, and finally a peak around 1035 cm^{-1} due to C–O stretch of carbohydrates (Schnitzer & Khan 1972; Inbar et al. 1990; Niemeyer et al. 1992; Stevenson 1994; Francioso et al. 1998).

The most appreciable changes caused by the composting process in the infrared spectra of the humic acids is the clear decrease in the intensity of the bands in the region of 3000–2850 cm^{-1} indicating the decrease of the aliphatic fractions in the humic structure, as has already been suggested by the elemental composition. Other changes were relatively more slight and were not so clear. There is a slight decrease in the intensity of the band at 1540 cm^{-1} region that may correspond to peptide groups, which could indicate the breakdown of the peptides into aminoacids for the ultimate incorporation in the humic "core". There is also an increase in the intensity of the 1420 cm^{-1} band which could correspond to C=C structures and would be in agreement with the chemical analysis, however

Table 4. Values of the K_{av} and areas of the excluded (A_1) and included (A_2) fractions (% of total area) in the gel permeation chromatograms of HA's extracted from the composts

	Excluded fraction (A_1)		Included fraction (A_2)	
K_{av}	0.0	0.8	0.9	1.0
Initial				
I	85.4	14.6	–	–
II	74.0	–	–	26.0
III	81.1	–	18.9	–
IV	90.7	9.3	–	–
V	94.1	–	5.9	–
VI	92.5	–	7.5	–
VII	83.8	–	16.2	–
Mature				
I	87.6	12.4	–	–
II	87.1	12.9	–	–
III	86.3	13.7	–	–
IV	92.0	8.0	–	–
V	93.2	6.8	–	–
VI	93.2	6.8	–	–
VII	91.3	8.7	–	–

K_{av}: $(V_e - V_0)/(V_t - V_0)$, where V_e, V_0 and V_t represent the eluted, column void and total permeation volumes, respectively.

this enrichment could simply be a consequence of the degradation of the more aliphatic fractions. It is remarkable that there were not important changes in the 1650 and 1035 cm^{-1} bands as has been found by others authors in humic acids extracted from municipal solid wastes (Sugahara & Inoko 1981) and sewage sludge composts (Miikki et al. 1997). Furthermore, the characteristic band of carbohydrates around 1035 cm^{-1} was very weak as compared with others humic acids extracted from composts, making difficult its use for monitoring structural changes.

Conclusions

Functional group analysis is the most sensible analytical method to study the changes produced on the humic acid structure, compared to the other analytical methods used in the present work such as elemental analysis, gel permeation chromatography and infrared spectroscopy. Based on the experimental results, the composting process yields humic acids with chemical and structural characteristics (mainly elemental and functional concentrations) that were closer to the more humified soil humic acids. The main effects of the composting process on humic acids can be summarised in the formation of a higher molecular weight structure with more aromatic characteristics and with higher oxygen and nitrogen concentrations and a higher amount of functional groups, which are in agreement with the generally accepted humification theories in soil.

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References

Ciavatta C, Govi M, Pasotti L & Sequi P (1993) Changes in organic matter during stabilization of compost from municipal solid wastes. Bioresource Technol. 43: 141–145.

Chefetz B, Hatcher PG, Hadar Y & Chen Y (1996) Chemical and Biological characterization of organic matter during composting of municipal solid waste. J. Environ. Qual. 25: 776–785.

Chefetz B, Adani F, Genevini P, Tambone F, Hadar Y & Chen Y (1998) Humic-acid transformation during composting of municipal solid waste. J. Environ. Qual. 27: 794–800.

Deiana S, Gessa C, Manunza B, Rausa R & Seeber R (1990) Analytical and spectroscopic characterization of humic acids extracted from sewage sludge manure and worm compost. Soil Sci. 150(1): 419–424.

Finstein MS, Miller FC, MacGregor ST & Psarianos KM (1985) The Rutgers strategy for composting: process, design and control. EPA project summary (EPA/600/S2–85/059). US Environmental Protection Agency, Washington DC.

Francioso O, Sánchez-Cortés S, Tugnoli V, Ciavatta C & Gessa C (1998) Characterization of peat fulvic acid fractions by means of FT-IR, SERS, and ^{1}H, ^{13}C NMR spectroscopy. Appl Spectrosc. 52(2): 270–277.

González-Vila FJ, Almendros G & Madrid F (1999) Molecular alterations of organic fractions from urban waste in the course of composting and their further transformation in amended soil. Sci. Total Environ. 236: 215–229.

He XT, Traina SJ & Logan TJ (1992) Chemical properties of municipal solid waste composts. J. Environ. Qual. 21: 318–329.

Hsu JH & Lo SL (1999) Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. Environ. Pollut. 104: 189–196.

Iglesias E & Pérez V (1989) Evaluation of city refuse compost maturity: a review. Biol. Wastes 27: 115–142.

Iglesias E & Pérez V (1992) Composting of domestic refuse and sewage sludge. II. Evaluation of carbon and some "humification" indexes. Resources, Conservation and Recycling 6: 243–257.

Inbar Y, Chen Y & Hadar Y (1990) Humic substances formed during the composting of organic matter. Soil Sci. Soc. Amer. J. 54: 1316–1323.

Kakezawa M, Nishida T & Takahara Y (1992) Structural Characteristics of humic acids extracted from woody composts by two-step composting process. Soil Sci. Plant Nutr. 38(1): 85–92.

Miikki V, Senesi N & Hanninen K (1997). Characterization of humic material formed by composting of domestic and industrial biowastes. Part 2: Spectroscopic evaluation of humic acid structures. Chemosphere 34(8): 1639–1651.

Niemeyer J, Chen Y & Bollag JM (1992) Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. Soil Sci. Soc. Amer. J. 56: 135–140.

Perdue EM (1985) Acidic functional groups in humic substances. In: Aiken GR, McKnight DR, Wershaw RL & MacCarthy PC (Eds). Humic Substances in Soil, Sediment and Water (pp 493–526). Wiley-Interscience, New York.

Prudent P, Domeizel M, Massiani C & Thomas O (1995) Gel chromatography separation and U.V. spectroscopic characterization of humic-like substances in urban composts. Sci. Total Environ. 172: 229–235.

Sánchez-Monedero MA, Roig A, Cegarra J, Bernal MP and Paredes C (2002) Effects of HCl–HF purification treatment on chemical composition and structure of humic acids. Eur. J. Soil Sci. 53: 375–381.

Schnitzer M & Khan SU (1972) Characterization of humic substances by chemical methods. In: McLaren AD (Ed). Humic Substances in the Environment (pp 29–51). Marcel Dekker, New York.

Schnitzer M, Dinel H, Mathur SP, Schulten HR and Owen G (1993) Determination of compost biomaturity. III. Evaluation of a colorimetric test by 13C-NMR spectroscopy and pyrolysis-field ionization mass spectroscopy. Biol. Agri. Hort. 10: 109–123.

Senesi N (1989) Composted materials as organic fertilisers. Sci. Total Environ. 81/82: 521–542.

Stevenson FJ (1994) Humus Chemistry: Genesis, Composition, Reactions. John Wiley & Sons, New York.

Sugahara K & Inoko A (1981) Composition analysis of humus and characterization of humic acid obtained from city refuse compost. Soil Sci. Plant Nutr. 27(2): 213–224.

Swift RS & Posner AM (1971) Gel chromatography of humic acids. J. Soil Sci. 22: 237–249.

Tsutsuki K & Kuwatsuka S (1984) Molecular size distribution of humic acids as affected by the ionic strength and degree of humification. Soil Sci. Plant Nutr. 30: 151–152.

Unsal T & Sozudogru Ok S (2001) Description of characteristics of humic substances from different waste materials. Bioresource Technol. 78: 239–242.

Veeken A, Nierop K, de Wilde V & Hamelers B (2000) Characterisation of NaOH-extracted humic acids during composting of a biowaste. Bioresource Technol. 72: 33–41.