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Elemental analysis, FTIR and ^{13}C -NMR of humic acids from sewage sludge composting

Soumia AMIR^a, Mohamed HAFIDI^{a*}, George MERLINA^b, Hassan HAMDI^a, Jean-Claude REVEL^b

^a Unité Sol et Environnement, Labo. Eco-Végét., Faculté des Sciences Semlalia, Dépt. de Biologie, BP 2390, Marrakech, Morocco

^b École Nationale Supérieure Agronomique, Auzeville-Tolosane, BP 107, Toulouse, France

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Abstract – The effect of composting on the chemical structure of humic acids extracted from a mixture of sewage sludge and straw at different times of treatment was investigated by elemental analysis, Fourier transform infrared and carbon 13-nuclear magnetic resonance. The starting material was composed of a heterogeneous mixture of etherified aromatic structures bearing peripheral long-chain lipidic, peptidic and carbohydrate structures. The results show a decrease in aliphatic compounds during composting, and an increase in aromatic structures – mainly those substituted by functional groups such as phenolic, methoxylic and carboxylic groups. These changes support the preferential oxidation of readily accessible moieties such as aliphatic side chains of lipidic and peptidic structures, leading to the production of highly functionalized aromatic macromolecules. The latter compounds are resistant to microbial degradation and are therefore preserved as part of the newly-formed humic acid structures.

sewage sludge / composting / humic acids / chemical structure / humification

Résumé – Caractérisation des acides humiques extraits au cours du compostage de boues résiduaires par analyse élémentaire, IRTF et ^{13}C -RMN. L'action du processus de compostage sur la structure chimique des acides humiques extraits de mélange de boues résiduaires de stations d'épuration et de paille a été suivie à différents stades du traitement par analyse élémentaire, spectroscopies infrarouge à transformée de Fourier et résonance magnétique nucléaire du carbone 13. Le matériel initial est composé d'un mélange hétérogène de structures aromatiques étherifiées sur lesquelles sont liées de longues chaînes lipidiques et des structures peptidiques et carbohydrates. Les résultats obtenus montrent une diminution, durant le processus de compostage, des composés aliphatiques, avec une augmentation relative des structures aromatiques principalement celles substituées par des groupements fonctionnels tels que phénols, méthoxyles, carboxyles. Ces transformations mettent en évidence l'oxydation préférentielle des composés facilement accessibles, comme, par exemple, les chaînes aliphatiques périphériques des structures lipidiques et peptidiques, ce qui conduit à une production de structures aromatiques riches en groupements fonctionnels. Ces dernières sont résistantes à la décomposition microbienne et sont donc préservées pour constituer une partie de la structure d'acides humiques néoformés.

boues résiduaires / compostage / acides humiques / structure chimique / humification

1. INTRODUCTION

Composting of sewage sludge has recently largely developed in order to obtain a more suitable fertilizer and a safer product for agricultural recycling than the starting material. This aerobic thermophilic biotreatment stabilizes organic matter and suppresses most pathogenic populations [11, 12]. In addition, it is thought to reduce the bioavailability of heavy metals, and enhance the biodegradation of organic pollutants.

However, the compost must reach stability and maturity in order to avoid possible adverse effects during storage and use. According to numerous studies, the amount and chemical structure of the humic substances, principally humic acids formed during composting, constitute the main index for assessing the stability and maturity of compost [9, 12, 19, 21,

28]. These humic substances originate from raw organic matter that has undergone a short period of biotransformation during composting compared with native humification processes in soil [24, 26].

The progress of humification processes is related to numerous factors, such as the nature of the starting materials, time and the oxidative conditions of treatment that influence microbial activities and thus the degradation of the composted material. Gerasimowicz and Byler [13] showed that the relative increase in the degree of aromaticity and unsaturation of the humic acids vary as a function of time and of the treatment used for sewage sludge (drying, anaerobic digestion, or composting with or without wood chips). Diaz-Burgos et al. [10] and Senesi et al. [26] showed that during composting the unstable organic compounds such as aliphatic and protein

* Corresponding author: hafidi@ucam.ac.ma

materials are transformed through intense microbial activities to more stable humic compounds with more polycondensed, oxidized, olefinic or aromatic structures. This is similar to the progressive aromatization, mainly the increase in oxidized aromatic structures during native humification processes occurring in soil, that has been evidenced by numerous authors [15, 27].

Other authors such as Almendros et al. [1] found that in most cases for composted forest and shrub biomass, the recalcitrant material accumulated during composting was not exclusively aromatic in nature and that the presence of tannins may contribute, through their selective preservation and condensation reactions, to limiting the decomposition of aliphatic biomacromolecules. This view was first proposed by Zech et al. [29], who considered that the hypothesis of increasing aromaticity during humification was questionable, and that this is not the dominant process. Bracewell and Robertson [5] and Baldock et al. [3] showed that during decomposition of natural organic materials, alkyl carbon is produced, whereas aromatic carbon, presumably derived from lignin structures, is accumulated as carbohydrates, cellulose and hemicelluloses are utilized, but disappears with further decomposition to leave a residue with high contents of alkyl carbon.

The objective of this study was to determine the influence of composting on the chemical structure of humic acids in sewage sludge. It has to be emphasized that the starting material has already undergone a long period of aerobic-anaerobic lagooning treatment, and is composed of various solid residues which come from urban discharge water.

Elemental analysis and spectroscopic techniques such as Fourier transform infrared (FTIR), and carbon-13 nuclear magnetic resonance have been widely used for similar studies. We applied these methods to the humic acids extracted from the sewage sludge at different stages of composting.

2. MATERIALS AND METHODS

Sewage sludge was taken from an anaerobic lagoon in an experimental wastewater treatment plant. Composting of a mixture of sewage sludge (90%) and straw (10%) was performed for 9 months on a composting platform. In order to provide aerobic conditions, the mixture was turned over every 15 days. The progress of composting was followed by monitoring temperature change [2]. Some previously determined physico-chemical characteristics of the compost at different stages of treatment (T0: initial time and T1, T6 and T9, corresponding to 1, 6 and 9 months of composting) are reported in Table I [2].

To extract humic substances, the samples (30 g) were first successively treated three times with 40 ml of distilled H₂O to remove non-humic, water-soluble substances (sugars and proteins), to avoid interference between these compounds and humic substances. The extraction of humic substances was then carried out with 40 ml of NaOH (0.1M). Extraction was repeated until uncolored solutions were obtained. Centrifugation was carried out at 4000 × g for 15 min, and the supernatants filtered through Whatman paper. The filtered solutions of each sample were combined and precipitated by acid treatment with 1.5M H₂SO₄ for 24 h at 4 °C. The precipitated humic

Table I. Change in various physico-chemical characteristics of the sewage sludge during composting.

Stages (months)	pH	C/N	O.C. % ^a	DEC %	ash % ^a	H.S. (mg g ⁻¹) ^a	H.A. (mg g ⁻¹) ^a
T0	7.30	24.00	17.74	/	65.90	19.10	9.94
T1	7.40	21.40	16.18	20.00	70.70	17.45	8.80
T6	6.73	16.40	14.26	40.00	76.30	20.80	11.60
T9	6.77	15.24	13.45	41.50	76.78	22.80	12.70

^a % of dry weight. O.C.: organic carbon. DEC: decomposition rate. H.S.: humic substances. H.A.: humic acids.

acids were separated from fulvic acid solution by filtration through Whatman paper and redissolved in 0.1M NaOH. The humic acid solution obtained was dialyzed with a Spectra-por membrane (7500 Da) to eliminate excess salts. The dosage of humic acid was done by KMnO₄ oxidation [6].

The lyophilized humic acids were analyzed by various chemical techniques:

- Elemental analysis was performed for C, H and N on a Fison Carlo Erba EA 1110, and for O on a Carbo Erba 1106.
- The E4/E6 ratio was determined as described by Chen et al. [7]. The absorbance was measured at 465 and 665 nm after dissolving 2 mg of freeze-dried humic acids in 25 ml of NaHCO₃ (0.025M).
- Infrared (FTIR) spectra were recorded as KBr pellets (250 mg of dried KBr and 2 mg of lyophilized humic acids pressed under vacuum according to Cross [8]) with a FTIR Perkin Elmer 1600 spectrophotometer over the 4000 to 400 cm⁻¹ range, at a rate of 16 nm/s.
- ¹³C-NMR spectra with ¹H broadband decoupling were recorded at 75.469 MHz on a Bruker AM WB 300 MHz Spectrometer. The solution for NMR examination was prepared by dissolving 100 mg of humic acids in 3 ml of NaOD/D₂O (0.5M). The spectrum was obtained using inverse-gated decoupling to suppress the nuclear Overhauser enhancement in order to obtain quantitative results [23]. Acquisition time was 0.98 s, relaxation delay was 1.8 s with a pulse of 35 degrees and the total acquisition time was 72 h. Free induction decays were processed by applying 50 Hz linebroadening and baseline corrections. The integrated areas of the spectrum integrated were: 0–110 ppm (aliphatic carbon), 110–165 ppm (aromatic carbon) and 165–200 ppm (carboxylic carbon).

3. RESULTS

3.1. Elemental analysis

Elemental analysis and the E4/E6 ratio of the humic acids extracted from the sewage sludge at different stages of composting, and the mean values for soil humic acids proposed by Schnitzer [25] are reported in Table II.

These results revealed a significant decrease in N content and an increase in the C/N ratio during composting, in parallel

Table II. Elemental composition and E4/E6 ratio of the humic acids extracted from the sewage sludge compost at different stages of treatment.

Stages (months)	C ^a	H ^a	O ^a	N ^a	atomic ratio			E4/E6
					C/N	C/H	O/C	
T0	48.90	6.87	38.60	5.61	10.00	0.59	0.59	3.70
T1	49.20	6.42	39.90	4.46	12.86	0.64	0.60	3.93
T6	47.84	6.50	41.00	4.58	12.18	0.61	0.64	4.00
T9	48.76	6.59	40.00	4.68	12.16	0.62	0.63	4.00
HAs ^b	56.20	4.80	35.50	3.20	20.50	1.00	0.50	5.00

^a % calculated on organic matter basis. ^b Has: humic acid of soil model [25].

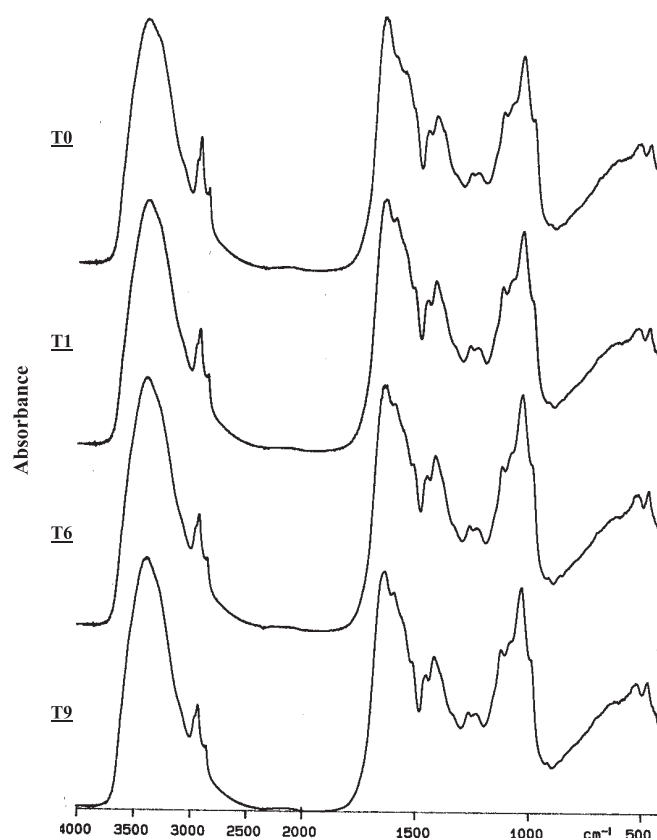
with a substantial increase in O content and a slight increase in O/C. However, no significant variations were observed for H and C contents. The above changes are attributed to the development of microbial activities, during composting, that use the readily available organic matter such as aliphatic and nitrogenous structures as an energy source. Thus, the oxidative microbial degradation of the side chains of nitrogen-containing components, linked or adsorbed to polymers in the starting material, lead to the increase in the relative abundance of more oxidized aromatic structures. Similar results were observed by other authors reporting a decrease in N content in humic acids of mature compost compared with uncomposted sludge and straw mixture [10, 12, 13]. These authors suggested a partial loss of N-containing groups and aliphatic side chains following intense mineralization through microbial activity during composting, and formation of more polycondensed, oxidized, olefinic and aromatic humic acids.

The E4/E6 ratio exhibited a slight increase during composting, probably originating from reduction of the molecular weight of the humic acids through loss of their aliphatic compounds or increase in their oxygen content as suggested by Chen et al. [7].

Compared with the humic acid model of soil proposed by Schnitzer et al. [25], the humic acids of the sludge compost are characterized by higher levels of H, N and O and higher O/C ratios, but lower C content, and C/N and C/H ratios. This can probably be attributed to the richness of the sludge that contains organic waste, in peptidic compounds, and also to the low degree of carbonization of this young humic material [24, 26]. On the other hand, in addition to their high oxygen content, the E4/E6 ratio of the humic acids in the sewage sludge compost was below that of soil humic acids at all stages of composting. This is consistent with the suggestion of Riffaldi et al. [24] and Piccolo et al. [22] about the occurrence of large oxidized macromolecular humic structures in sewage sludge wastes.

3.2. FTIR spectra

The FTIR spectra of the humic acids extracted at various stages of composting exhibit the same absorbances, indicating that no noticeable qualitative changes occurred during process-

**Figure 1.** FTIR spectra of humic acids extracted from the composted sewage sludge at different stages of treatment. Numbers (T0, T1 ... T9) refer to composting time: 0, 1 ... 9 months.

ing, but these spectra differ in the relative intensity of some bands (Fig. 1). The assignments of the main recorded bands reported in numerous studies [4, 13, 17, 19, 25] are: a broad band at 3600–3200 cm⁻¹ corresponding to H-bonded OH groups of alcohols, phenols and organic acids, as well as H-bonded N-H groups; a peak at 2930–2920 cm⁻¹ from C-H stretching of alkyl structures; a broad band at 1650–1630 cm⁻¹ with a first shoulder at 1600 cm⁻¹ produced mainly by aromatic C=C, C=O in amide (I), ketone and quinone groups; the second less marked shoulder at 1540–1550 cm⁻¹ characterizes amide (II); a less intense band between 1460–1380 cm⁻¹ corresponds to several chemical groups such as CH₃, OH of phenols, COO⁻ and/or ortho-disubstituted aromatic rings; a weak band at 1265–1223 cm⁻¹ produced by amides or ethers, and a broad band between 1120 and 980 cm⁻¹ with a sharp peak centered near 1034 cm⁻¹, which generally characterizes aromatic ethers, but carbohydrates and silicates also absorb in this zone.

All spectra showed a high intensity for three bands: 3400, 1650 and 1034 cm⁻¹. The first corresponds to H-bonded OH groups, OH...OH...OH and/or N-H groups, the second one arises mainly from benzoic compounds and the third one is produced mainly by ether linkages C-O-C. These characteristics confirm the presence of highly oxidized polymerized humic acid structures in the sewage sludge.

During composting, the spectra exhibit a significant decrease in the relative absorbance of aliphatic compounds, mainly lipid chains around $2930\text{--}2920\text{ cm}^{-1}$, with an increase in absorbance at 1460 cm^{-1} and a strong increase at 1034 cm^{-1} , as well as the appearance of new small peaks around 1600 , 1510 and 1125 cm^{-1} attributed mainly to aromatic ethers from lignin-like structures. This confirms the decrease, during composting, in aliphatic structures and the increase in more oxidized, poly-condensed aromatic structures.

Similar results were obtained by Garcia et al. [12] and Gerasimowicz and Byler [13]: a decrease, during sludge composting, in the intensity of bands corresponding to aliphatic components, amides, carboxyl, ketone groups and carbohydrates and an increase in bands assigned to aromatic structures.

3.3. ^{13}C -NMR spectra

Humic acids generally exhibit similar resonance in liquid-state ^{13}C -NMR spectra at different times of composting; however, the intensities of these resonances change significantly during composting (Fig. 2). The interpretations of the spectra are based on numerous studies [1, 3, 12, 13, 14, 22]. The signals at 14.6, 16.7, 19.2, 22.9, 25.2, 29.4, 33.5, 37.3, 41.4 and 46.2 ppm are attributable to paraffinic C in alkyl chains. The main signal at 29.4 ppm is characteristic of $(\text{CH}_2)_n$ fatty acid chains [16, 20]. The aliphatic carbons substituted by oxygen and nitrogen are usually observed in the 50 to 105 ppm region. The prominent signal around 56.5 ppm is mainly produced by methoxyl groups of aromatic ethers [22]. Amino acids generally exhibit a signal around 61.5 ppm. Carbohydrates appear at 73 ppm, frequently around 70–90 ppm and at 102, 106 and 111.5 ppm, which corresponds to anomeric carbons. Unsubstituted aromatic C shows signals at 118.9 and at 128 ppm. Olefinic C may also be included in this region. Carbon-substituted aromatic carbons appear around 134.6, 137.5 and 139.7 ppm. The signal at 152.7 ppm is mainly produced by phenolic carbon, aromatic ethers and/or N-substituted aromatic carbons. The high resonances at 169 and 174 ppm may arise from phenolate, carboxylic and amide groups.

^{13}C -NMR spectroscopy indicates the presence of heterogeneous structures in the humic acids extracted from the sewage sludge. During composting, the aliphatic area shows a significant decrease in resonance in the 15–45 ppm interval, corresponding to long aliphatic chains. Substituted aliphatic carbons usually exhibit high resonance, and are mainly attributed to methoxyl groups of aromatic ethers at around 56 ppm. Thus, the latter structures are highly resistant to microbial degradation compared with other alkyl groups. In fact, Huang et al. [18] observed the persistence of a peak at 57 ppm in *Caluna vulgaris* litter after natural degradation for 23 years. In the aromatic area, the resonance of substituted aromatic structures around 130–165 ppm shows an increase compared with unsubstituted aromatic signals around 110–130 ppm.

Comparison of integrated areas: aliphatic (0–110 ppm), aromatic (110–165 ppm) and carboxylic (165–200 ppm) regions (Tab. III) shows a high proportion of aliphatic carbon compared with aromatic and carboxylic carbon. The aliphatic character still remains after composting of the sludge. The significant contribution, in the aliphatic area, of methoxyl

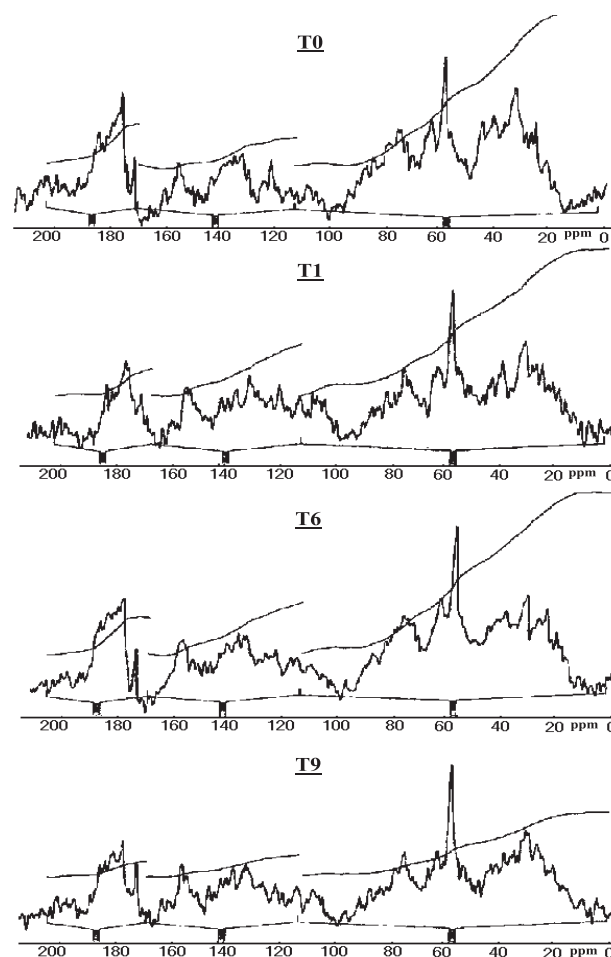


Figure 2. ^{13}C -NMR spectra of humic acids extracted from the composted sewage sludge at different stages of treatment. Numbers (T0, T1 ... T9) refer to composting time: 0, 1 ... 9 months.

groups of aromatic ethers and their persistence to microbial degradation could explain that only a small decrease in aliphatic carbon takes place. This may also explain the slight decrease in H and C contents, observed above by elemental analysis, despite the significant decomposition of the aliphatic structures of lipidic or nitrogen-containing compounds. Aromatic carbon shows a high increase, doubling the original amount. Carboxylic carbon shows a decrease during the stabilization phase (1 month), and then increases during the maturation phase. The decrease in resonance in the carboxylic area during the stabilization phase may originate from the intense degradation of lipids. The subsequent increase during the maturation phase is probably related to the increase in the proportion of benzoic sub-units. These findings are in agreement with those of Garcia et al. [12] and Gerasimowicz and Byler [13].

Taken together, all these results show the heterogeneous composition of the humic acids extracted from the sewage sludge. There is a strong contribution of long-chain lipids and recalcitrant etherified aromatic structures, besides peptidic

Table III. Changes of carbon distribution in humic acids of the sewage sludge during composting.

Stages (months)	Aliphatic ^a	Aromatic ^a	Carboxylic ^a
T0	69.61	12.35	18.03
T1	64.43	24.28	11.29
T6	64.64	20.65	14.70
T9	63.31	21.56	15.13

^a Values are expressed as percentages of the whole spectrum area.

and carbohydrate structures. The sewage sludge originates from various liquid wastes, which have undergone microbial degradation during treatment in the wastewater plant. So, they are composed mainly of residues of dead algae, fungi and bacteria, resulting in peptides and lipidic chain presence, besides material recalcitrant to microbial degradation such as the resistant etherified structures.

During treatment by composting, proliferating microbial populations use the readily degradable aliphatic compounds, primarily lipidic and peptidic molecules, as a source of energy. In parallel, aromatic structures, mainly those substituted by phenolic, methoxylic and carboxylic functional groups, show a relatively abundant increase with treatment time. The results obtained indicate that with the duration of composting, the starting material and mainly readily degradable compounds such as aliphatic side chains linked to polymers can be oxidized, leading to the production of more highly functionalized aromatic structures that contain a greater proportion of hydroxyl, methoxyl, carboxyl and carbonyl groups. These resistant structures are thus preserved to become part of the newly-formed humic acids. Contents of humic acids show, during composting, an increase from 9.94 to 12.70 mg per g of dry compost (Tab. I). Thus, these findings are consistent with the increase in the degree of polymerization and aromaticity, during composting, resulting from the humification process. The duration of treatment, the structure of the starting material and aerobic conditions have a clear influence on the humification processes occurring during composting.

4. CONCLUSION

Structural changes of the humic acids extracted from a mixture of sewage sludge and straw during composting were followed using various analytical techniques (elemental analysis, Fourier transform infrared spectroscopy and ¹³C-NMR). The results obtained indicate that the starting material is a heterogeneous mixture of etherified aromatic structures bearing peripheral long-chain lipidic, peptidic and carbohydrate structures. During composting, through the development of intense microbial activity, oxidative degradation mainly of readily accessible compounds occurs, such as that of aliphatic side chains of lipidic and peptidic structures. This leads to the production of highly oxidized and polycondensed aromatic macromolecules. The latter compounds, as biopolymers resistant to microbial decomposition, are preserved to become part of

the newly-formed humic acids. These findings show that humification occurs during composting, hence the stability and maturation of the end product.

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