



Physico-chemical analysis of tannery solid waste and structural characterization of its isolated humic acids after composting

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ABSTRACT

In Marrakech, solid by-products from tanneries are highly polluting, generating large amounts of nitrogenous and organic matter. In the present study composting is tested as a cost-effective method for waste management to overcome many of the environmental hazards and produce a stable, rich material for soil fertilization. Two composting trials were conducted after neutralization by ammonia or lime. The aim of the neutralization was to avoid the antimicrobial effects of the acidity in the tannery waste, thus ensuring correct composting. Different techniques such as elemental analysis and ¹³C NMR spectroscopy were applied to analyse humic acids isolated from raw and composted materials, and to monitor the process of tannery waste composting, and the stability and maturity of the final product according to the means of neutralization. Comparison of data showed similar behaviour in both trials, but the composting process appeared to be more complete following neutralization with lime. The C, H and N content decreased, while the O increased. The FTIR and ¹³C NMR spectra show the decrease of aliphatic compounds demonstrated by the reduction of absorbance around 2922 cm⁻¹ and of the resonance in the C-alkyl area around 0–55 ppm. The humic acids newly formed during composting were richer in the O–N alkyl and oxidized aromatic structures that increased almost twofold on composting after neutralization with lime. The first principal component axis PC1 (54%) separated C-aliphatic, C-carboxylic and other less stable and less polycondensed compounds such as polyphenols from the more polycondensed O–N alkyl and oxidized C-aromatic compounds.

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1. Introduction

Leather processing or tanning consists in the transformation of animal skin to leather using different chemical and mechanical processes to clean the skin of meat, fat and hair [1]. Pre-tanning involves numerous operations, such as curing (dehydration)–soaking (rehydration), liming (swelling)–deliming (deswelling), pickling (acidification)–depickling (basification) [2]. Furthermore, it subjects the skins or hides to wide variations in pH [3]. Such changes in pH demand the use of acids and alkalis. These operations lead to the generation of salts and result in a net increase in chemical oxygen demand (COD), total dissolved solids (TDS), chlorides, sulfates, Cr³⁺ or tannins and other minerals in tannery wastewaters [1]. In Marrakech city, traditional and industrial tanneries are a great environmental hazard, because of the difficulty in disposing of the highly polluting liquid and solid by-products

[4–6]. The solid by-products are estimated to amount to around 280 kg per tonne of treated skins for tanned waste and 330 kg per tonne for non-tanned wastes [7]. As they are discharged into the environment without previous treatment, the solid waste causes extensive harmful effects due to: (i) inorganic pollution especially by Cr, (ii) the presence of pathogens—mainly of faecal origin and (iii) organic pollution due to the high organic load [5,8]. The solid waste originating from Cr tanning plants is mainly composed of tanned residue, hair, lime, plus chromium sulfide which makes their treatment by biological processes difficult [9]. In contrast, the waste originating from traditional units using vegetable tanning are composed of tannin residues, hair, wheat bran and lime, but are exempt of Cr [6]. Thus, the latter wastes could be used in agricultural recycling considering that they may provide a lot of nitrogen and organic matter [7,8]. Among the techniques used to prepare waste for agricultural applications, composting is a useful way to produce a stable product that overcomes many of the problems encountered during spreading or in the future [10–13]. Furthermore, composting is recognized as a cost-effective method for waste management, it has been updated to process organic

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Table 1

Physico-chemical characteristics of elements of waste produced during tanning and of both mixtures M1 and M2 prepared for composting

	pH	Moisture (%/fw)	TOC (%/dw)	TKN (g/kg dw)	C/N	Ash (%)
Tanned residues	3.56 ± 0.17	60.8 ± 0.8	37.7 ± 0.54	13.5 ± 0.2	27.9 ± 0.43	16.9 ± 0.78
Wheat bran	4.60 ± 0.43	87.6 ± 1.4	46.5 ± 0.28	26.2 ± 0.27	17.7 ± 0.11	12.8 ± 0.53
Hairs	8.40 ± 0.66	64.3 ± 2.1	28.9 ± 0.4	67.2 ± 1.05	4.3 ± 0.0	49.3 ± 0.29
Mixture M1	6.8 ± 0.13	60.9 ± 0.08	42.3 ± 0.82	13.02 ± 0.26	32.5 ± 0.79	15.0 ± 0.24
Mixture M2	6.2 ± 0.46	61.8 ± 0.18	39.5 ± 0.89	12.05 ± 0.39	32.8 ± 0.73	14.4 ± 0.36

TOC: total organic carbon; TKN: total Kjeldhal nitrogen, fw: fresh weight; dw: dry weight.

waste of different origins, such as sewage sludge, animal manure and agro-industrial waste [14–16].

Composting is commonly defined as aerobic biological decomposition in which part of the organic matter is biotransformed to yield stable humic-like substances (HS) [17–19]. These substances allow a well-balanced release of nutrients upon their slow decomposition, and improve soil structure and cation exchange capacity.

Accordingly, many authors consider the amount and chemical structure of the humic material as important indices for assessing the stability and maturity of the compost and hence its agronomic value [20–22]. However, many difficulties arise due to the stochastic nature of humification, the formation of HS from plant and animal residues follows no genetic code and occurs stochastically. It produces an immense structural heterogeneity and polydispersity of humic substances [23].

This study aimed to investigate the potential of solid tannery waste compost and the possible means of neutralization of the acidity of the tanning residues using lime or ammonia to avoid antimicrobial effects. Accordingly, various physico-chemical and spectroscopic methods have been used to analyse the humic acid (HA) fraction isolated at initial and final times of composting.

2. Materials and methods

The waste used was from a traditional unit in the old town of Marrakech city after some preliminary treatment to remove hair and fatty materials. The waste was tanned residues, wheat bran and the remaining hair. The physico-chemical properties of the waste and of both mixtures M1 and M2 to be composted are presented in Table 1. Each mixture is composed of 93% tanned residues, 5% wheat bran and 2% hair to obtain an optimum C/N ratio for microbial activity (near to 30). The trials on both mixtures (M1 and M2) differed only in the way the acidity of the mixture was neutralized. The first trial (M1) was neutralized by carefully mixing 3.27 g of lime (slightly crushed) to 10 kg of the above mixture, the second (M2) by adding 3 ml of a 26% solution of ammonia to another 10 kg of the above mixture [24]:

- M1: 93% tanned residues + 5% wheat bran + 2% hair + 3.27 g lime.
- M2: 93% tanned residues + 5% wheat bran + 2% of hair + 3 ml ammonia to achieve pH values of about 6.81 and 6.22, respectively. The pH was measured from suspensions of 10 different sub-samples of mixtures homogenized manually several times to obtain representative readings.

To start composting of the two mixtures, 10 g of mature compost was added as an inoculum to facilitate proliferation of microbial activities. The composting was conducted in PVC mini-reactors with a capacity of 3.4 l (diameter: 10 cm; height: 50 cm), equipped with permanent airflow for composting under aerobic conditions. During the 20-day period of reactor incubation, CO₂ release was monitored and the temperature reached a maximum of 55 °C indicating good development of biological activity in the course of the stabilization phase of the composting process. When the tem-

perature of the mixtures returned to ambient values of about of 25 ± 3 °C, the product was stored for maturation in perforated plastic bags for about 70 days. The mixtures were turned manually each week to avoid the slowing down of microbial activity. Samples were taken at start and end of composting (M1 or M2 = raw tannery waste, CM1 or CM2 = the waste after 90 days of composting).

Various preliminary analyses were performed to investigate the maturity of the compost for both mixtures (Table 1): pH was measured in a 1/10 (fresh waste/water) aqueous extract [14]. The moisture was calculated after drying samples at 105 °C for 24 h. Total organic carbon (TOC%) was measured according to the method described by Aubert [25] and organic matter was calculated using the formula OM% = TOC% × 1.72. Total nitrogen was determined by the Kjeldahl method [26]. The inorganic nitrogen NH₄⁺-N and NO₃⁻-N was analysed according to method described by Bremner [26]. After ignition of the dry sample at 550 °C (16 h) the level of decomposition (Dec) was calculated according to the formula [14,27]:

$$\text{Dec}(\%) = \left[\frac{100 \times (A_f - A_i)}{A_f \times (100 - A_i)} \right] \times 100$$

where A_f is final ash and A_i is initial ash.

To assay the polyphenols, extraction was carried out with ethyl acetate on 1 g of ground up fresh sample taken at different stages of composting, which had been previously treated cold with: (1) methanol, (2) ammonium sulfate 40% + metaphosphoric acid and (3) petroleum ether. The extract obtained was dehydrated at 35 °C, and the residue taken up in 2 ml of pure methanol. The polyphenol concentration was proportional to the intensity of reduced color of added Folin reagent, measured at 760 nm [24,28].

2.1. Extraction of humic fractions

The HS were extracted after three treatments with 40 ml of distilled H₂O to remove the non-humic or water-soluble substances (sugars, proteins), to avoid interference between these compounds and actual humic substances. The extraction of HS was then carried out with 40 ml of NaOH (0.1 M). Extraction was repeated several times until colorless solutions were obtained. Centrifugation was carried out at 4000 rpm for 15 min to recover the supernatants, which were filtered through Whatman paper (125 mm). The filtered supernatants of each sample were combined and precipitated by acid treatment with 1.5 M H₂SO₄ for 24 h at 4 °C. The precipitated HA were separated from the fulvic acid (FA) solution by filtration and redissolved in 0.1 M NaOH. The HA solution obtained was dialyzed with a Spectra-por membrane (1000 Da) to eliminate the excess of salts. The proportions of HA and HS were calculated by weight difference after freeze-drying of a known volume of humic solution. The FA was calculated by subtracting the amount of HA from the amount of HS. The humification ratio (HR) and humification index (HI) were determined as follows:

- HR = [(HA + FA)/TOC] × 100.
- HI = (HA/TOC) × 100.
- Degree of polymerisation = HA/FA.

2.2. Chemical and spectroscopic analysis of HA

The freeze-dried HA were analysed by various chemical techniques, the samples analysed were: HM1, humic acids extracted from raw tannery waste neutralized by lime; HCM1, humic acids extracted from the final composted tannery waste pre-neutralized with lime; HM2, humic acids extracted from raw tannery waste neutralized with ammonium hydroxide NH_4OH ; HCM2, humic acids extracted from final composted tannery waste pre-neutralized by ammonium hydroxide.

2.3. Elemental analysis was carried out for C, H, O and N using a Carlo Erba EA 1112

Fourier transform infrared (FTIR) spectra were recorded on KBr pellets using a FTIR PerkinElmer 1600 spectrophotometer over the $4000\text{--}400\text{ cm}^{-1}$ range, at a rate of 16 nm/s . The KBr pellets were made by pressing, under vacuum, a mixture of 2 mg of freeze-dried HA with 250 mg of dried KBr.

^{13}C NMR spectra with ^1H broadband decoupling were recorded at 75.469 MHz on a Bruker AM WB 300 MHz Spectrometer. For solution state ^{13}C NMR, samples were prepared by dissolving 80 mg of freeze-dried HA in 2 ml of $\text{NaOD/D}_2\text{O}$ (0.5 M). The spectrum was obtained using inverse-gated decoupling to suppress nuclear Overhauser enhancement to obtain quantitative results. Acquisition time was 0.98 s , relaxation pause was 1.8 s , pulse of 35° , total acquisition time was 72 h . Free induction decays were processed by applying 50 Hz line broadening and baseline corrections. The integrated areas of the spectra were: $0\text{--}50$; $50\text{--}110$; $110\text{--}165$ and $165\text{--}200\text{ ppm}$.

Principal component analysis (PCA) was used to correlate all the parameters studied in both raw and composted mixtures using the package SPSS 11.5 for Windows.

3. Results and discussion

3.1. Progression of composting

The various preliminary physico-chemical parameters used to supervise the composting trials are presented in Table 2. During composting, both trials showed the same evolution, but with differences in the degree of decomposition of the starting organic carbon, which was greater in the trial conducted after lime neutralization—CM1. Decomposition reached over 27% in the latter case, while it remained close to 18% in the trial with ammonia—CM2. The increase of moisture is due to decomposition, or “mineralization”, reactions in the stabilization phase that release water. This occurred in both composts. The decrease of organic matter and carbon reached 35% in CM1, but nearly 28% in CM2. In a previous study, Hafidi et al. [24] showed that the pH neutralization of olive mill wastewater before aerobic digestion enhanced the development of microbial activity and the humification process and that the degree of oxidation seems greatly influenced by the means of neutralization. In both trials, the pH was successfully neutralized, although it then showed a slow increase to 7.15 and a decrease to 5.8 in M1 and M2, respectively, after 15 days of composting (Table 2). The reacidification of the pH in the M2 trial could be explained by the fact that ammonia proved to be volatile as shown by a decrease of its amount from 1.15 to 0.07 g/kg dw during composting of M2, which was not conserved in any other forms such as organic nitrogen or nitrate. The added ammonia could have had toxic effects on the microorganisms, which probably hindered the progress of M2 composting. Similar observations have been reported by Paredes et al. [29–30]. These authors found in composting trials of different organic wastes mixed with lignin-cellulose wastes, that the lower N_2 fixation may be due to the presence of higher $\text{NH}_4^+\text{--N}$ concen-

Table 2
Physico-chemical analysis of raw and composted tannery waste after neutralization by lime or ammonium hydroxide

Sample	pH	Moisture (%/fw)	TOC (%/dw)	OM (%/dw)	Dec (%/dw)	TKN (g/kg dw)	N. org (g/kg dw)	$\text{NH}_4^+\text{--N}$ (g/kg dw)	$\text{NO}_3^-\text{--N}$ (g/kg dw)	C/N	Polyphenols (mg/g fw)
M1	6.8 ± 0.13	60.9 ± 0.08	42.3 ± 0.82	72.9 ± 0.82	n.d.	13.02 ± 1.26	12.9 ± 0.17	0.12 ± 0.00	0.06 ± 0.00	32.5 ± 0.79	0.54 ± 0.04
CM1	6.7 ± 0.46	61.1 ± 0.32	27.4 ± 0.33	47.3 ± 0.33	27.4 ± 0.47	14.04 ± 1.15	13.9 ± 1.3	0.14 ± 0.00	0.17 ± 0.00	19.5 ± 0.74	0.01 ± 0.00
M2	6.2 ± 0.46	61.8 ± 0.18	39.5 ± 0.89	68.0 ± 0.89	n.d.	12.05 ± 1.39	10.9 ± 0.7	1.15 ± 0.08	0.06 ± 0.00	32.9 ± 0.73	0.54 ± 0.06
CM2	6.1 ± 0.08	66.4 ± 0.53	28.5 ± 0.94	49.2 ± 0.94	18.5 ± 0.31	12.37 ± 1.19	12.3 ± 1.3	0.07 ± 0.00	0.15 ± 0.00	23.0 ± 0.55	0.02 ± 0.00

TOC: total organic carbon; OM: organic matter; TKN: total Kjeldahl nitrogen; N. org: organic nitrogen, fw: fresh weight; dw: dry weight; n.d.: not determined.

Table 3

Changes in the amounts of humic substances, their fractions (fulvic and humic acids) and the humification indices (HR, HI, HA/FA) during the composting of tannery sludge after neutralization by lime or ammonia

Sample	HA ^a	FA ^a	HS ^a	HR	HI	HA/FA
HM1	11.3 ± 0.2	11.2 ± 0.13	22.5 ± 1.5	53.2	26.7	1.01
HCM1	14.9 ± 0.34	8.4 ± 0.27	23.3 ± 1.9	85.0	54.4	1.77
HM2	11.8 ± 0.29	12.4 ± 0.30	24.2 ± 2.7	61.3	29.9	0.95
HCM2	13.2 ± 0.14	6.8 ± 0.32	20 ± 1	70.2	46.3	1.94

^a mg/g dw.

trations, which are recognized to reduce the progress of composting [31]. NH₃ volatilisation could also be favoured by high NH₄⁺-N concentrations as reported by Paredes et al. [30]. Thus, during composting the total proportion of nitrogen increased significantly in CM1, but only showed a slight increase in CM2. The increase could have originated from the nitrogen or from the decrease of substrate carbon resulting from CO₂ loss [32,33]. In both trials, the C/N ratio decreased and the ratio (final C/N)/(initial C/N) was 0.6 and 0.7 in CM1 and CM2, respectively, values similar to the 0.6–0.75 reported for mature compost [34].

The variations recorded are similar to previous composting experiments for tannery waste [8] and other organic wastes [16,32,35] indicating that tannery waste is suited to composting after neutralization, particularly by lime.

However, many authors consider compost maturity with respect to its content in humic fractions and its degree of humification and polymerisation [35,36]. Table 3 illustrates the amount of humic matter (fractions HS, HA and FA) and different indices of humification after tannery sludge composting. In the finished compost, the amount of FA was reduced, and this could be attributed to the high FA content of easily bio-degradable organic compounds (sugars, amino acids, etc.). The other fraction, HA, that is known to be more polymerised, increased during the process. Thus, the HA/FA ratio increased in the course of composting in both M1 and M2. The high ratio in compost M2 can be explained by the presence of high levels of easily bio-degradable structures in the FA fraction of M2.

The increase of this ratio known as the “degree of polymerisation”, reflects the formation of complex molecules (HA) from simpler molecules (FA) and a diminution in the non-humic components of the fulvic acid fraction which are the most easily degraded by micro-organisms. Sanchez-Monedero et al. [36] also reported, during composting of different organic-waste mixtures, an increase of the degree of polymerisation from 0.65 in raw MSW mixtures to final values of 1.86–2.00 in composts. This ratio has been proposed as an indicator of maturity by authors such as Iglesias Jómenez and Perez Garcia [37].

The other ratios (HR and HI) also became higher with the composting process (Table 3), and indicate the humification of the organic structures producing large amounts of humic-like substances in the course of composting. Greater changes occurred in compost CM1 than in compost CM2. This confirms that the OM humification process is strongly linked to the stabilization and maturity of compost.

A similar evolution of this ratio has been reported by other authors such as Tomati et al. [10] who showed that during the composting of olive mill wastewaters the increase in the humification ratio rose from 25.9% at 35 days to 41.6 after 140 days. High values have also been reported in a study by Sanchez-Monedero et al. [36], who found, after composting of three different mixtures, an increase of HAC/EXC (HA/HS) from an initial 68 ± 71% to final values of 75 ± 80%. Those authors explain that the high values recorded for these indices may have been due to the joint extraction or removal of other materials such as lignin residues which would behave in a similar way to the humic acids and precipitate at pH 2, leading to values unusually high for residues of a vegetable nature.

Table 4

Elemental composition of humic acids isolated from raw and composted tannery sludge after neutralization by lime or ammonia

Sample	C ^a	H ^a	N ^a	O ^a	Atomic ratio		
					C/H	C/N	O/C
HM1	49.1 ± 0.36	5.6 ± 0.09	2.9 ± 0.2	42.5 ± 0.7	0.73	19.75	0.65
HCM1	42.5 ± 0.24	4.7 ± 0.1	2.5 ± 0.1	50.3 ± 0.35	0.75	19.83	0.89
HM2	49.2 ± 0.37	5.3 ± 0.17	3.1 ± 0.3	42.4 ± 0.33	0.77	18.51	0.65
HCM2	49.5 ± 0.18	5.1 ± 0.12	2.4 ± 0.0	43.0 ± 0.1	0.82	24.06	0.65

^a% Calculated on organic matter basis.

3.2. Study of the humic acids isolated from uncomposted and composted solid tannery wastes by various chemical techniques (elemental analysis, FTIR, ¹³C NMR)

The results obtained from elemental analysis show that the composition of humic components in solid tannery waste changed more on composting when neutralized by lime (HCM1) than when neutralized by ammonia HCM2 (Table 4). The content of elements decreased by about 13.5% for C and N and 16% for H. The level of oxygen showed a relative increase of about 18.4% on composting after neutralization by lime and the HA content also showed a strong increase. The C/H and C/N ratios remained relatively stable, while the O/C ratio increased by about 37%. This can be attributed to the active decomposition of aliphatic and peptidic structures and the neo-formation of more oxidized humic structures during composting of M1. During composting of olive mill wastewater, Tomati et al. [10] found an increase of the ratio between oxygen and carbon showing that the process evolved towards oxidation. Hafidi et al. [24] showed that with neutralization by lime, intense oxidation of organic compounds occurred and humification involved polyphenol condensation. This indicates that the composting process occurred satisfactorily, as described in previous works showing humification during composting [17,19,20,22,38].

The FTIR spectra of HA isolated from uncomposted and composted solid tannery wastes are presented in Fig. 1. Table 5 reports the assignments of the main absorbance bands in the spectra by reference to previous works [22,24,39–41]. The HA are mainly composed of tannin and protein structures as indicated by the absorbance bands around 3400, 1600 and 1395 cm⁻¹, respectively. Composting did not actually change initial structures, but it varied their intensities. In the course of composting a decrease occurred in the aliphatic compounds absorbing around 2922 cm⁻¹, mainly after neutralization by lime (HCM1). The 1598/2922 ratio,

Table 5

The main absorbance bands in FTIR spectra and their assignments

Absorbance area (cm ⁻¹)	Assignments
3400	H-bonded OH groups of alcohols, phenols and organic acids, as well as H-bonded N–H groups
2922	C–H stretching of alkyl structures
1598	Aromatic and olefinic C=C, C=O in carboxyl; amide (I), ketone and quinone groups
1395	OH of phenols, COO ⁻ , –CH ₃
1159–1033	–C–O–C of carbohydrates, aromatic ethers, Si–O–C groups

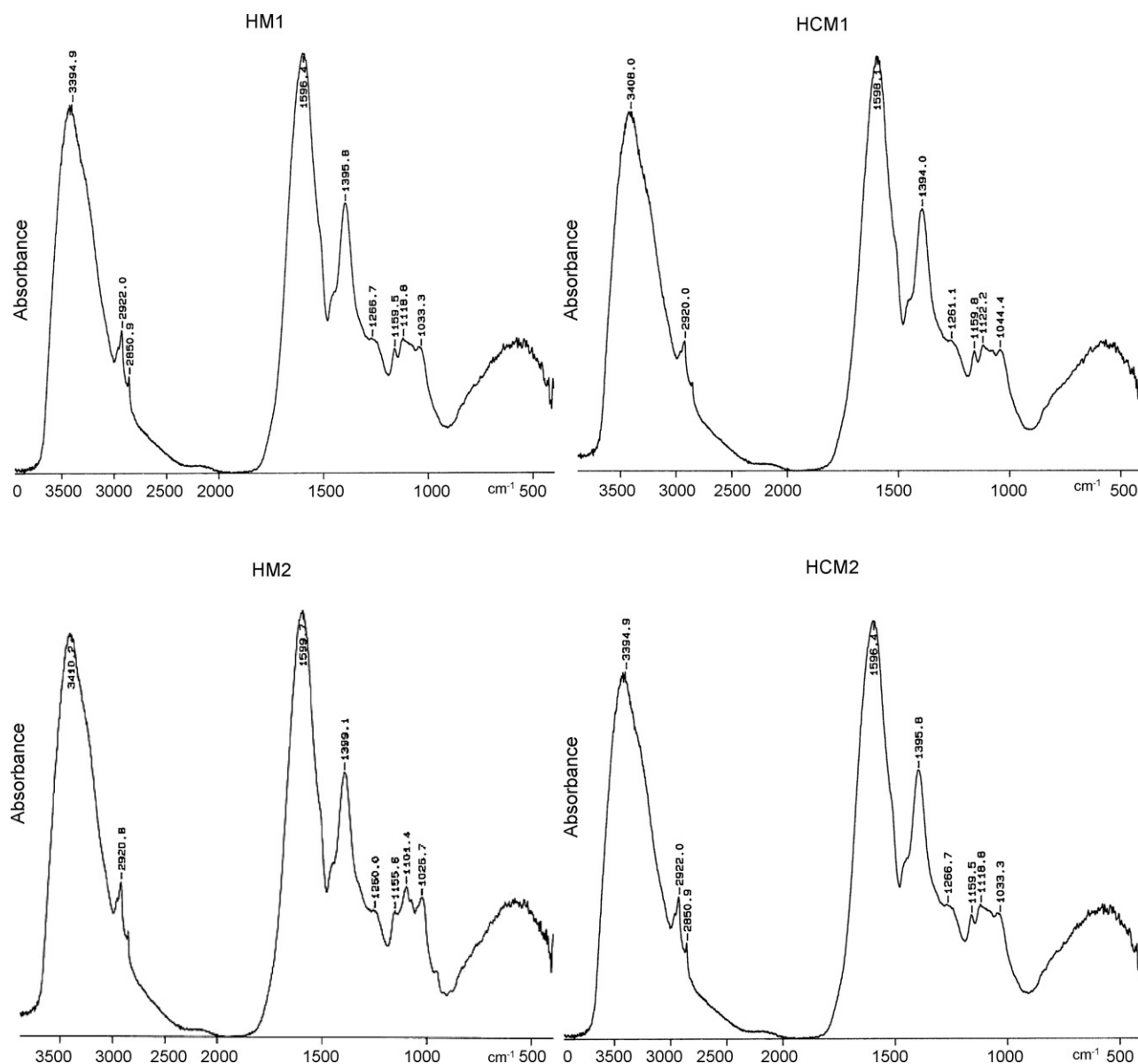


Fig. 1. FTIR spectra of humic acids extracted from raw tannery waste neutralized by lime (HM1) or ammonium hydroxide (HM2) and from their end compost (HCM1) and (HCM2), respectively.

i.e. the ratio of aromatic to aliphatic structures increased from 2.92 in HM2 to 3.26 in HCM1 and from 2.75 in HM2 to 2.88 in HCM2. This suggests that the microbial communities that developed during composting had greater access to aliphatic structures, hence increasing the intensity of other structures such as the aromatics. Similar results have been reported by Hsu and Lo [35] who found, during composting of separated pig manure, an increase in the 1650/2930 ratio (aromatic C/aliphatic C) from 1.04 to 1.68. They too explained the change by the idea that easily degradable OM constituents, such as aliphatic and amide components, polysaccharides, and alcohols, are chemically or biologically oxidized and, therefore, the mature compost contains more aromatic structures of higher stability. Castaldi et al. [42] propose that an increase in the ratio 1650/2930 (aromatic C/aliphatic C) in the FTIR spectra of HA from 0.922 to 1.104 could be caused by the formation of humic polymer and/or by a reduction in aliphatic C.

The ^{13}C NMR spectra of HA extracted from uncomposted and composted tannery waste after both lime and ammonia pre-treatment are presented in Fig. 2. Table 6 reports the interpretations of the main resonance areas in all spectra [22,24,40,43,44]. According to these assignments, the humic acids extracted in the initial raw material present a high resonance in the aliphatic carbon, O–N alkyl and carboxyl carbon areas. During composting, an increase

Table 6
Resonance signals in ^{13}C NMR spectra and attributed chemical groups

Signal (ppm)	Attributed chemical groups
0–50	Paraffinic C in alkyl chains
50–110	Aliphatic carbons substituted by oxygen and nitrogen, around 78 ppm mainly arising from carbohydrates
110–130	Olefinic carbons; unsubstituted aromatic C
130–145	Carbon-substituted aromatic carbons
145–160	Oxygen or nitrogen substituted aromatic carbons
160–200	Carboxylic carbons, ester or amide

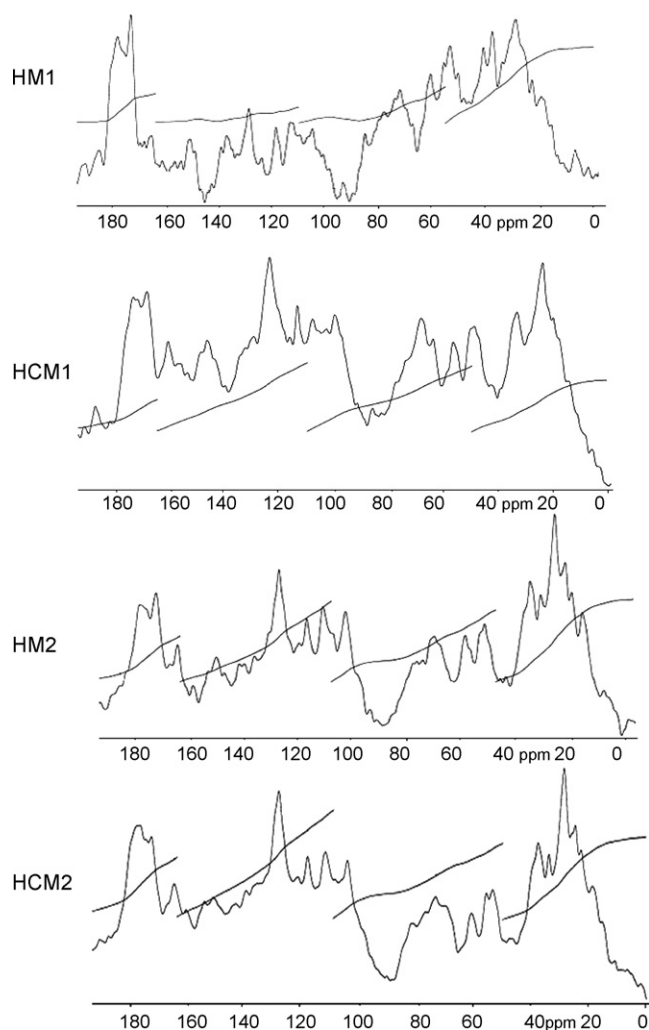


Fig. 2. ^{13}C NMR spectra of humic acids extracted from raw tannery waste neutralized by lime (HM1) or ammonium hydroxide (HM2) and from their end compost (HCM1) and (HCM2), respectively.

occurred in the aromatic carbon area with a decrease in resonances of aliphatic carbon and carboxyl carbon (173 ppm) particularly in HCM2. Thus, the distribution of carbon among the different structures was determined through the integration of areas of the whole spectrum (Table 7). The data obtained from integration showed a reduction in aliphatic carbon in the HA isolated after both composting trials of about 50.6% after lime neutralization (HCM1) and 13.3% after adding ammonium hydroxide (HCM2). The higher level of organic carbon decomposition was observed in CM1 compared to CM2. The intensity of O–N alkyl and aromatic carbon increased in both trials, but more so on composting after liming (HCM1), to reach almost double the initial amount. The increase of O–N alkyl and aromatic carbon did not exceed 2% in HCM2 compost. This is correlated with the amount of HA, which showed a strong increase

Table 7

Changes in carbon distribution in humic acids extracted from raw and composted tannery after neutralization by lime or ammonia

Samples	C-alkyl ^a	O–N alkyl + C–aromatic ^a	C-carboxylic ^a
HM1	48.6 ± 3.5	33.5 ± 2.4	17.9 ± 1.1
HCM1	24.0 ± 1.7	61.7 ± 1.3	14.3 ± 0.9
HM2	29.4 ± 4.7	54.3 ± 2.5	16.3 ± 0.8
HCM2	25.5 ± 0.3	56.3 ± 2.1	18.2 ± 1.4

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

from 11.3 to 14.5% in CM1, but changed less strongly (from 11.8 to 13.2%) in CM2 (Table 3). Thus, the changes in HA structures during maturation are highly correlated with the processes taking place in the bulk compost as reported by Zbytnewski and Buszewski [45] from spectroscopic characterization (^{13}C NMR) of humic acids during sewage sludge composting.

The decrease in the level of aliphatic structures and increase in the intensity of aromatic compounds has also been found to occur during humification in the course of composting in previous studies [20,22,39]. Jerzykiewicz et al. [46] showed an increase in the aromaticity index from 14% to 25–28% in the humic acids extracted from municipal waste after 5 months of composting. They suggest that the humic acids at the beginning of the process contain six aliphatic carbons for each aromatic carbon, whereas after 5 months there may be five aliphatic carbons for every two aromatic carbons. Castaldi et al. [42] also found an increase in the intensity of the aromatic C peak (130 ppm) and in the percentages of unsaturated C (160–101 ppm) by 10.7% during the composting process. The intensity of the aliphatic C region (100–0 ppm) spectra decreased from 69.3 to 63.2% in the final compost. These results are in agreement with the FTIR spectra, which indicate a loss of aliphatic structures through degradation and synthesis and partial neo-formation of aromatic polymers during the process, so the HA in final composts are enriched with aromatic structures. The carboxyl carbon decreased in the humic acid structures extracted from compost neutralized by lime, but increased in the trial after adding ammonia. This could be explained by the strong contribution made by carboxyl functions to the oxidative polymerisation reaction in the neo-formed HA [19,42].

To correlate the above parameters from the two bulk composts and those of the humic acids isolated from them, PCA was applied (Fig. 3). The results show that the parameters fall into two groups A and B along axis 1 that represent 54% of the total variability. The parameters of the two groups are negatively correlated and prove to diverge and represent different tendencies. In contrast, the elements within each group proved to be strongly correlated and present the same patterns.

The left part of the loading plot gathers parameters that indicate the presence of fewer polycondensed aliphatic structures rich in functional groups such as carboxyl and phenolic moieties. In addition, the elements that indicate the probable presence of toxicity caused by volatile and less stable components like NH_4^+ and phenols are found in this part. The section to the right of the loading

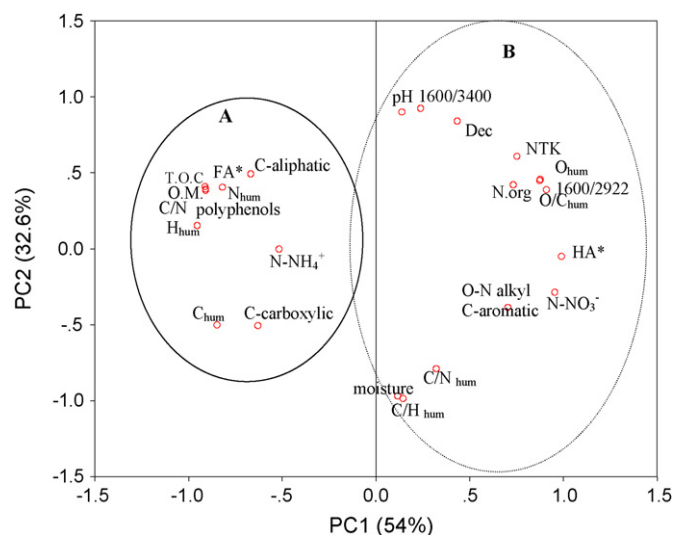


Fig. 3. Principal component analysis among all studied parameters at different times of composting of both mixtures M1 and M2.

plot gathers the parameters that indicate the stabilization and maturation of compost such as TKN, N–NO₃–, N–Org and O content as well as humic structures rich in aromatic components (C-aromatic, 1600/3400 ratio) that are more polycondensed and rich in carbon and O with regard to H and N elements, which are presented by C/N hum, C/N hum and O/C hum ratios.

Group A thus gathers the parameters concerning compost substrate in a state of decomposition. Group B gathers parameters of the new restructured material rich in stable humic structures. Hence, the first component, PC1, accounts well for the evolution of the material being composted from the decomposition phase (group A) towards a restructuring phase (group B). PCA analysis of different composts reported by Suzuki et al. [47] show the division of the composts into different groups according to their maturity and their chemical composition. Zbytyniewski and Buszewski [45] showed, also on the basis of PCA on chemical analyses, the occurrence of three phases during 53 days of composting of sewage sludge: (i) domination of rapid decomposition of non-humic, easily biodegradable organic matter (2–3 weeks), (ii) domination of organic matter humification and formation of polycondensed, humic-like substances (the next 2 weeks), (iii) stabilization of transformed organic material and weak microbial activity.

The second component, PC2, that represents 32.6% of the variance, presents the least polycondensed compounds in the HA structures. This can be seen in the % O and O/C humic, O/N alkyl and C-aromatic that are very near to zero on the PC2 axis in the zone of restructuration (in right part of the loading plot), which supports the high level of oxidative polycondensation in this group. The C/N humic, C/H and 1600/3400 ratios are higher on axis PC2 which indicates the presence of peptide compounds that are free or less bound. These data suggest the formation of oxidative bonds as the HA become restructured during composting [19,24]. Castaldi et al. [42] also reported that during composting, a reduction of C aliphatic with progressive transformation of the polysaccharides into other oxygenated compounds, particularly carboxylic and ester groups, takes place with a parallel increase in aromatic structures suggesting the partial formation of neo-humic polymers.

4. Conclusion

To achieve good composting of solid tannery waste, neutralization by lime or ammonia was carried out to overcome the antimicrobial effects of the original acidity of the waste. The results obtained from the various analyses, FTIR and ¹³C NMR spectroscopies, indicated satisfactory stabilization of the waste through oxidation and humification when the composting followed liming. This can be attributed to the creation of a suitable environment for the intense activity of a wide class of microorganisms, able to benefit from the readily available aliphatic compounds (C-alkyl). The HA newly formed during composting are richer in stable oxidized O–N alkyl and aromatic structures. These findings confirm data obtained previously concerning the progress of humification during composting.

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