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Structural characterization of fulvic acids, extracted from sewage sludge during composting, by thermochemolysis–gas chromatography–mass spectrometry

S. Amir [a], M. Hafidi [a,*], L. Lemee [b], J.-R. Bailly [c], G. Merlina [c], M. Kaemmerer [c], J.-C. Revel [c], A. Ambles [b]

[a] *Laboratoire d'Ecologie Végétale et Environnement, Faculté des Sciences Semlalia, Département de Biologie, BP/2390, Marrakech, Morocco*
[b] *Laboratoire Synthèse et Réactivité des Substances Naturelles (UMR 6514), Université de Poitiers, 40 avenue du Recteur Pineau, Poitiers 86022, France*
[c] *Equipe Agronomie, Environnement et Ecotoxicologie (A2E), Ecole Nationale Supérieure Agronomique, Auzeville-Tolosane, BP/107, Toulouse, France*

Abstract

Thermochemolysis coupled with gas chromatography and mass spectrometry were applied to determine the chemical structure of fulvic acids (FA) extracted from a sewage sludge and straw mixture at different steps of composting. The FA starting structures were composed mainly of mono-, di- and tri-methoxy (alkyl) benzoic acids representing lignin derivatives in an advanced stage of oxidation, as well as of methylated polyphenols and a series of fatty acids saturated C15, C16, C18, unsaturated and branched C18:1. Besides, there are many unidentified structures suspected to be N-containing compounds. During the composting process, the fate of the three structural typical monomer units of lignin was followed. The *p*-hydroxyphenyl units showed a strong relative decrease especially at the beginning of composting. The guiaicyl units showed a steady increase in course of composting. A relative decrease of syringyl units was noted at the beginning of composting, but they then relatively increased towards the end of composting. These changes support the formation during composting of more oxidized units. All subunits composing the fulvic acid structures have been subdivided into main five groups of similar chemical structure. The lignin-like C6–C3 subunits showed a relative decrease during composting attributed to microbial oxidation, but there was a relative increase during the intermediate step of composting related probably of an enhancing of lignin solubility. The C6–C1 subunits were reduced at the beginning of composting, which mainly attributed to the oxidation of 4-methoxybenzaldehyde to hydroquinones derivatives under the composting conditions. Although, the relative increase of these subunits (C6–C1) at the end of composting originates from oxidation of C6–C3 lignin side-chains or could be partly attributed to microbial neosynthesis. The unidentified N-containing compounds increased strongly during course of composting. The rise in the level of fatty acids at the beginning of composting is attributed to an increase of branched-chain fatty acids such as C18:1 commonly used as bacterial biomarkers. Their amounts were greatly reduced at the end of composting. Both the Shannon–Weaver and similitude indices show a relative increase in structural diversity at the start of composting conditions following the appearance of hydroquinone derivatives and unidentified nitrogen compounds in the FA network. But, the produced FA structure reaches a certain level of homogeneity at the end of composting through self-polycondensation or recombination of C6–C1 subunits and hydroquinones derivatives with N-containing compounds.

Keywords: Thermochemolysis–gas chromatography–mass spectrometry; Composting; Sewage sludge; Fulvic acids

1. Introduction

Recycling sewage sludge in agriculture as fertiliser and as an organic amendment in intensively cropped and organic matter-depleted soils is classed as an important environmental strategy [1]. However, pre-treatment is needed to avoid numerous harmful effects caused by the application to soil of non-matured composts [2]. Composting is an aerobic thermophilic biotreatment leads to the biotransformation of raw organic matter into a more stable product rich in humic substances. The amounts and chemical structures of the humic material constitute, according to numerous studies, the main indices to assess the stability and maturity of compost [1,3,4]. Humic substances consist of two main fractions

* Corresponding author.
E-mail address: hafidi@ucam.ac.ma (M. Hafidi).

''humic acids'' and ''fulvic acids''. Fulvic acids have proved to be of great interest in chelating mineral elements and in enhancing plant growth. Due to their smaller size and high carboxyl group content, they are much more chemically reactive than humic acids [5]. As a consequence, the determination of their chemical composition and structure at a molecular level is of importance for soil scientists. The structural nature of humic material is still controversial with many factors possibly involved in the contradictions such as the origin of the humic material investigated, the extraction techniques used, the purification methods and the analysis techniques. In previous works using FT-IR and ^{13}C NMR spectroscopy to study changes in chemical structure of fulvic acids isolated from composted sludge at different degrees of maturity [6], the presence of large amounts of etherified/esterified aromatic structures was revealed by intense bands around 1600 and 1140 cm^{-1} in FT-IR spectra and high ^{13}C NMR signals at 74 and 174 ppm corresponding to alkyl ether and ester bonds. However, in these areas arise also the polysaccharides.

Thermochemolysis has proved to be a good method to obtain detailed structural information on the building blocks of organic macromolecules. It is based on the thermal breakdown of humic macromolecules into various subunits; which are subsequently isolated and identified.

Numerous authors have used simple pyrolysis or TMAH–GC–MS analysis to develop a better understanding of how humification occurs in soil by supervising biotransformation of aromatic entities from lignin derivatives [7–10]; as well as by characterizing humic acid material isolated from compost at different degrees of maturity [11,12]. In contrast to simple pyrolysis–GC/MS that could involve decarboxylation and formation of very polar products, thermochemolysis with tetramethyl ammonium hydroxide (TMAH) selectively cleaves ester and certain ether linkages (such as B–O–4 aliphatic-aryl bonds) in macromolecular organic matter [13]. The latter chemolytic procedure hydrolyses and methylates ester and ether linkages, and assists in the depolymerization and methylation of organic structures [14–16]. It is especially useful for detecting polar compounds such as long-chain fatty acids and benzenecarboxylic acids [17–19].

In this work, we used TMAH–GC–MS approach during composting to follow the structural changes of the units making up the fulvic acids structure as a whole.

2. Material and methods

2.1. Composting

Sewage sludge was taken from an anaerobic lagoon in an experimental wastewater treatment plant. A mixture of fresh sewage sludge (90%) and straw (10%) mixture was composted on a purpose-built platform at 54% of moisture and supervised for 9 months. To provide aerobic conditions for the composting, the mixture was turned every 2 weeks. Some preliminary physico-chemical characteristics of the mixture at different stages of composting (RM = raw mixture; 30 days = stabilization phase; 90; 180; 270 days of composting = maturation phase) are illustrated in Table 1 [20].

Table 1
Change of some physicochemical characteristics of sewage sludge and straw mixture during composting

Stages of composting (days)	pH	C, %[a]	N, %	C/N	DEC, %	Ash, %[a]
RM	7.30	17.74	0.742	24.00	–	65.90
30	7.40	16.18	0.756	21.40	20.00	70.70
90	6.80	15.01	0.812	18.48	36.50	75.30
180	6.73	14.26	0.868	16.40	40.00	76.30
270	6.77	13.45	0.882	15.24	41.50	76.78

C: organic carbon N: total Kjeldahl nitrogen DEC: decomposition rate.
[a] Result expressed by dry weight.

2.2. Fulvic acid isolation

In order to extract humic substances (HS), the fresh sample (30 g) is firstly treated three successive times with 40 ml of H_2O to remove non-humic, water-soluble substances (sugars, proteins), and so avoid interference between these compounds and humic compounds [21]. The extraction was then made on a pre-treated sample with 40 ml of 0.1N NaOH solution. Extraction was repeated several times until uncoloured supernatant was obtained. Centrifugation was then carried out at 4000 × *g* for 15 min. The filtered solutions underwent acid treatment with 3N H_2SO_4 until pH 2 and preserved for 24 h at 4 °C. The fulvic acids (FA) supernatants were separated from the precipitates ''humic acids (HA)'' through filtration. The FA were then dialyzed through a 100 Da Spectra Por membrane to eliminate excess salts then lyophilized. The FA content in course of composting has been determined by $KMnO_4$ oxidation method [22].

2.3. Pyrolysis–gas chromatography–mass spectrometry

About 4 mg of lyophilized FA were dissolved in chloroform and mixed with 10 μl of a methanol solution containing 50% (w/w) of tetramethyl ammonium hydroxide (TMAH) (Aldrich). The samples were heated for 5 min to 40 °C to evaporate the methanol. The remaining solid was transferred to a 25 × 1.9 mm (i.d.) quartz tube filled with quartz wool.

The tube was then heated from 350 to 650 °C at 5 °C ms^{-1} and maintained at 650 °C for 10 s. Thermochemolysis products were carried into the GC or the GC–MS by He gas.

GC separations were done on a Varian 3900 gas chromatograph using a BPX (SGE) capillary column (30 m long, 0.25 mm i.d.). Column temperature was programmed from 60 to 300 °C at a rate of 5 °C min^{-1} and held for 30 min at 300 °C. GC–MS analysis was performed in similar conditions on a Varian 3400 chromatograph coupled with a Finigan Incos 500 mass spectrometer. The compounds were identified on the basis of their GC retention times and by comparisons of their mass spectra with standards and with data from the literature.

3. Results and discussion

The evolution of the amount of the fulvic acids during composting is presented in Fig. 1. The fulvic acid level shows an increase of about 55% initial amount, which is more important in first 90 days of composting (37%) and reduced in the later phase (18%). In fact, this increase rate of fulvic acids is in agreement with the importance in the first phase of composting of the isolation of oxidized organic compounds through biodegradation, although the later period is considered primarily as a phase of humification by a further polycondensation of these organic structures and the others preserved recalcitrant organic structures to microbial biodegradation [23,24].

The chromatograms of TMAH–GC/MS of the fulvic acids (FA) isolated at different stages of composting are illustrated in Fig. 2. Generally, the product distribution is similar to that obtained by Hatcher and Clifford [25]; the first portion of the chromatogram mainly contains the peaks of aromatic compounds. The second portion consists of peaks that represent aliphatic compounds, consisting mostly of fatty acid methyl esters. Table 2 lists the main aromatic compounds presenting significant surface areas in the chromatograms. The results show that various aromatic compounds are thermally released from FA, which is mainly composed of methyl esters of mono-, di- and tri-methoxy (alkyl)-bezencarboxylic acids. These entities seem to present oxidized degradation products derived from lignin [15,26]. There are classified into three main groups presenting typical monomer units of lignin: *p*-hydroxyphenyl, guaiacyl or syringyl methylated skeletons. Besides, other aromatic structures occur such as 4-methoxyphenol. It should be noted that, the methoxy groups could correspond to those originally present in the molecule or to free hydroxyl groups that become methylated during the pyrolysis reactions.

The fatty acids methyl esters (FAMEs) yielded from FA thermochemolysis are mainly composed of saturated linear C15, C16, C18 and branched unsaturated C18:1 (the peaks of fatty acids are labelled with a star (*)) (Fig. 2). Besides, there are numerous unidentified structures such as (7) which are suspected to be nitrogen-containing aliphatic structures. The S-containing structure has not been identified. Lehtonen et al. [27] also reported that the unambiguous identification of nitrogen and sulphur containing degradation products proved to be quite difficult. In other study, Lehtonen et al. [28] suggest that the presence of sulphur containing substructures in humic substances can be slightly underestimated when using TMAH as alkylating reagent and too high lower limit for *m*/*z* ranges.

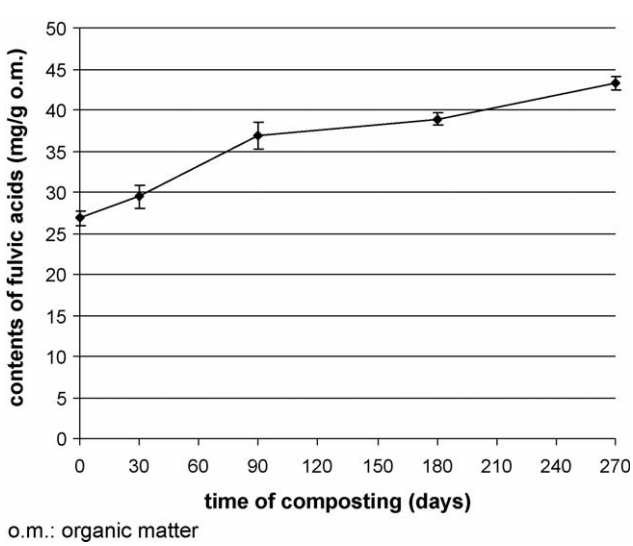


Fig. 1. Contents of fulvic acids isolated from sewage sludge during composting.

The absence of polysaccharides may be explained by they are not structural entities of fulvic acids and could be easily removed during the first step of water extraction [29]. However, according to Clifford et al. [30], the lack of carbohydrate-derived peaks in the TMAH chromatogram could be due to the poor sensitivity of this technique to carbohydrates.

The large contribution of more oxidized lignin-derived compounds may be explained by the fact that they are the compounds most resistant to microbial degradation among various organic wastes during wastewater lagooning. Schnitzer and Khan [29] and Ertel and Hedges [31] found also that following oxidative degradation by CuO, lignin phenols in FA are more highly oxidized or altered.

Fatty acids C15, C16, C18:1, C18 which trapped within the fulvic structure or cross-linked through ester bonds, could be originated from residues of microorganisms or plant [17–19,32,33].

By considering, in this study that the experiment conditions of TMAH–GC/MS to be applied as a quantitative tool has been difficult to be provided, the evolution of these various structural subunits of fulvic acids during composting, was followed through the comparison between their abundances in the thermograms at different steps of composting. The relative abundance "Pi" of each thermochemolysis product from FA is calculated as the ratio between the areas of the corresponding peak a_i to the sum of the areas of all the peaks considered in the pyrogram [34].

$$\mathrm{Pi} = \frac{a_i}{\sum_1^n a_i}$$

The values obtained are presented in Table 3. The results show that during the stabilization phase of composting, 4-methoxybenzaldehyde (10) disappeared with the appearance of 4-methoxytoluene (1), 4-methoxyphenol (2), unknown nitrogen substances (7) and 3,4-dimethoxybenzoic acid methyl ester (30). The relative abundance of the latter compounds increase progressively during composting. The mono-, di-methoxy alkyl-bezencarboxylic acid methyl esters (37) and (39) show a relative increase in the intermediate phase and then a relative decrease in the final step of composting. The results are in agreement with the theory of side-chain oxidation of lignin structures as one of the major humification processes [17,35]. Indeed, the relative increase of (37) and (39) is explained by the fact that they are the intermediate products in the oxidation sequence of the structural monomers of lignin such as *p*-hydroxycinnamyl alcohol and coniferyl alcohol [36–38]. While, 3,4-dimethoxybenzoic acid methyl ester (30), which highly relatively increased in the end of composting, could be

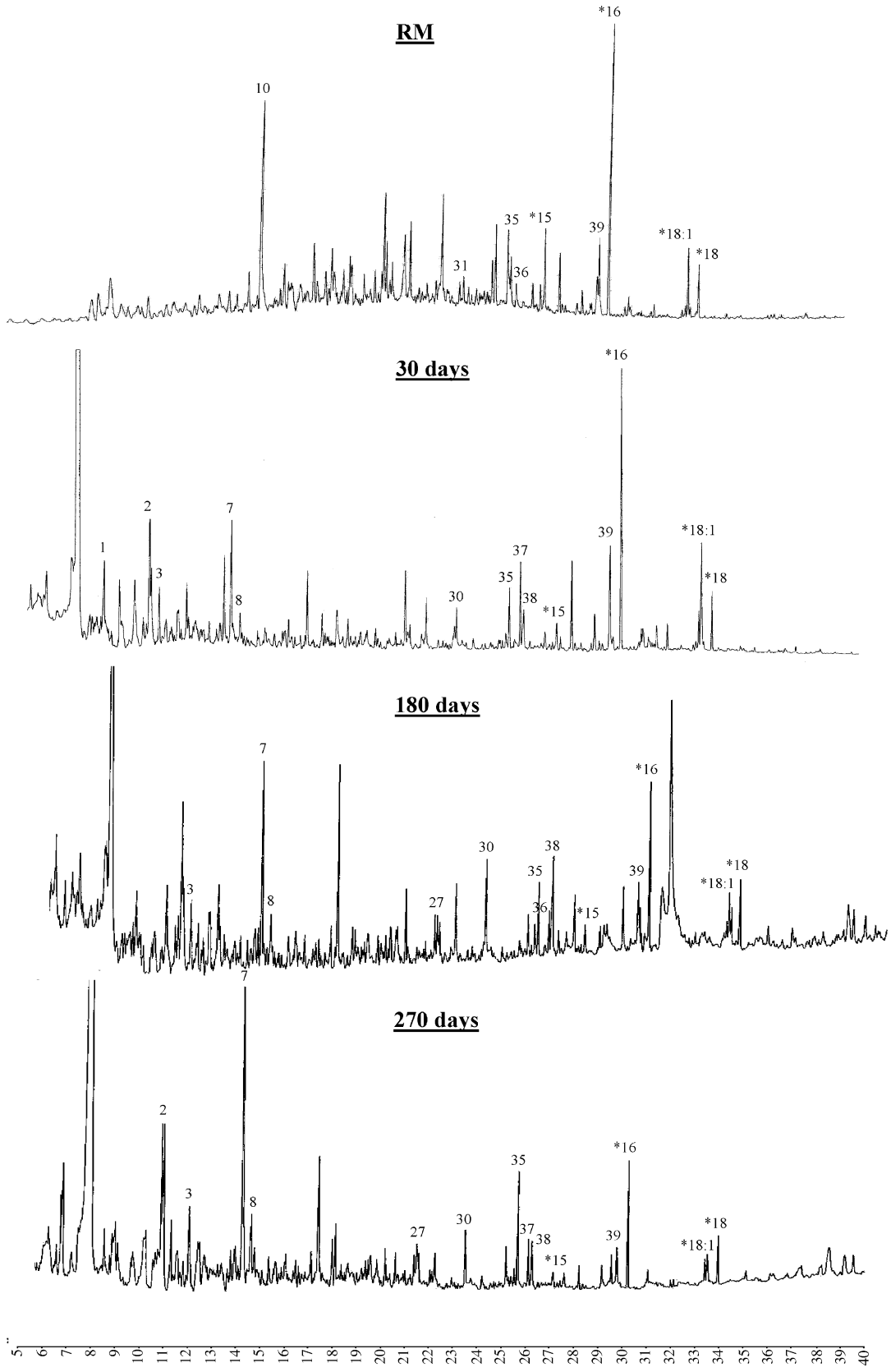
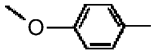
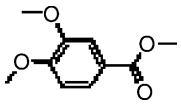
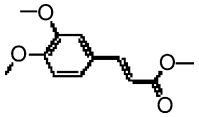
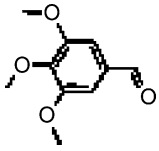
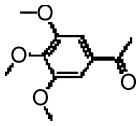
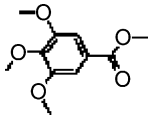
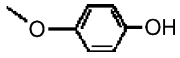
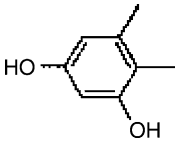
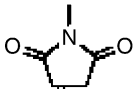


Fig. 2. Thermochemolysis–gas chromatogram of fulvic acids (FA) extracted from sewage sludge at different stages of composting.

Table 2
Peak identification of main aromatic compounds significantly present in thermochemolysis chromatograms of fulvic acids (FA) isolated from sewage sludge during composting

Compounds derived from *p*-hydroxyphenyl structures		
1		4-Methoxytoluene
10		4-Methoxybenzenaldehyde
37		3-(4-Methoxyphenyl)-prop-2-propenoic acid methyl ester
Compounds derived from guaiacyl structures		
30		3,4-Dimethoxybenzoic acid methyl ester
39		3-(3,4-Dimethoxyphenyl)-2-propenoic acid methyl ester
Compounds derived from syringyl structures		
31		3,4,5-Trimethoxy benzaldehyde
35		3, 4, 5-Trimethoxyacetophenone
38		3,4,5-Trimethoxybenzoic acid methyl ester
Other aromatic compounds		
2		4-Methoxyphenol
8		4,5-Dimethoxybenzene-1,3-diol
Nitrogen-containing compounds		
3		1-Methyl-2,5-pyrrolidinedione
7	Unidentified aliphatic N compounds	
Fatty acids methyl esters		

*15: *iso*-Pentadecanoic acid, methyl ester
*16: Hexadecanoic acid, methyl ester
*18:1: *iso*-Octadecenoic acid, methyl ester
**18:1: Anteiso-octadecenoic acid, methyl ester
*18: Octadecanoic acid, methyl ester

Table 3
Changes in the relative abundance of the main products thermally released from fulvic acids (FA) of sewage sludge at different stages of composting

Major identified compounds[a]	RM	30 d	90 d	180 d	270 d
1	nd	8.3	5.8	7.4	3.3
2	nd	11.1	11.1	11	13.9
7	nd	9.9	8.1	14.3	29.2
10	24.5	nd	nd	nd	nd
30	nd	3.5	3.2	6.9	5.3
35	8.0	4.3	3.1	4.4	9.7
37	9.7	5.3	7.7	9.4	3.5
38	7.5	4.5	3.1	10.0	4.7
39	6.9	7.9	10.8	5.2	4.7
*15	8.2	2.5	2.4	3.6	2.2
*16	27.0	24.3	25.1	9.6	11.0
*18:1	nd	2.7	3.0	6.3	3.1
**18:1	4.7	10.3	11.4	4.3	4.0
18	3.4	5.2	5.1	7.6	5.2

[a] See Table 2.

originated from side-chain oxidation of di-methoxy alkyl-benzene carboxylic acid methyl esters (39). According to Guggenberger and Christensen [35] and Chefetz et al. [17], the high benzenecarboxylic acids content of the TMAH products indicates a developed stage of lignin side-chain oxidation by microorganisms. Commonly the side-chain oxidation of mono-methoxy alkyl-bezencarboxylic acid methyl esters (37) must yield its corresponding product 4-methoxybenzenaldehyde or acid (10). However, even the initial amount of this compound (10) disappeared after the stabilization phase, which could be attributed to its ready oxidation or biotransformation to hydroquinone derivatives (2) in the composting conditions. Numerous previous studies suggest that phenols can be spontaneously oxidized to quinones and that the conversion is more likely carried out by polyphenoloxidase enzymes [39]. Indeed, 4-methoxyphenol (2) or hydroquinone derivatives show an important relative increase at the end of composting.

Concerning, the syringyl-derived structures, 3,4,5-tri-methoxyacetophenone and 3,4,5-trimethoxybenzoic acid methyl ester (35, 38) show a relative decrease in the intermediate steps of composting and then a relative increase at the end of composting. The ratio of acid- to aldehydes-containing derivatives (acid/aldehyde ratio) is commonly used to assess the degradation stage of lignin. High acid/aldehyde ratios represent a developed stage of lignin side-chain oxidation by microorganisms [17,35]. In this study, 3,4,5-trimethoxy-ybenzoic acid methyl ester (38) show a high amount at 180 days of composting, but relatively decreased in the end of composting when 3,4,5-trimethoxyacetophenone (35) rela-tively increased. This evolution is in agreement with the increase of acid-containing derivatives following the intense oxidation of lignin side-chains during the course of compost-ing. But, the relative decrease of this acid to aldehyde ratio at the end of composting could be attributed to process of oxidative polymerization of polyphenol acid units through ester/ether linkages [40,41]. The oxidative polymerization of polyphenols was previously proposed in numerous studies as important mechanism involving the formation of humic substances in soil and sediments [39,42–44].

However, Tanczos et al. [45] recommend caution in the use of acid/aldehyde ratios in TMAH pyrolysis as a parameter to measure degradation of lignin compounds. They show that aldehydes can undergo a Cannizzaro reaction (disproportion/methylation) producing their corresponding carboxylic acids.

Among other parameters to follow process of humification, Chefetz et al. [10] suggested the S/G ratio (syringyl to guaiacyl) as an index of evolution based on the assumption that condensed G-type lignin units are more resistant to biological degradation than lignin with a larger proportion of methoxyl groups S-type. These authors report that when the S/G ratio is decreased it suggests preferential degradation of syringyl units by microorganisms. In our case, the S/G ratio showed a decrease at the beginning of composting, but relatively increased at the end of the process. Fig. 3 presents the evolution of various lignin-derived units in fulvic acids during composting. Syringyl units show at the beginning of compost-ing a relative decrease that could be attributed to their decomposition by the intense activity of the thermophilic populations that develop in the stabilization phase. However, these structures then relatively increased at the end of composting. The proportion of guaiacyl units showed a steady increase during the course of composting. The *p*-hydroxyphe-nyl units showed a strong relative decrease especially at the beginnig of composting. Therefore, these results suggest the relative increase of guiacyl units and syringyl derivatives at the end of composting, which indicate the increase of more oxidized units during composting. The G/C ratio (guaiacyl to *p*-hydroxyphenyl) showed a progressive relative increase during composting. Indeed, lignin side-chains are oxidized, methoxy groups removed and hydroxylation occurred, resulting in an increase of more oxidized polyphenolic acids [46]. The relative increase of methylated derivative of hydroquinone, 3,4-dimethoxybenzoic acid methyl ester, 3,4,5-trimethoxybenzoic

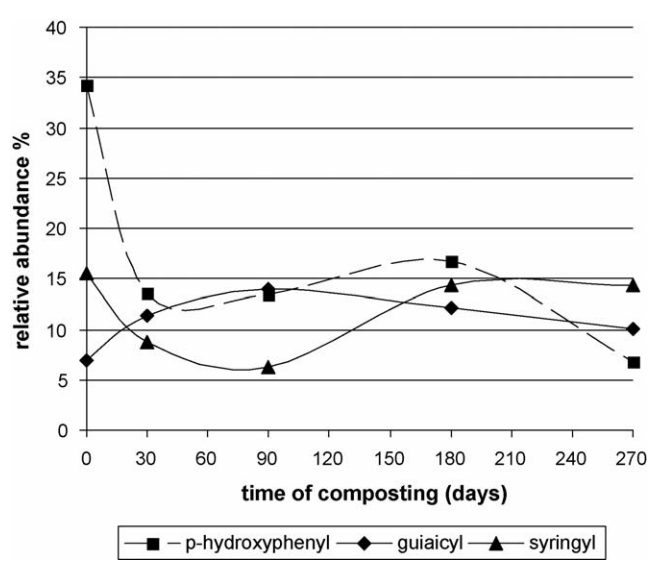


Fig. 3. Evolution of various lignin-derived units in fulvic acids of sewage sludge during composting.

acid methyl ester or 3,4,5-trimethoxyacetophenone at the end of composting is an agreement with that they constitute the last products to be formed or also possibly preserved in the lignin degradation sequence [10,17,47]. These products lead to the formation of humic substances following self-polycondensation and/or combination with other nitrogen compounds [13,14].

Fatty acids C15 and C16 show a relative decrease in course of composting, while branched C18:1 relatively increased during the stabilization phase and then decreased in the final stages of composting (Table 3). The relative decrease of fatty acids could be attributed to their use by microbes as a source of energy, but the relative increase of branched C18:1 in the intermediate step of composting could originated from residues of microbial activity. Branched-chain fatty acids are commonly used as bacterial biomarkers [48].

To determine the main changes occurring in the chemical structure of the fulvic acids during treatment, the evolution of five subunit families grouping compounds of similar chemical structure in the fulvic acid network was monitored in the course of composting (Fig. 4): C6–C3 (three carbons in the side-chain bound to the benzene unit); C6–C1 (benzoic acids), hydroquinone derivatives, nitrogenic structures and fatty acids.

During the stabilization phase there occurs relatively a slight decrease of C6–C3, but a strong relative decrease of C6–C1 subunits. In parallel, there was a relative increase in the hydroquinone derivatives and unidentified structures of aliphatic nitrogen-containing compounds. The great decrease of C6–C1 can mainly be attributed to the oxidation of 4-methoxybenzenaldehyde into hydroquinones derivatives following the start of composting conditions. The relative increase

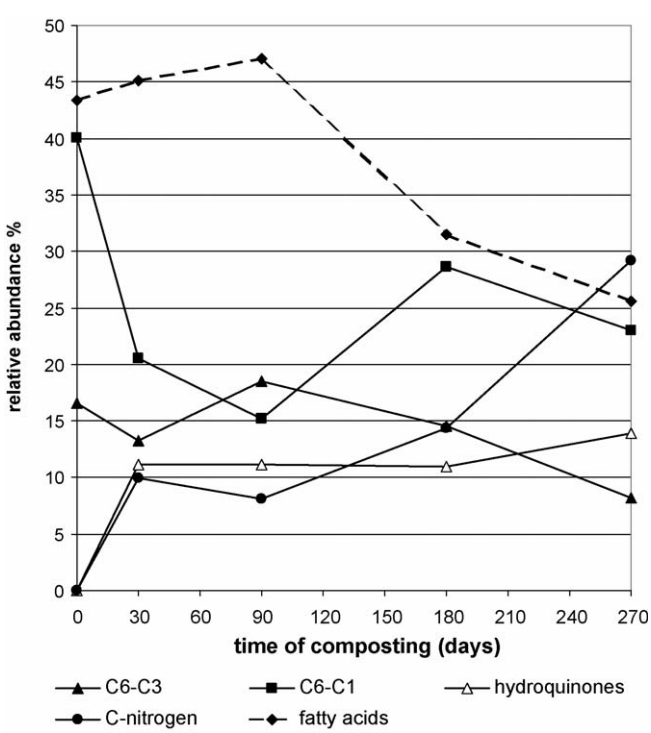


Fig. 4. Changes of five structural groups composing thermochemolysis products released from fulvic acids of sewage sludge during composting.

of intensity of numerous unidentified nitrogenous structures may derive from residual plant compounds or fungal and microbial remains that have been physically or chemically protected in humic structures against further microbial degradation [49]. Koch [50] showed that bacterial cell walls are formed of peptidoglucan (murein), which contain carbohydrate as well as amino acid elements composing *N*-acetylglucosamine and *N*-acetylmuramic acid. Knicker et al. [51] and Gomes et al. [52] investigated also the melanins in various actinomycetes and fungi. These authors indicated by IR study the high aliphaticity of these melanins than humic structure from the same soils [32]. In fact, González-Vila et al. [53] show the preferential incorporation of carbohydrate, possibly low molecular weight polysaccharides (hemicelluloses) and additional amount of N-containing compounds into fulvic acids.

On the other hand, the parallel in the relative increase of the proportion of nitrogenous structures with that of hydroquinones at the beginning of composting supports the Bremner [54] scheme of nitrosation of phenolic moieties, and the high affinity of hydroquinone for ammonia (NH_3) [39]. Some authors show that the contribution of nitrosation of organic matter as mechanism to immobilisation of N can occur in soil environments upon normal nitrification at neutral to slightly acidic pH values [55,56]. However, this parallel increase could be also explained by that these structures are from microbial origin, Butler and Day [57] suggest that melanin in fungi contain protein, carbohydrates, lipids and a polymeric core that consists of various types of phenolics, indolic, quinone, hydroquinone, semi-quinone monomers. They reported that ligninase enzymes from white-rot fungi are able to completely degrade fungal and bacterial melanins.

In the beginning of the maturation phase, the relative increase of proportion of C6–C3 may be attributed to an increase in the solubility of lignin-like compounds in the presence of the mesophilic populations that develop during this phase. This community is recognized by its strength to attack more resistant structures. In the late steps of composting, it is mainly C6–C1 subunits and the unidentified nitrogen structures that are relatively increased, while the C6–C3 subunits are relatively decreased. The large relative increase in C6–C1 could due to the oxidation of the side-chain of C6–C3 compounds, but could partly be attributed to microbial neosynthesis. Fatty acids increased during the stabilization phase of composting when microbial activities were intense, and then decreased after the death of the majority of the microbial populations in the final stage of composting.

Therefore, these findings allow us to suggest that the increase of level of fulvic acids, during composting, is originating from self-polycondensation or recombination mainly of C6–C1 subunits and hydroquinones derivatives with unknown aliphatic structures N-containing compounds. Numerous authors show the presence in soil or compost of a considerable proportion of "unidentified nitrogen" that constituted the hydrolysis-resistant structures [58,59].

This is in the agreement with the common models of formation of refractory organic nitrogen through depolymerization and recondensation pathways [51,58,60]. The latter

involves microbial degradation of organic macromolecules such as lignin and proteins to oligomers and monomers. Part of the oligomers and monomers recombine by random condensation or 1, -4 additions of ammonium and amino groups on phenols or quinone to form complex macromolecular N-containing structures. Jokic et al. [61] showed that the Maillard reaction and polyphenol model, into one combined humification pathways incorporating carbohydrates, amino acid and polyphenol moieties into the humic structure as would likely occur in nature.

Therefore, all above observations support the formations of fulvic acids in the course of composting through polycondensation of monomers already biotransformed or preserved during microbial metabolism rather than strict biodegradation of starting polymers. To assess the importance of the structural changes produced, two indices were applied to follow the distribution of various thermochemolysis subunits in the FA network during composting.

The index of Shannon–Weaver (Ish) exhibit the degree of diversity inside fulvic polymer. This ecological index is usually used to express the degree of organisation of an ecosystem [63]. Low values correspond to low diversity, while a high index corresponds to a complex system. This index has been used by Alcaniz et al. [62] and Ayuso et al. [34] to examine the organisation of soil and humic acids. Alcaniz et al. [62] suggest that a high diversity index Ish in soil implies the presence of fragments that occur in quantitatively equal amounts, while a low index indicates the occurrence of a selective process. Ayuso et al. [34] show that the low Ish of the leonardite soil pyrogram must be due to a selective process such as oxidation occurring in the course of humification. This index is calculated as follows:

$$\mathrm{Ish} = -\sum_{i}^{n} \mathrm{Pi} \log_2 \mathrm{Pi}, \quad 0.5 < \mathrm{Ish} < 4.5$$

where Pi is the relative abundance of each product considered in the thermochemogram [63]. Our results are illustrated in Fig. 5

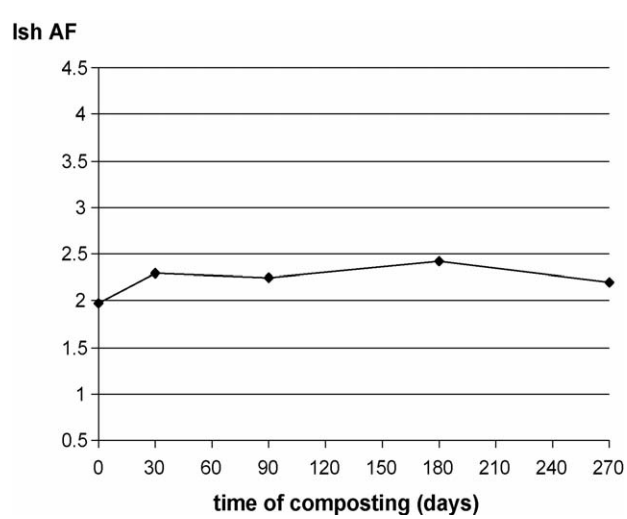


Fig. 5. Index of Shannon–Weaver for fulvic acids (FA) of sewage sludge during composting.

and show a relative increase of Ish at the start of composting and a slight relative decrease at the end. The initial augmentation arises from the relative increase in structural diversity of fulvic acids through formation of hydroquinone derivatives and other unidentified aliphatic N-containing structures following the change of medium conditions as the composting process starts (aeration, self-heating, intense microbial activity...).

A similitude index S_{ij} compares two thermograms and gives the degree of resemblance between the chemical structural composition of fulvic acids at different steps of composting. This index has been used by Ceccanti et al. [64] and Ayuso et al.

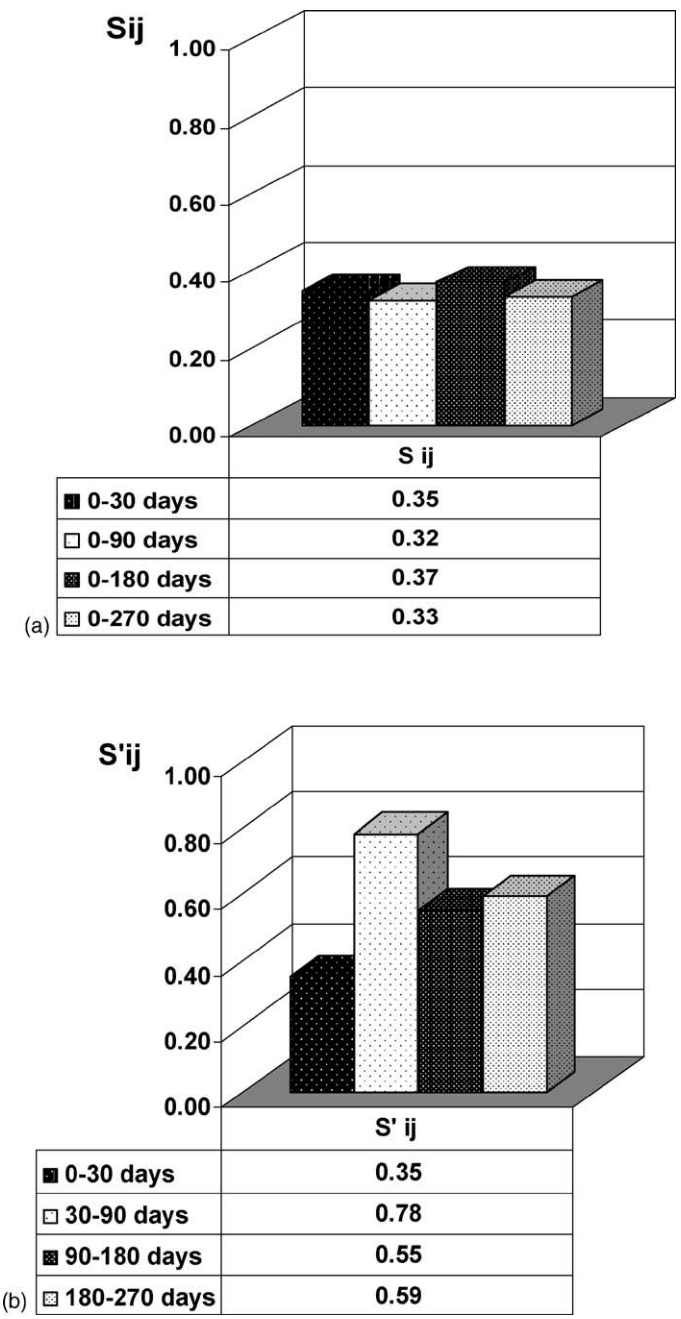


Fig. 6. (a) Similitude index between thermochemolysis chromatogram of fulvic acids at each stage of composting and the chromatogram at the start of composting. (b) Similitude index between two thermochemolysis chromatograms of fulvic acids at successive stages of composting.

[34] to determine the degree of similarity between two pyrograms of different organic materials (soil, compost, sewage sludge...). It is calculated as follows:

$$S_{ij} = \frac{\sum_1^n P_i^k / P_j^k}{n}, \quad P_i^k < P_j^k$$

P^k is the relative abundance of product in thermogram i and j and n is the number of peaks (products) considered.

S_{ij} is the degree of similitude between the thermogram at each stage of composting and thermogram at start of composting, which is presented in (Fig. 6a). S'_{ij} is the degree of similitude between two thermograms at successive steps of composting, which is illustrated in (Fig. 6b).

The index of similitude $S_{0/1}$ shows a low value of about 0.35 between the thermogram at 30 days and that obtained before composting. This value remains relatively constant in comparison between each thermogram of other stages 90; 180 and 270 days, with the thermogram before composting. The low similarity indice between the composted and uncomposted substrate implies great changes in the mechanism of formation of FA with the changes in conditions at the beginning of the composting process. Indeed, with the onset of the composting conditions, new structures appeared in the FA network such as hydroquinones and nitrogen-containing compounds. S'_{ij} increased to 0.78 between 30 and 90 days, but relatively decreased and remained constant near a value of 0.6, between 90 and 180 days and between 180 and 270 days of treatment. The relative increase in the index of similarity suggests that FA produced is constituted from similar chemical subunits. The slight relative decrease of indice value at the end of composting could be attributed to the importance of selective processes or oxidation of the side-chains of lignin-like compounds C6–C3 producing C6–C1 subunits.

4. Conclusion

The evolution of the chemical structure of fulvic acids isolated from a sludge and straw mixture in the course of composting was monitored using thermochemolysis coupled with gas chromatography and mass spectrometry. The starting structures were composed mainly of mono-, di- and tri-methoxy (alkyl) benzoic acids. Besides, methylated polyphenols and a series of fatty acids. During composting, a relative increase of intensity is shown for the more oxidized units C6–C1 such as 3,4-dimethoxybenzoic acid methyl ester, 3,4,5-trimethoxybenzoic acid methyl ester or 3, 4, 5-trimethoxyacetophenone following the side-chain oxidation of the lignin-like structure C6–C3. Besides, the appearance and the relative increase of level of hydroquinone derivatives and unidentified aliphatic structures mainly N-containing compounds occurred with the entrance in the composting conditions. The level of fatty acids shows a relative increase during composting which is related to the presence of intense microbial activities and it then decreases after the microbial populations die in the final step of low respiration rate. These findings allow us to suggest that fulvic acids produced during composting is originating from self-polycondensation or recombination mainly of C6–C1 subunits and hydroquinones derivatives with unidentified N-containing compounds. Therefore, there is polycondensation of monomers already biotransformed by microbial metabolism during composting rather than strict biodegradation of starting polymers.

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