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

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## Abstract

Thermochemolysis coupled with gas chromatography and mass spectrometry were applied to determine the structure of humic acids (HA) extracted from a sewage sludge and straw mixture at different steps of composting. The HA extracted from sludge mixture released various compounds, such as mono-, di-, tri-methoxy (alkyl) benzene and (alkyl) benzoic acids, which originated from lignin like derivatives of *p*-hydroxyphenyl, guaiacyl and syringyl units. In addition, other aromatic non-lignin derived structures were found along with series of branched C15, linear C16, C18 fatty acid methyl esters. The follow-up of various lignin-derived units during composting shows a decrease in *p*-hydroxyphenyl type-compounds (C) after the stabilisation phase. In parallel the more oxidized units, derived from guaiacyl (G) and syringyl (S) units, corresponding mainly to methylated derivatives of caffeic acids, protocatechuic acids, gallic acids and aldehydes, significantly increased in comparison with the other aromatic structures. Various ratios commonly used as parameters to determine the degree of lignin decomposition during humification were followed to monitor the chemical structure changes of the HA extracted from sludge mixture during composting. In the present case, the S/G ratio did not present significant changes during composting. The acid/aldehyde ratio was supervised using the ratio of gallic acids to gallic aldehyde methylated derivatives and showed an increase from 0.73 to 2.13 after the stabilisation phase but a decrease to 0.93 at the end of composting. This evolution may be explained by the increase of acid-containing derivatives following the intense oxidation of lignin side-chains during the stabilisation phase. But, the decrease of the acid/aldehyde ratio during the maturation phase could be attributed to a decrease in acid units by polymerisation of benzoic acid type-compounds through ester/ether linkages. The follow up of six families of compounds of similar chemical structures during composting showed a decrease of lignin-type compounds C6–C3 and that C6–C1 units predominated in the humic acid isolated from end compost sludge. The fatty acid methyl esters showed an increase in the intermediate phase of composting probably originating from the activities and tissues of microorganisms, which are numerous during the process. The final decrease in the amount of fatty acids may be explained by the death of most of the microbial population at the end of composting typified by a low respiratory rate. The index of Shannon-Weaver (Ish) remained constant at about 3 in course of composting indicating the neoformation of HA from subunits of similar chemical nature. A similitude index ( $S_{ij}$ ,  $S'_{ij}$ ) showed a split between 30 and 90 days of composting indicating a change in the rate of neoformation of HA after a stabilisation phase. Afterwards, the rate of HA neoformation varied linearly with the duration of composting.

**Keywords:** Thermochemolysis–GC–MS coupling; Sludge composting; Humic acids; Benzenecarboxylic acid units; Condensation; Lignin degradation; Fatty acids

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## 1. Introduction

Composting of organic waste has become widely developed to yield a product that provides more fertilization and is safer than the raw material for agricultural recycling. This aerobic thermophilic biotreatment leads to the biotransformation of raw organic matter into a more stable product, rich in humic substances. The amounts and chemical structure of the humic material constitute, according to numerous studies, the main indices for assessing the stability and maturity of the compost [1–3]. The humic substances play a fundamental role in conditioning soil properties, since they are directly involved in the slow release of nutrients, high cation exchange capacity, pH, buffer capacity and heavy metals and xenobiotic organic molecule retention [4]. However, the actual structure of humic material is still controversial [5,6]. Some studies suggest the predominance of aromatic units in humic substances, whereas other groups have shown that many humic extracts contain largely aliphatic structures. Many factors could cause these contradictions, such as the origin of the humic material, the extraction technique, the purification method and the analysis techniques [7]. Previous works using FTIR and  $^{13}\text{C}$  NMR spectroscopy to study changes in the chemical structure of humic acids (HA) [8] and fulvic acids (FA) [9] isolated from composted sludge at different degree of maturity, showed the presence of abundant etherified/esterified aromatic structures by intense bands around 1600 and 1140 or 1034  $\text{cm}^{-1}$  in FTIR spectra and a strong and persistent  $^{13}\text{C}$  NMR signal, at different stages of composting, around 56, 74 and 174 ppm corresponding to ether and ester bonds. The thermochemolysis technique 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 break down of humic macromolecule into various subunits; which are subsequently isolated and identified [10,11]. In contrast to simple pyrolysis, this chemolytic procedure hydrolyses and methylates ester and ether linkages, and assists in the depolymerisation and methylation of lignin or other organic structures [12,13]. Moreover, the procedure avoids decarboxylation and produces the methyl esters of carboxylic acids and methyl ethers of hydroxyl groups, rendering many of the polar products volatile enough for GC analysis [14]. Numerous authors have used this chemical approach to develop a better understanding of how humification occurs in soil by following the biotransformation of aromatic entities from lignin derivatives [15,16]; as the approach has also been used to characterize humic material isolated from compost at different degrees of maturity [17]. Hatcher and Clifford [18] and Del Rio et al. [15] have shown that the TMAH thermochemolysis of HA in soil yields mono-methoxy, dimethoxy, and trimethoxy benzenecarboxylic acid methyl esters and fatty acid methyl esters. Chefetz et al. [16] showed that the ratio of syringyl to guaiacyl compounds (S/G) decreased suggesting a preferential degradation of the

syringyl units by microorganisms, and explained this by humification with increasing depth. Del Rio et al. [15] suggest the formation of benzenecarboxylic acid moieties during the coalification process. Miikki et al. [17] observed, with increasing maturation time in the course of composting, an increase in the relative amounts of alkylbenzenes and a decrease in the relative amounts of alkylphenols. Veeken et al. [19] showed that during composting, there was an increase of aromatic compounds mainly methoxy-phenols with respect to other compounds suggesting an increased solubility of lignin. These authors report the significant contribution of fatty acids C16, C18:1, C18 to the humic acids of biowaste compost. Miikki et al. [17] and Réveillé et al. [20] reported also that fatty acids constitute an important fraction in the pyrograms of the humic acids in sewage sludge even on exhaustively pre-extracted samples. These entities could have a positive effect on soil by influencing the water retention capacity, the structural stability and the biodegradation–humification balance in amended soils. However, they could be unfavourable in large quantities and induce phytotoxicity problems [21]. Numerous authors show that part of these fatty acids seem to be bound chemically to the macromolecular structure network of HA because they are not recovered in the lipid extract [20,22–24]. They explained this, either by the fact that solvent extraction was not complete, only partial removal of lipids from HA being allowed, or that these lipids were more difficult to extract. Réveillé et al. [20] suggested that even with additional lipid extraction carried out directly on the purified humic acids, some lipids would remain associated with humic acids by covalent bondings or by simply being trapped in their structure. Grasset et al. [24] used TMAH and tetraethylammonium acetate (TEAAC) to discriminate between free and esterified fatty acids for a better understanding of the humification process and the capacities of soils to retain or release auto-chthonous or allochthonous (xenobiotic) compounds. Accordingly, considering their importance as entities tightly trapped within humic structures or cross-linked through ester bonds in the matrix, the removal of lipid could destroy or disturb cross-linking bounds between molecular units and thus some entities may become more easily released through pyrolysis from molecular structures leading to an overestimation of their presence compared to others. In this work, we used TMAH-GC–MS analysis to follow during composting the structural changes of the units making up the humic acid structure as a whole, without previous or later lipid removal.

## 2. Materials and methods

### 2.1. Composting

Sewage sludge was taken from an anaerobic lagoon in an experimental wastewater treatment plant. Composting of a fresh sewage sludge (90%) and straw (10%) mixture at

Table 1

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

Stages of composting (days)	pH	OC (%) <sup>a</sup>	NTK (%)	C/N	DEC (%)	Ash (%) <sup>a</sup>
RM	7.3	17.7	0.74	24.0	–	65.9
30	7.4	16.2	0.76	21.4	20.0	70.7
90	6.8	15.0	0.81	18.5	36.5	75.3
180	6.7	14.3	0.87	16.4	40.0	76.3
270	6.8	13.5	0.88	15.2	41.5	76.8

OC: organic carbon; NTK: total Kjeldahl nitrogen; DEC: decomposition rate.

<sup>a</sup> Results expressed by dry weight.

54% of moisture was followed over 270 days on a composting platform. To provide aerobic conditions, the mixture was turned every 2 weeks. Some preliminary physical–chemical characteristics of compost at different stages of treatment are illustrated in Table 1 (RM: initial raw mixture; after 30 days “stabilization phase”; 90; 180; 270 days of composting “maturation phase”).

## 2.2. Humic extraction

To extract humic substances (HS), fresh sample (30 g) was washed with 3 × 40 ml of H<sub>2</sub>O to remove non-humic, water-soluble substances (sugars, proteins), and so avoid interferences between these molecules and humic compounds [25]. The extraction was then carried out on a pre-treated sample with 40 ml of 0.1 N NaOH. Extraction was repeated several times until colourless supernatants were obtained. Centrifugation is then done at 4000 × *g* for 15 min. The filtered solutions were then acidified with 3 N H<sub>2</sub>SO<sub>4</sub> until pH 2 and kept for 24 h at 4 °C. The precipitates, or humic acids were separated from the fulvic acids remaining in solution and then dissolved in 0.1 N NaOH. The HA fraction was dialyzed with a 1000 Da Spectra Por membrane to eliminate excess salts, and then was freeze-dried. The HA dosage were assayed by KMnO<sub>4</sub> oxidation [26].

## 2.3. Pyrolysis–gas chromatography–mass spectrometry

About 2 mg of freeze-dried humic acids 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 then heated for 5 min to 40 °C to evaporate the methanol. The remaining solid was transferred to 25 mm × 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<sup>-1</sup> and maintained at 650 °C for 10 s. Thermo-chemolysis products were carried into the GC or the GC–MS by a flow of 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 5 °C min<sup>-1</sup> and held at 300 °C for 30 min. 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 data from the literature.

## 3. Results and discussion

The chromatograms of TMAH-GC–MS of the humic acids isolated at different stages of composting are illustrated in Fig. 1. Table 2 lists the main compounds identified in the chromatograms, which can be classified into the following major groups: lignin derived and non-lignin derived aromatic compounds, heterocyclic N-containing compounds, fatty acid methyl esters (FAMES). The TMAH thermochemolysis of HA at different stages of composting yielded various compounds: mainly mono-, di- and tri-methoxy (alkyl)-benzenecarboxylic acid methyl esters. These entities seem to present oxidized degradation products derived from lignin as methylated cinammyl, guaiacyl and syringyl units. It should be noted that the methoxyl groups detected could correspond to the methoxyl groups originally present in the molecule or to free hydroxyl groups that become methylated after pyrolysis methylation.

Besides these lignin-derived structures, there are other non-lignin aromatic structures and series of FAMES composed of branched iso and anteiso C15, C18:1 and linear C16, C18 (fatty acid peaks are labelled by star (\*)). These fatty acids have often been shown to predominate amongst homologous series of fatty acids in HA [12,18,20].

The nitrogen-containing structures are mainly present as heterocyclic compounds, such as indol. But, the presence of other unknown aliphatic nitrogenic structures (7) should be mentioned. The heterocyclic structure could originate from biological precursors, such as plant and microbial residues. However, the absence of amino acids or other peptide compounds could be explained by their intermolecular condensation to form heterocyclic structures as indol under pyrolysis conditions [27–29]. The absence of carbohydrates and polysaccharides may be attributed to the fact that they are not structurally humic compounds and they are only physically adsorbed, which

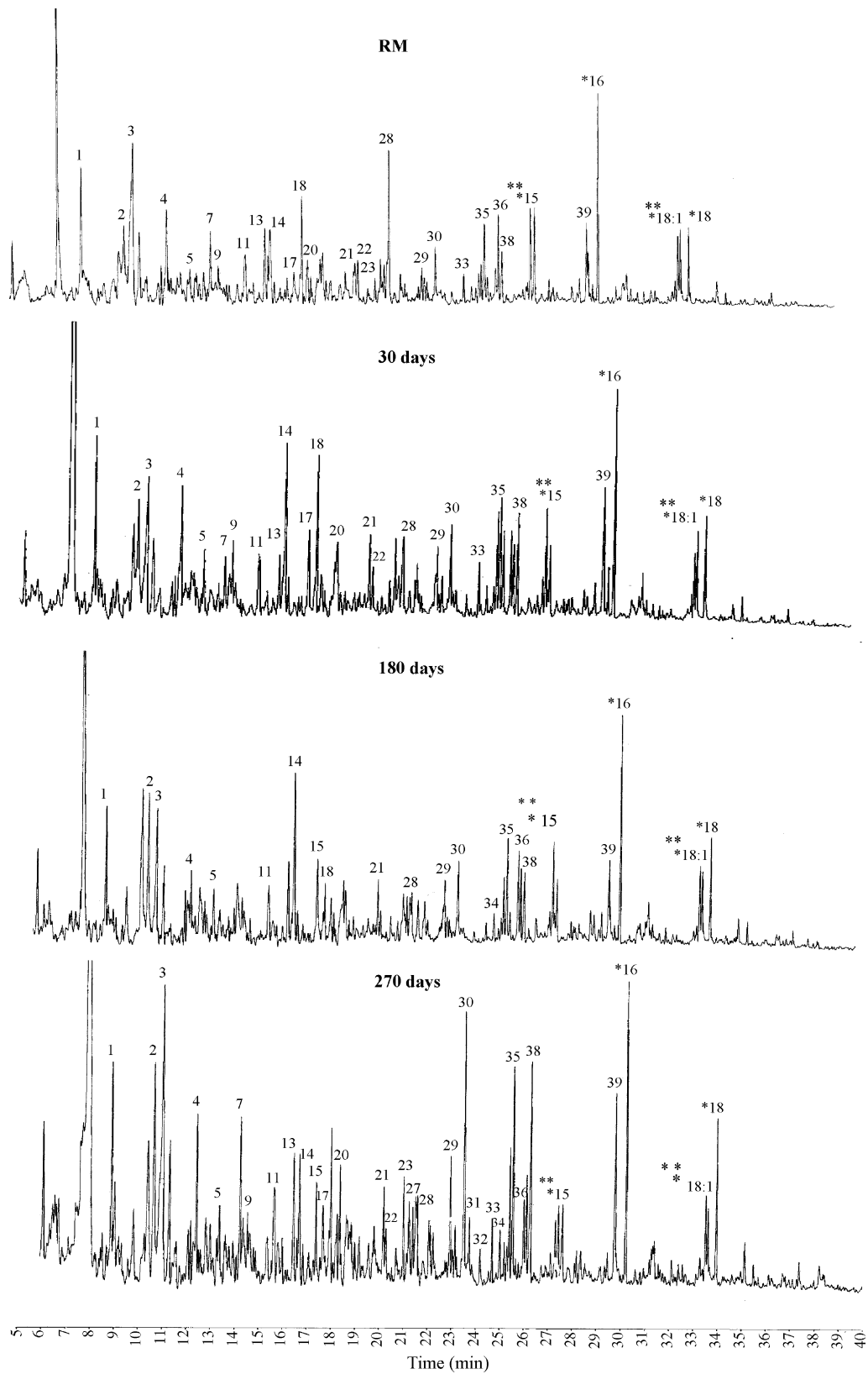







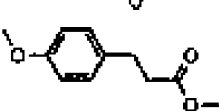

Fig. 1. Thermochemolysis-gas chromatogram of humic acids of sewage sludge during composting.

Table 2

List of the main aromatic compounds related to peaks presenting significant surface area in the chromatograms obtained from thermochemolysis of humic acids of sewage sludge during composting

Lignin-derived compounds

Compounds derived from *p*-hydroxyphenyl (cinnamyl) structures

1		4-Methoxytoluene
4		1-Methoxy-4-vinyl benzene
10		4-Methoxybenzaldehyde
19		4-Methoxy acetophenone
20		4-Methoxybenzoic acid, methyl ester
28		3-(4-Methoxyphenyl)-propanoic acid, methyl ester
37		3-(4-Methoxyphenyl)-2-propenoic acid, methyl ester

Compounds derived from guaiacyl structures

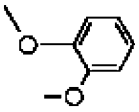
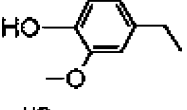

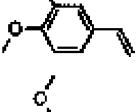




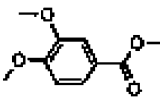
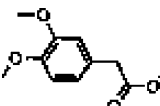
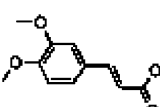
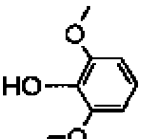
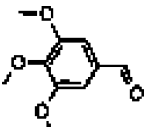
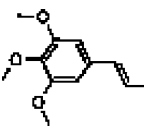
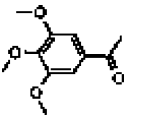
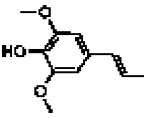
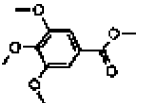
6		1,2-Dimethoxybenzene
12		4-Ethyl-2-methoxyphenol
17		2-Methoxy-5-vinylphenol
18		3,4-Dimethoxystyrene
23		3,4-Dimethoxybenzaldehyde
26		4-Hydroxy-3-methoxybenzoic acid methyl ester
27		1-(4-Hydroxy-3-methoxyphenyl)-propan-2-one
29		1-(3,4-Dimethoxyphenyl)-ethanone

Table 2 (Continued)

## Lignin-derived compounds

30		3,4-Dimethoxybenzoic acid methyl ester
32		2-(3,4-Dimethoxyphenyl)-ethanoic acid methyl ester
39		3-(3,4-Dimethoxyphenyl)-2-propenoic acid methyl ester

## Compounds derived from syringyl structures

15		2,6-Dimethoxyphenol
31		3,4,5-Trimethoxy benzaldehyde
33		1,2,3-Trimethoxy-5-propenylbenzene
35		3,4,5-Trimethoxyacetophenone
36		2,6-Dimethoxy-4-propenylphenol
38		3,4,5-Trimethoxybenzoic acid methyl ester

## Other non-lignin derived compounds


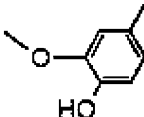
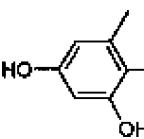
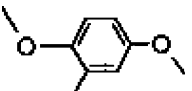
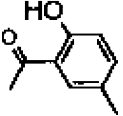
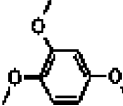

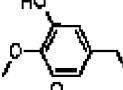
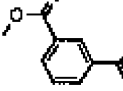
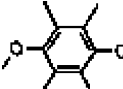
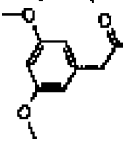
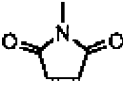
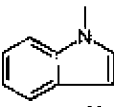
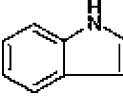
2		4-Methoxyphenol
5		2-Methoxy-4-methylphenol
8		4,5-Dimethoxybenzene-1,3-diol
9		1,4-Dimethoxy-2-methylbenzene

Table 2 (Continued)

Lignin-derived compounds		
14		2-Hydroxy-5-methylacetophenone
16		1,2,4-Trimethoxybenzene
21		2-Hydroxy-3-isopropyl-6-methylcyclohex-2-enone
22		5-Allyl-2-methoxyphenol
24		Isophthalic acid, dimethylester
25		1,4-Dimethoxy-2,3,5,6-tetramethylbenzene
34		1-(3,5-Dimethoxyphenyl)-propan-2-one
Nitrogen-containing compounds		
3		1-Methyl-2,5-pyrrolidinedione
7		unidentified aliphatic N compounds 7
11		1-Methyl-1H-indol
13		1H-Indol
Fatty acids methyl esters		
*15		<i>iso</i> -Pentadecanoic acid, methyl ester
**15		anteiso-Pentadecanoic acid, methyl ester
*16		Hexadecanoic acid, methyl ester
*18:1		<i>iso</i> -Octadecenoic acid, methyl ester
**18:1		anteiso-Octadecenoic acid, methyl ester
*18		Octadecanoic acid, methyl ester

lead to their easy removal by the first water extraction step [30]. Some authors suggest that the lack of carbohydrate-derived peaks in the TMAH chromatogram is due to poor sensitivity of this technique to carbohydrates [31]. Recently, Fabbri and Helleur [32] reported that TMAH thermochemolysis of cellulose and starch yields 1,2,4-trimethoxybenzene (compounds labelled 16).

Accordingly, humic acids isolated from sludge compost are composed of heterogeneous structures. In fact, the

sludge material originates from various residues carried by domestic, industrial, and agronomic liquid-waste effluents and other sources. Similar observations have been reported by Garcia et al. [1] who show that gas chromatogram features of products methylated after persulfate and alkaline permanganate oxidation of HA isolated from uncomposted and composted mixtures of sludge and grape debris are mainly composed of linear *n*-fatty acids and phenolic acids, such as methoxy-, dimethoxy- and trimethoxy-benzoic acids.



The presence of fatty acids C15, C16, C18:1, C18 is often attributed to residues from microbial activity [14,33]. The high contribution of lignin-like compounds may be explained by the fact that, of all the organic compounds present in organic waste, they are the most resistant to microbial degradation during wastewater lagooning. Since, lagooning sludge consists mainly of components of dead algae, fungi and microorganisms and material recalcitrant to microbial degradation.

During composting, the proportion of structural units varies between the chromatograms at different steps of composting and more oxidized lignin-derived units prevail at the end of process. Thus, it is important to follow the chemical transformation of lignin by-products since they are subject to humification and they can become incorporated into the macromolecular structure of HS. Accordingly, structural changes of HA during composting were followed through comparison between the relative abundances of the subunits making up their chromatograms at different stages of composting. The relative abundance  $\ll P_i \gg$  of each pyrolysis product from HA is calculated as the ratio between the area of the corresponding peak to the sum of areas of all the peaks considered in the pyrograms [34]

$$P_i = \frac{a_i}{\sum_{i=1}^n a_i}$$

$a_i$  is the area of each peak in the pyrogram.

The relative abundance of various pyrolysis products released from HA are presented in Fig. 2. The follow-up during composting of fatty acid methyl esters shows an increase, after the stabilization phase, mainly of branched C15, linear C16 and C18 fatty acids, which then decreased at the end of composting. Concerning the aromatic compounds, there was a significant decrease in the proportion of methylated *p*-hydroxyphenyl derivatives (28) and also of syringyl derivatives (36), with the progressive increase in the proportion of methylated protocatechuic acid (30), gallic aldehyde (35), gallic acid (38) and of caffeic acid (39). These transformations of lignin-like structures during composting are consistent with those of the pathway proposed by Martin and Haider [35] for the production of polyphenolic acids, such as protocatechuic acid and gallic acid from biotransformations of coniferyl alcohol or *p*-hydroxycinnamyl alcohol by three fungi of Imperfecti group (*E. nigrum*, *S. atra*, and/or *A. sydowi*) during lignin biodegradation. Lignin side chains are oxidized and methoxy groups removed or hydroxylation occurs, resulting in an increase of more oxidized polyphenolic acids. The protocatechuic acid and gallic acid derivatives always constitute the last lignin-degradation product leading to the formation of humic substances following self-polycondensation and/or combination with other nitrogen-containing compounds, while caffeic acid (39) constitutes the intermediate products during lignin biodegradation [35,36].

Therefore, to understand the humification process, recently, numerous authors have monitored lignin decom-

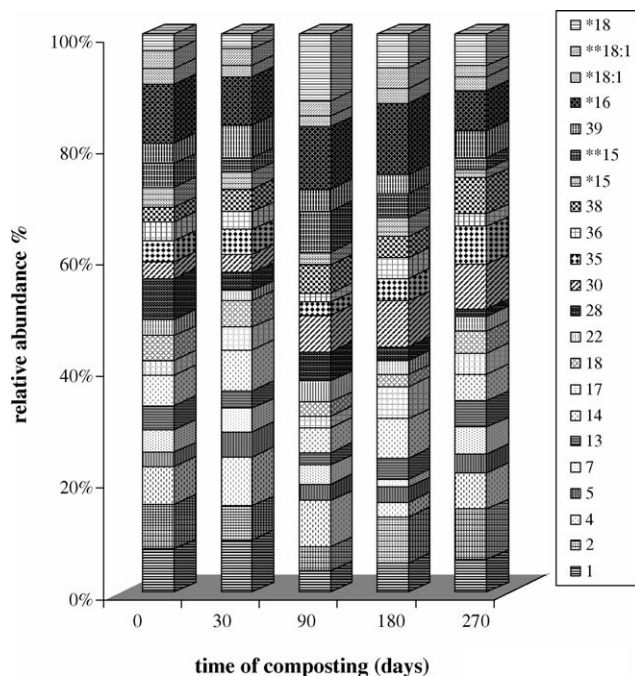


Fig. 2. Changes of the relative abundance of main products thermally released from humic acids (HA) of sewage sludge at different stages of composting.

position and its structural changes by following various indexes. Chefetz et al. [16] use the S/G ratio (syringyl to guaiacyl) as a parameter to gauge evolution based on the assumption that condensed lignin G-type units are more resistant to biological degradation than lignin with a larger proportion of methoxyl groups. These authors report that the S/G ratio decreases suggesting preferential degradation of syringyl units by microorganisms. In our case, the S/G ratio did not present significant changes, it decreased from 0.59 to 0.49 in the intermediate step of composting and increased to 0.66 at the end of the process. The follow up of various lignin-derived units shows a decrease of *p*-hydroxyphenyl type-compounds after 30 days of composting, while in parallel the guaiacyl and syringyl units significantly increase at the end of process (Fig. 3). Guaiacyl and syringyl units both show only a slight decrease at the beginning of the maturation phase. Indeed, the increase of guaiacyl and syringyl units at the end of composting is in agreement with strong production of protocatechuic acid and gallic acid derivatives during composting.

In fact, other authors, such as Guggenberger et al. [37] and Chefetz et al. [14] base their reasoning on the theory of side-chain oxidation of lignin structures as one of the major humification processes. They have reported that a decrease in the content of lignin compounds and an increase in the acid/aldehyde ratio occurs with humification in the soil. Hatcher et al. [38] also suggested that benzenecarboxylic acids in TMAH products are predominantly derived from lignin units during wood decomposition, where the  $\alpha$ -C of the side chain has been oxidized to a carboxyl group. The

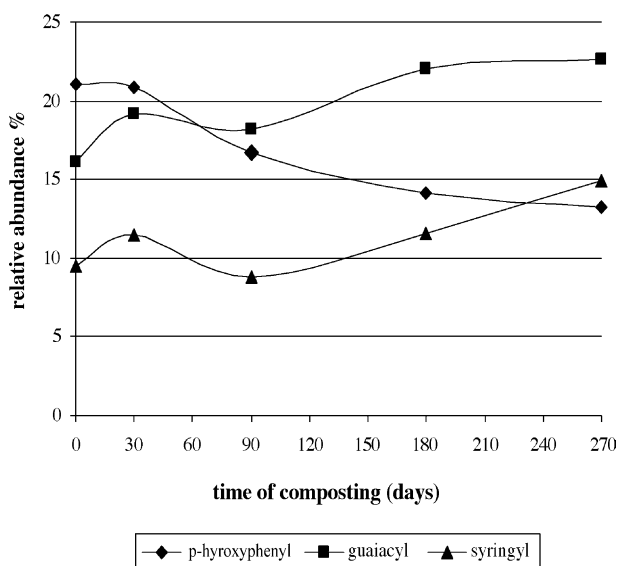


Fig. 3. Evolution of various lignin-like derived units in humic acids of sewage sludge during composting.

ratio of acid-containing derivatives to aldehydes containing derivatives (acid/aldehyde ratio) is commonly used to assess the stage of lignin degradation. High acid/aldehyde ratios represent an advanced stage of lignin side-chain oxidation by microorganisms. In the present study, using the ratio of gallic acids (38) to gallic aldehydes (35) methylated derivatives showed an increase from 0.73 to 2.13 after the stabilisation phase and decreased to 0.93 at the end of composting. This evolution is in agreement with the increase of acid-containing derivatives following the intense oxidation of lignin side-chains during the stabilisation phase. But, the decrease of the gallic acid to aldehyde ratio during the maturation phase could be attributed to a decrease of acid units by polymerisation of benzoic acids compounds through ester/ether linkages. The later mechanism was previously proposed in numerous studies involving the formation of humic substances [8,39,40]. However, according to some authors, caution must be taken when using the acid/aldehyde ratio in TMAH pyrolysis as a measure of lignin degradation [41]. They show that aldehydes can undergo a Cannizzaro reaction (disproportion/methylation) producing their corresponding carboxylic acids.

Therefore, to determine the degree of decomposition of the side-chains of lignin structures and the evolution of compounds with similar chemical structures, in this study six families are grouped from various pyrolysis products released from humic acids at each steps of composting (Fig. 4): C6–C3 (three carbons in side-chain of aryl unit); C–C2 (two carbons in side-chain of aryl unit); C6–C1 (benzoic acids compounds), hydroquinones, nitrogenous structures and fatty acids.

The initial organic material was composed of 25–28% of C6–C1 units, the same proportion of fatty acid methyl esters; 13–17% of each C6–C3 or C6–C2; 7–8% of hydroquinones and similarly the nitrogenous compounds. During compost-

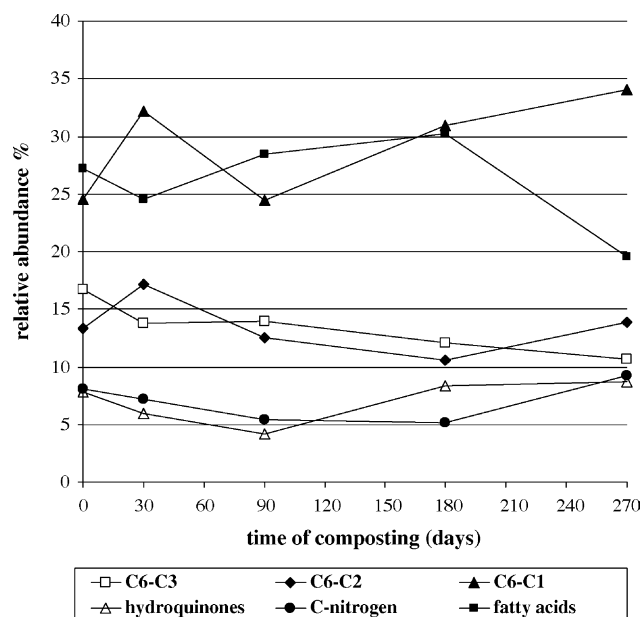


Fig. 4. Changes of six structural groups composing thermochemolysis products released from humic acids of sewage sludge during composting.

ing, the humic structures show a decrease in the proportions of C6–C3 or lignin-like compounds from 16.7 to 10.7%, in parallel C6–C2 and mainly C6–C1 compounds tend to increase during composting. The increase in the proportions of the latter groups was partly related to the microbial decomposition of the side-chains (C3) of lignin-like compounds C6–C3. However, the decrease recorded mainly in both groups C6–C2 and C6–C1, and hydroquinone and nitrogen compounds that occurred at the beginning of the maturation phase may be attributed to their absorption into the cells of the mesophilic microorganisms, which proliferate in this step of composting. According to Varadachari and Ghosh [42], lignin is first degraded by extracellular enzymes to smaller units, which are then absorbed into microbial cells where they are partly converted and discharged into the surrounding medium where they polymerise.

In the final stage of composting, the predominant compounds were the C6–C1 unit-like, which rose from 24.5% to 34%. The hydroquinones and nitrogenous structures did not show significant increases in comparison with the initial amounts. As reported above, the C6–C1 compounds, such as methylated protocatechuic acid and gallic acid derivatives constitute the last subunits yielded from the biotransformation of lignin compounds and leading through autopolycondensation or recombination with nitrogenous structures to the neof ormation of humic acids during composting. The great increase in the proportion of these units during composting could be also partly attributed to microbial neosynthesis rather than only to the biotransformation of lignin structures.

The fatty acids present within the humic acid structures, can be divided into the unbound or “free” lipids, lipid

components that are physically or otherwise strongly trapped within the macromolecular network, and finally those that are cross-linked to the matrix through ester bonds. During composting, the relative abundance of total fatty acids decreased from 27.2% to 19.5%. This variation could be explained by their decrease following the decomposition during the stabilisation phase of plant and animal residues present in the original waste. However, the increase in the amount of fatty acid in the intermediate step could originate from algal and microbial activity, which intensely developed during composting. The final decrease may be attributed to the death of most microbial populations at the end of the composting process [43].

In summary, all the above observations support the formation of humic substances during the course of composting through polycondensation of monomers already biotransformed during microbial metabolism rather than just strict biodegradation of starting polymers. The microbial populations that developed during composting used the starting organic material as a source of energy. The microbial metabolism probably involved  $\beta$ -keto-adipate where protocatechiuc acid and catechol are the two main probable entrance points for opening the aromatic ring [44,45]. The polyphenols arising from oxidation or microbial biotransformation of raw materials, could be subjected to ultimate microbial decomposition producing  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Alternatively, a great part of these polyphenols may undergo recombination, either alone or with other metabolites (amino acids and peptides). Therefore, the data obtained here support the notion that the humification process occurring during composting follows a polyphenolic model. The relative importance of sources of polyphenolic acids C6–C1 units from degradation of starting organic wastes or from microbial neosynthesis is unknown.

However, the continued presence of lignin materials in the humic acids and the high levels of production of benzenecarboxylic acids show that oxidative degradation acts partly on side chains and monomers linked to or adsorbed to a macromolecular starting polymer. In contrast, if microbial oxidation acts on free monomers, a great part of the monomers would be ultimately subjected to decomposition and few benzenecarboxylic acid derivatives would be produced. Saiz-Jimenez et al. [46] attributed the greater part of poly-methoxy-phenol – and hence lignin residues – in the pyrolysis product of HA to the incomplete degradation or selective preservation of grass lignin during earthworm composting.

Veeken et al. [19] suggest that the main route of HA formation during composting of biowaste is most likely the degradation pathway (lignin theory). But, the significant contribution of condensation route (or polyphenol theory) is, according to these authors, also possible following the increase of solubility of lignin- and polyphenol-derived compounds, as well as the appearance of N-type compounds in the HA fraction after 35 days of composting.

Thus, oxidized lignin-derivatives always constitute the “core” of humus, mainly for material in which microbial decomposition is still incomplete or not sufficiently advanced.

To assess the importance of structural changes that occur during composting, two indices were applied to follow the distribution of various pyrolysis subunits.

The index of Shannon-Weaver (Ish) indicates the degree of diversity inside the humic polymer. This ecological index is usually used to express the degree of organisation of an ecosystem. Low values correspond to low diversity, while a high index corresponds to a complex system. This index has been used by Alcaniz et al. [47] and Ayuso et al. [34] to examine the organisation of organic compounds in soil and humic acids. Alcaniz et al. [47] suggest that a high diversity index Ish in soil implies that the abundance of fragments is quantitatively equal, while a low index indicates the existence of a selective process. Ayuso et al. [34] show that the low Ish seen in the leonardite soil pyrogram must be due to a selective process, such as oxidation followed by humification. This index is calculated as follows:

$$\text{Ish} = - \sum_{i=1}^n P_i \log_2 P_i \quad 0.5 < \text{Ish} < 4.5$$

where  $P_i$  is the relative abundance of each pyrolysis product considered. The results show a high index of about 3.00. This may be related to the great heterogeneity of the organic material making up sludge compost. During composting, the index value remains relatively constant in spite of a high neoformation of humic acids during composting (Fig. 5). This shows the resemblance and continuity of the same mechanism of their neoformation during composting, plus their neoformation from subunits of similar chemical nature [48].

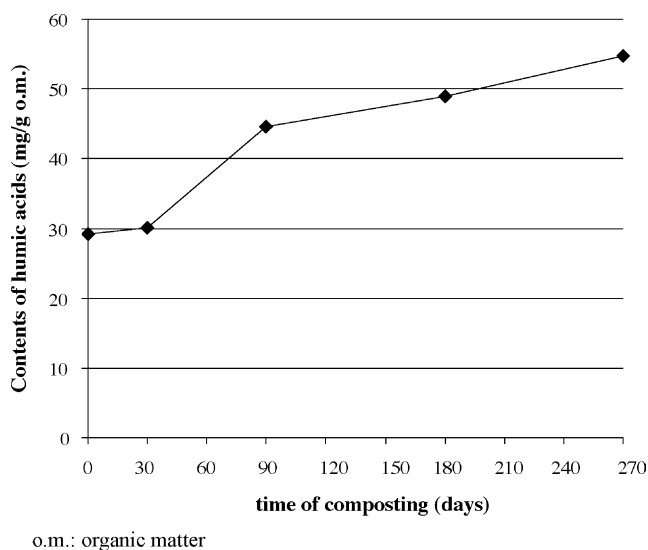
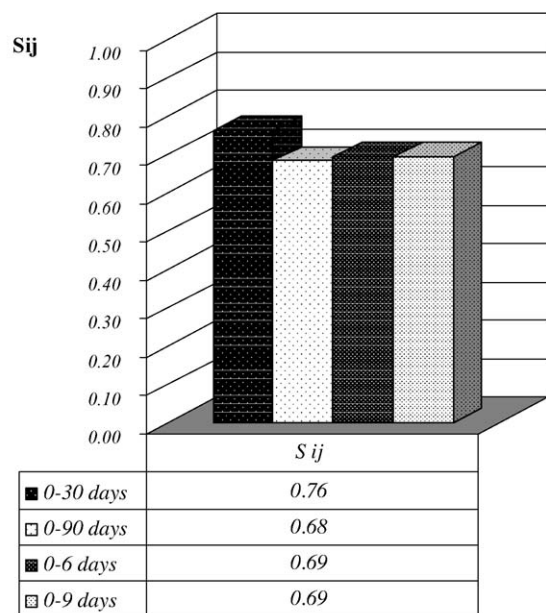


Fig. 5. Contents of humic acids isolated from sewage sludge during composting.

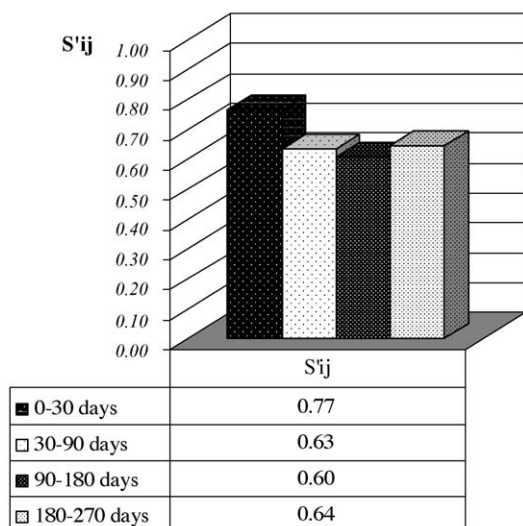
A similitude index  $S_{ij}$  compares two pyrograms and gives the degree of resemblance between their chemical-structural compositions. This index has been used by Ceccanti et al. [49] and Ayuso et al. [34] to determine the degree of similarity between two pyrograms of different organic materials (soil, compost, sewage sludge . . .). It is calculated as follows:

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

$P^k$  is the relative abundance of the subunits making up the  $i$  and  $j$  pyrograms considered;  $n$ , the number of peaks.



(a)



(b)

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

$S_{ij}$  is the degree of similitude between the pyrogram of humic acids at each stage of composting and their pyrograms at the start of composting (Fig. 6a).  $S'_{ij}$  is the degree of similitude between two pyrograms of humic acids at successive stages of composting (Fig. 6b).

$S_{0/1}$  between pyrograms of HA at 30 days of composting and of the initial mixture before composting (RM) shows a great value of about 0.76. The index value then decreased and remained constant near 0.68 in comparison with each pyrogram of the other stages of treatment 90; 180 and 270 days with the pyrogram of the initial mixture before composting (RM).  $S'_{ij}$  decreases to about 0.63 between pyrograms at 30 and 90 days of composting. But, remains relatively constant in comparison between pyrograms of other successive stages, between 90 and 180 days and between 180 and 270 days of treatment. All these changes show a split between 30 and 90 days of composting indicating a change in the rate of neoformation of HA after the stabilisation phase (Fig. 5). Afterwards, this rate of neoformation varied linearly with the time of composting.

#### 4. Conclusion

The results obtained from thermochemolysis coupled with gas chromatography and mass spectrometry show that the organic material isolated from the initial sewage sludge (90%) and straw (10%) mixture is composed mainly of various lignin-derived compounds constituting organic waste recalcitrant to microbial decomposition in the wastewater plant. Moreover, the presence of fatty acid methyl esters was attributed to residues of dead algal, fungal and microbial organisms remaining in the lagooning sludge. The evolution of lignin-derived compounds during composting shows that *p*-hydroxyphenyl units decreased after the stabilisation phase. In parallel the more oxidized units of guaiacyl (G) and syringyl like-structures (S) significantly increased corresponding mainly to methylated derivatives of caffeic acids, protocatechuic acids, gallic acids and aldehydes. In the chemical structure of humic acids isolated from mature sludge compost, C6-C1 type units or polyphenolic acids are highly predominant. These units originate partly from the oxidation of the side-chain of C6-C3 lignin-like compounds, which is considered as the main process occurring during the biotransformation of lignin to form humic substances. Microbial neosynthesis could also be suggested to explain the great amounts of these units present at the end of composting. Indeed, the index of Shannon-Weaver supports the neoformation of HA during composting, from subunits of similar chemical nature. The similitude index indicates a change in the neoformation rate of humic acids during maturation phase, which is commonly known as composting phase where much humification occur. The all variations are consistent with the “condensation” pathway and that neoformation of humic acids during composting seems to occur through polycondensation of

monomers already biotransformed during microbial metabolism rather than just strict biodegradation of starting polymers. However, the continued presence of lignin materials in humic acids and the high levels of benzenecarboxylic acid production show that oxidative degradation acts partly on side chains and monomers linked or adsorbed to the macromolecular starting polymer. The oxidized lignin-derivatives always constitute the “core” of humic structures, especially for material in which microbial decomposition is still incomplete. The fatty acids within the humic acid structures show an increase during the composting process attributed to the development of intense microbial activity and then the amounts decrease after the death of most of the microbial communities in the final stage of low respiration rate.

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