



Elimination of polyphenols toxicity from olive mill wastewater sludge by its co-composting with sesame bark

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ABSTRACT

Olive mill wastes represent a significant environmental problem in Mediterranean areas where they are generated in huge quantities in a short period of time. Their high phenol, lipid and organic acid concentrations turn them into phytotoxic materials, but these wastes also contain valuable resources such as a large proportion of organic matter and a wide range of nutrients that could be recycled. Composting is one of the technologies used for the valorization of this effluent, producing a fertilizer useful for poor soils. The present work deals with the changes that occur in the content of phenolic compounds and the biotoxicity of the oxidized substrate which result from the composting of olive mill wastewater (OMW) sludge with sesame bark. The total organic matter decreased 52.72% while water-soluble phenol degradation decreased 72% after 7 months of processing. Gas chromatography coupled with mass spectroscopy was used to confirm the elimination of polyphenols during composting. Initially, the analysis showed three abundant polyphenolic compounds, one of which was identified as the 4-hydroxyphenyl-ethanol (tyrosol), a well-known antioxidant in OMW. After 7 months of composting, all of the phenolic compounds disappeared. The phytotoxic effects of OMW sludge, assessed by the plant index germination, increased during the composting to reach 80% after 210 days. This trend was confirmed by the correlation between physico-chemical and toxicity parameters. The results obtained confirmed the stability of the compost prepared from OMW sludge with sesame bark and indicated a gradual detoxification as the compost matured.

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1. Introduction

During the second half of the last century, the industrialisation of agriculture has led to an increase in the production of organic wastes. Olive mill wastewater (OMW), a by-product of olive oil processing, is one of these wastes produced mainly in Southern Mediterranean countries characterized by a semi-arid climate, where it accounts for 95% of the global OMW production [1]. Tunisia is the fourth largest olive oil producer in the world [2], and its industrial activity generates a huge volume of OMW which varies from 70% to 170%, respectively, in the discontinuous and the continuous process [3]. This industrial wastewater includes polyphenolic compounds which are hazardous materials and a serious environmental

problem. The abundant phenol in olives is oleuropein (formed from elenolic acid, glucose and hydroxytyrosol), then dimethyl-oleuropeine and ligstroside, containing tyrosol and verbascoside that includes hydroxytyrosol and caffeic acid as well as flavonoids [4,5]. In ripe olives, hydroxytyrosol is present in quantities ranging from 10% to 30% of dried weight and under mechanical processing about 1% of the total phenols present in the olives is found in its oil. Indeed, the majority of olive phenols remain in the wastewater and in the solid waste [4,5].

The nature and the complexity of OMW compounds vary according to variable parameters and to date, more than 1000 references on the various treatment methods have been published worldwide [1]. The phenolic fraction accounts for most of the problems associated with OMW pollution. First, it is intensively coloured with a dark red to black colour, which is attributed to its high molecular weight phenolic compounds [6]. Second, the phenolic compounds are responsible for antibiosis and phytotoxicity and other biological effects [6].

Several works have been carried out dealing with the treatment of this effluent [3,7–9]. However, these conventional treatments

Abbreviations: OMW, olive mill wastewater; COD, chemical oxygen demand; SB, sesame bark; EC, electrical conductivity; OM, organic matter; N_T, total nitrogen; C_{org}, organic carbon; C_{EX}, extractible carbon; C_{FA}, fulvic acid carbon; C_{HA}, humic acid carbon; HR, humification ratio; TOC, total organic carbon; GI, germination index.

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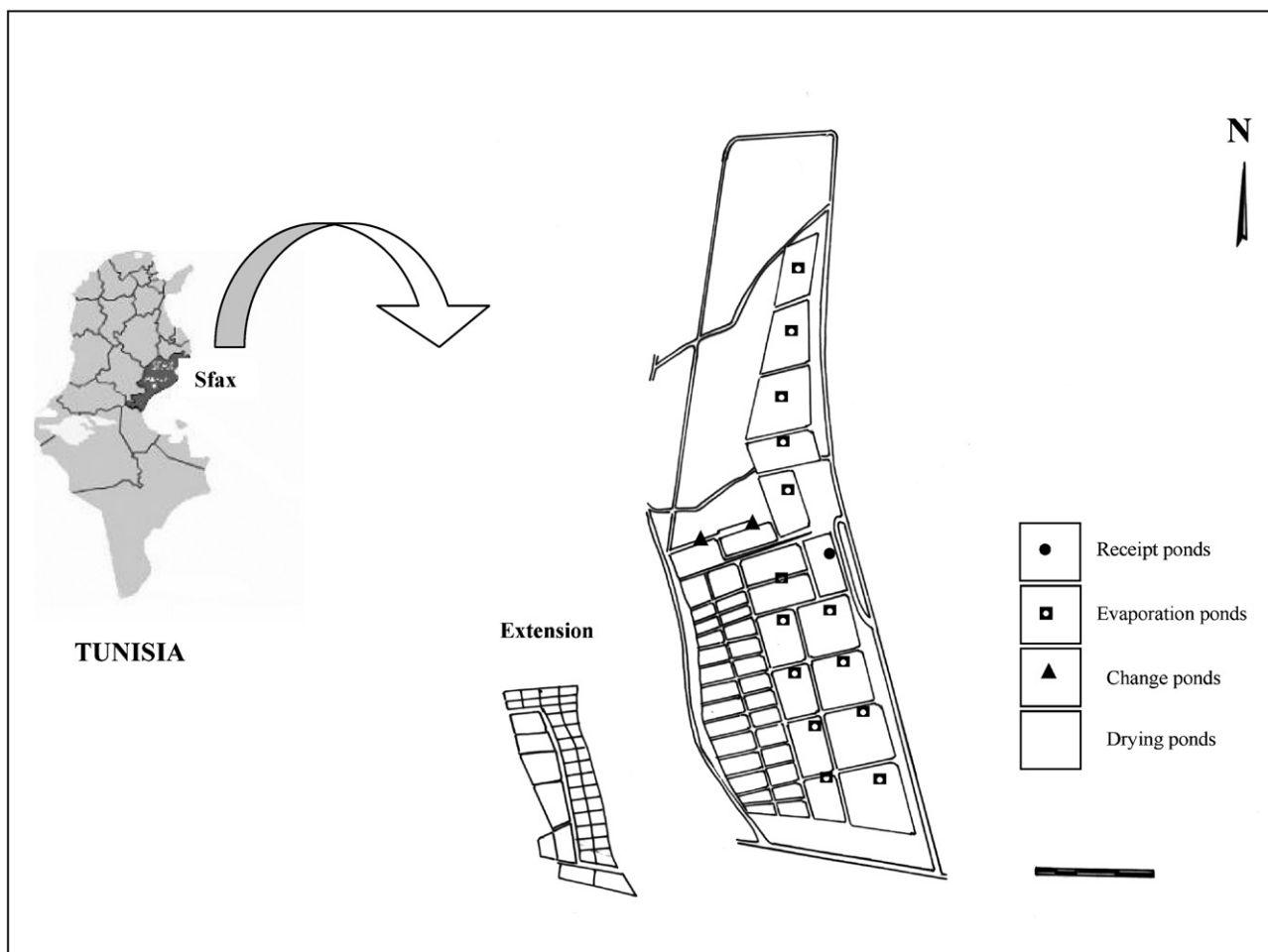


Fig. 1. OMW storage ponds through Geographic Information System in Sfax, Tunisia.

have been hindered by the high organic load of OMW, expressed in terms of chemical oxygen demand (COD) and the presence of polyphenols, which are hazardous contaminating compounds [10]. Furthermore, the real problem encountered is the financial cost necessary for the implementation of such processes. An environmentally safe and a cost-effective solution to OMW treatment has yet to be found.

At present, a cost-effective practice of OMW management is its storage in evaporation ponds [11]. The effluent is collected and stored in ponds where it undergoes natural evaporation until becoming completely dry during the hot period of the year. As a consequence, the polluting effluent is transformed into solid hazardous waste, where polyphenolic compounds are concentrated. To our knowledge, few works have dealt with the management of this solid waste.

Because of the high organic load and the substantial amounts of plant nutrients (N, P, K, Ca, Mg and Fe) available in OMW sludge [12], composting such sludge for the production of organic fertilizer seems to be an appropriate solution. Several authors have studied the co-composting of olive mill by-products with other agricultural wastes. Some suitable materials used as bulking agents were straw [13], cotton waste [14] and poplar sawdust with bark chips [15]. Recently, poultry manure was used as a major co-substrate during composting [9]. In all cases, the final product showed a high degree of humification, no phytotoxic effect and improved mineral nutrients content.

The present work was carried out to test the feasibility of co-composting OMW sludge with sesame bark; these solid materials were watered with a mixture of OMW and confectionary wastewater. The efficiency of this biological treatment was evaluated mainly by measuring the degradation of phenolic components, thereby reducing the toxicity of both the sludge and the effluent.

2. Materials and methods

2.1. Raw materials

A windrow of 60 tons was prepared by mixing sesame bark (SB) collected from a confectionary (Triki-Le Moulin, Sfax) with OMW sludge carried out from the evaporation basins (Fig. 1) located in Sfax city (Tunisian Enterprise of Services, STS, Tunisia), in the following composition, on a fresh weight basis: 60% OMW sludge and 40% SB.

Physico-chemical characteristics of the solid wastes used are presented in Table 1 as an average value with standard deviation. These homogenised materials were humidified by a mixture of OMW and the effluent from a confectionary (Table 2) [16]. The total volume of both effluents equally mixed, used during all the process, was 2.1 m³/ton of composted solid materials.

Table 1

Physico-chemical characterization of the raw materials: OMW sludge and sesame bark before composting (dry weight basis)

Parameters	OMW sludge	Sesame bark
pH	9.69 ± 0.03	4.22 ± 0.07
EC (mS/cm)	4.84 ± 0.04	2.11 ± 0.03
TOC (%)	23.120 ± 0.310	35.560 ± 0.234
N _T (%)	0.780 ± 0.011	1.470 ± 0.017
C/N	30.35	25.90
WSC (%)	3.590 ± 0.009	3.200 ± 0.049
Polyphenols (%)	0.920 ± 0.014	0.480 ± 0.001
Carbohydrates (%)	0.520 ± 0.009	1.700 ± 0.003
Potassium (ppm)	23.20 ± 0.01	5.30 ± 0.01
Magnesium (ppm)	5.000 ± 0.015	4.600 ± 0.013
Sodium (ppm)	4.000 ± 0.005	0.007 ± 0.002
Calcium (ppm)	12.800 ± 0.017	58.300 ± 0.014
Phosphorus (ppm)	15.200 ± 0.028	14.400 ± 0.024
Copper (ppm)	0.02 ± 0.012	0.0 ± 0.015
Nickel (ppm)	<0.088	<0.088
Zinc (ppm)	0.05 ± 0.01	0.04 ± 0.01
Lead (ppm)	<0.041	<0.041
Iron (ppm)	0.87 ± 0.042	0.80 ± 0.025
Cadmium (ppm)	<0.004	<0.004
Manganese (ppm)	0.100 ± 0.034	0.030 ± 0.021
Chromium (ppm)	<0.015	<0.015

EC: electrical conductivity; TOC: total organic carbon; N_T: total nitrogen; C/N: carbon/nitrogen ratio; WSC: water-soluble carbon.

2.2. Composting process

The OMW sludge was mixed with SB and the mixture was humidified with OMW and confectionary wastewater. The composting process was held over 7 months. During the biological transformation, moisture was maintained around 45–60% by adding the mixed effluents. The windrow was turned mechanically every 3 or 4 days during the most active bio-oxidative phase, and once a week throughout the maturation period.

Samples were collected at every turning and consisted of a homogeneous mixture of six sub-samples taken at six different heights of the windrow, spanning the whole according to ISO 8633 [17]. Temperatures were measured on a daily basis at different positions in the core of the windrow, and the average of all the measurements was recorded.

Table 2

Physico-chemical characterization of OMW and confectionery wastewater used for watering raw materials during the composting process

	OMW	Confectionery effluent
Dry matter (%)	10.00 ± 0.1	13.50 ± 0.15
pH	5.30 ± 0.212	5.05 ± 0.35
EC (mS/cm)	20.10 ± 1.55	22.00 ± 1.70
Suspended solids (g/L)	17.00 ± 3.04	8.10 ± 2.70
Organic matter (% dry matter)	78.60 ± 0.50	99.57 ± 0.65
COD (g/l)	178.40 ± 8.95	100.58 ± 6.25
BOD (g/l)	25.60 ± 5.28	24.30 ± 4.65
TOC (g/l)	30.60 ± 2.35	21.50 ± 1.63
Total polyphenols (g/L)	10.5 ± 1.43	Not determined
Carbohydrates (g/l)	0.32 ± 0.05	10.4 ± 0.04
Total nitrogen (ppm)	975.30 ± 0.12	66.14 ± 0.35
Phosphorus (ppm)	260.00 ± 0.15	350.50 ± 0.20
Potassium (ppm)	91.70 ± 0.35	76.00 ± 0.24
Magnesium (ppm)	298.50 ± 0.24	54.00 ± 0.34
Sodium (ppm)	280.4 ± 0.18	516.00 ± 0.47
Calcium (ppm)	72.40 ± 0.14	128.00 ± 0.25

EC: electrical conductivity; COD: chemical oxygen demand; BOD: biological oxygen demand; TOC: total organic carbon.

2.3. Analytical methods

2.3.1. Physico-chemical parameters

Physico-chemical characterization of the two effluents was made according to French standards [16]. Electrical conductivity (EC) and pH were analysed in a 1:10 (w/v) water-soluble extract, the dry matter content was assessed by drying at 105 °C for 12 h and organic matter (OM) by determining the loss-on-ignition at 430 °C for 24 h [18].

Total nitrogen (N_T) and total organic carbon (TOC) were determined by automatic microanalysis [19].

Water-soluble carbon (WSC) was determined by extracting 1 g of compost in 20 mL of deionized water (w/v), then a measurement of the WSC was made as previously mentioned [19].

Extractible carbon (C_{EX}) was measured in a 0.1 M NaOH compost extract (20:1, w/v) and the fulvic acid carbon (C_{FA}) was measured after precipitation of the humic acid at pH 2 by H₂SO₄ in the supernatant solution. The humic acid carbon content (C_{HA}) was calculated by subtracting fulvic acid carbon from the extractible carbon according to the following formula:

$$C_{HA} = C_{EX} - C_{FA}$$

The humification ratio (HR) was calculated as $HR = (C_{EX}/TOC) \times 100$; humification index (HI) as $HI = (C_{HA}/TOC) \times 100$ and humic acid percentage (P_{HA}) as $P_{HA} = (C_{HA}/C_{EX}) \times 100$.

Losses of (OM) and (N_T) were calculated from the initial (X₁) and final (X₂) ash contents and the initial (N₁) and final (N₂) N_T concentrations, according to the following equations [20]:

$$OM \text{ loss (\%)} = 100 - 100 \frac{X_1(100 - X_2)}{X_2(100 - X_1)}$$

$$N \text{ loss (\%)} = 100 - 100 \frac{X_1 N_2}{X_2 N_1}$$

Modelling of OM loss was made using Curve Expert 1.3.

Total fats content was determined by extracting 2.5 g of the sample in a Soxhlet with 200 mL of diethyl ether after which the dried organic fraction was weighed. Water-soluble phenolic substances (WSPH) were determined by a modified version of the Folin method for chemical quantification [21]. Carbohydrates were analysed in the water extract (1:10, w/v) by the anthrone method [12].

Macro- and micro-elements were brought into solution by acidic digestion (2 g of compost digested with HNO₃ and HCl), then analysed by an atomic absorption spectrophotometer. Phosphorus was determined colorimetrically at 430 nm as a molybdo-vanadate phosphoric acid [16].

2.3.2. GC-MS analysis

Polyphenols in the compost were monitored as follows: 5 g of compost were washed three times with 20 mL of hexane and then the polyphenols were extracted twice with 20 mL of ethyl acetate. The organic phase was concentrated at 40 °C to about 1 mL and converted into trimethylsilyl ethers with a silylation mixture made up of N,O-bis(trimethylsilyl)acetamide (BSA) and pyridine (2:2:1) for 1 h at room temperature. The solvent was removed under a stream of nitrogen and trimethylsilyl derivatives were analysed by gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS analyses were carried out with gas chromatography (GC, Hewlett-Packard 6890 Series, Agilent Technology) equipped with a mass spectrometer (MS, Hewlett-Packard 5973 Mass Selective Detector, Agilent Technology). A HP-5 MS fused silica capillary column (60 m × 0.25 mm i.d., 0.25-μm film thickness, Agilent Technology) was used. The GC oven temperature was programmed

as follows: 100 °C hold for 2 min, raised at 5 °C/min to 290 °C (held for 20 min).

Helium was the carrier gas at a flow rate of 1.07 mL/min. The injection was set on a splitless mode at 250 °C. The volume injected was 1.0 µL, the solvent delay was 6.00 min and total run time was 60 min.

Detection was conducted by a mass selective detector with electron impact ionization at 70 eV, in selected ion monitoring mode. MS transfer line temperature was at 280 °C. The MS was operated in full scan in electron ionization mode with an electron multiplier voltage of 2200 V. The mass scanning ranged from m/z 50 to 550.

Mass spectra were compared to the reference compounds in Wiley 275L mass spectral library.

2.3.3. Phytotoxicity test

The compost phytotoxicity was evaluated according to Albuquerque et al. [22], and seed germination as well as root elongation of cress (*Lepidium sativum* L.) measurements was combined in the germination index (GI). Samples (4 g of dry material) were moistened up to 60% and after 30 min, more deionized water (54 mL) was added and the samples were stirred mechanically for 30 min and then filtered through a 0.45-µm Whatmann membrane filter. Eight cress seeds were evenly distributed on filter paper in a Petri dish (10-cm diameter) and moistened with 1 mL of the water extract from the 4-g compost sample. Ten replicates per sample were incubated for 48 h at 27 °C in the dark. Later, the number of germinated seeds was divided by the number of sown seeds which grew on the control (paper moistened with distillate water). The GI was calculated according to the following formula:

$$\%GI = \frac{\%G \times \%L}{100}$$

where: %G: the growth index; %L: the root length percentage, $L = (\text{root length of treated plant}/\text{root length of control plant}) \times 100$.

Results presented in this work are the main values of ten separate measurements at $p < 0.05$ significance level.

2.4. Statistical analysis

Basic statistical analyses of data and correlation coefficient were made using SPSS 11.0 for Windows. Analysis of variance and the least significant differences (Lsd) were calculated for the composting samples to determine changes in the parameters with time ($p < 0.05$).

3. Results and discussion

3.1. Composting progress

3.1.1. Physico-chemical characterization

The changes of the physico-chemical characteristics of the fermented materials (OMW sludge with SB) during composting are presented in Table 3.

Table 3
Evolution of physico-chemical parameters during composting OMW sludge with sesame bark (dry weight basis)

Composting time (days)	pH	EC (mS/cm)	OM (%)	TOC (%)	WSC (%)	Carbohydrates (%)	Fats (%)	C/N
0	6.03 ± 0.00	7.85 ± 0.13	55.06 ± 0.04	33.63 ± 0.67	3.44 ± 0.01	0.77 ± 0.00	16.41	26.54
30	6.15 ± 0.02	7.22 ± 0.02	48.46 ± 0.33	27.71 ± 0.28	2.67 ± 0.03	0.87 ± 0.03	10.75	23.64
60	6.06 ± 0.03	7.99 ± 0.03	41.77 ± 0.36	24.80 ± 0.24	2.83 ± 0.19	0.69 ± 0.02	6.82	22.28
90	6.28 ± 0.07	8.01 ± 0.05	39.80 ± 0.05	24.00 ± 0.37	2.88 ± 0.04	0.64 ± 0.00	3.38	20.37
150	7.21 ± 0.03	7.18 ± 0.03	39.13 ± 0.01	20.70 ± 0.61	2.26 ± 0.03	0.29 ± 0.00	0.95	18.51
210	8.71 ± 0.02	5.70 ± 0.01	36.68 ± 0.22	20.50 ± 0.20	1.12 ± 0.22	0.20 ± 0.01	1.22	18.75

EC: electrical conductivity; OM: organic matter; TOC: total organic carbon; WSC: water-soluble carbon.

The pH of composted materials increased relatively slowly because of humidification with acidic OMW and confectionary wastewater (Table 2). The pH variations also resulted from the metabolism of organic acids and ammonia production during the microbial hydrolysis [23]. Indeed, pH increased as a consequence of the degradation of acidic compounds, such as carboxylic groups and phenolic acids, and the mineralization of proteins, amino acids and peptides into ammonia. Moreover, the pH increase during the composting of OMW could be related to the alkaline hydrolysis of K and Na salts from this liquid waste, as mentioned by Parades et al. [20]. The final alkali pH (8.7) reflects a usual pH of mature compost, as previously found [9,24,25]. Furthermore, at 150 days of composting, the pH of 7.21 indicates a classical evolution of compost under aerobic conditions and also suggests the formation of humic substances. The initial high EC was essentially due to the raw materials used for composting (OMW sludge) and the effluents used for humidification (OMW and confectionary wastewater). During the process, the EC variation described a leaching phenomenon, explaining the decrease of its value and revealing a migration of the soluble salts into the leachate, a phenomenon not assessed in this work.

Similar results were obtained by Parades et al. [26] during the biodegradation of olive mill wastewater sludge by its composting with cotton gin and maize straw and also by Said-Pullicino et al. [27] while composting urban solid waste. Moreover, Huang et al. [28] explained that the volatilization of ammonia and the precipitation of mineral salts could be possible reasons for the decrease of EC at the later stage of composting.

As composting progressed, the organic matter was bioconverted. Table 3 shows a substantial decrease in organic matter during the process. The OM degradation during composting, as determined by the OM loss (Fig. 2), followed a first-order kinetic equation, which is widely observed in carbon mineralization studies in soils [20]:

$$\text{OM loss} = A(1 - e^{-kt})$$

where A is the maximum degradation of organic matter (%), k the rate constant (days^{-1}) and t the composting time (days).

Curve fitting of the experimental data gave the following parameter values (standard deviation in brackets):

$$A = 51.906[2.2]; \quad k = 0.00229[0.00074]; \quad R^2 = 0.9942.$$

OM loss was 23.26% during the first month of the biological process and it exceeded 50% at the end of composting (Fig. 2). The high OM degradation can be attributed to the longer thermophilic phase (4 months) recorded in the windrow where the temperature increased at values exceeding 65 °C (Fig. 2(a)). Furthermore, we noticed that the decomposition of the organic matter which occurred during the first 3 months was more significant than that achieved at the end of the process (Fig. 2(b)); this fact is explained by the intense activity of microorganisms that used a large quantity of easily available compounds. Later, the degradation of organic matter was restricted by the high complexity of the composting substrate represented by lignin, cellulose and hemicellulose [29].

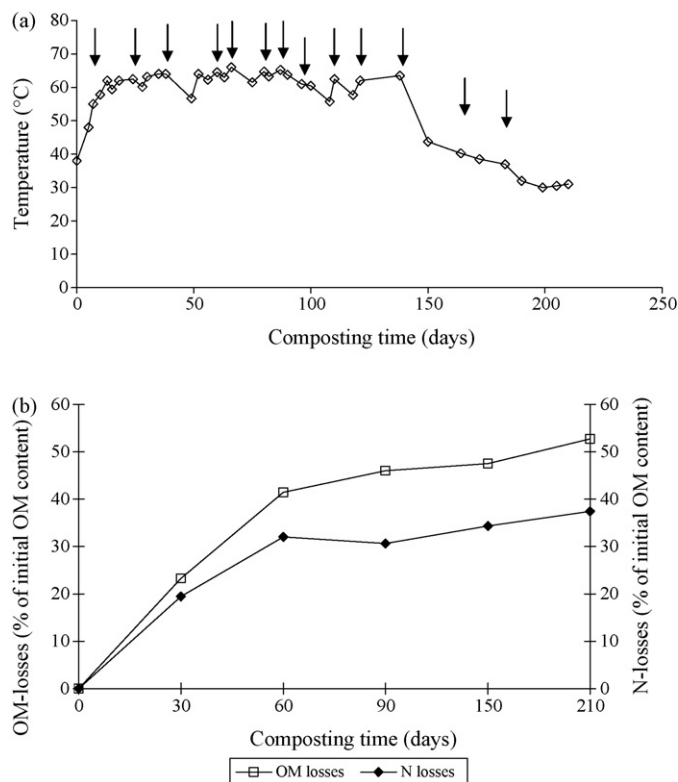


Fig. 2. (a) Temperature profile (arrows indicate turnings) and (b) organic matter losses and nitrogen losses of the experimented windrow during composting.

This fact was noticed by García-Gómez et al. [30] while supplying urea as a nitrogen source during composting treated OMW with olive leaves as a bulking agent. More recently, Paredes et al. [31], found higher organic matter degradation in the mixture of solid waste with OMW while composting cotton gin waste and sewage sludge.

The percentage of OMW sludge seems to have an optimal value. Indeed, while composting a mixture of 80% OMW sludge with 20% cotton waste, the OM losses found by Paredes et al. [26] did not exceed 66% at maturation phase (>182 days). However, the nature of the bulking agent plays an important role in composting. Indeed, the use of maize straw did not exhibit a usual OM loss (zero-order kinetic) and the thermophilic phase was very short, with a ceiling value of 55 °C. In our study, the percentage degradation of OM losses reached 53% with a lower content of OMW sludge (60%) and temperature exceeded 65 °C during the 4 months thermophilic phase.

The same behaviour was observed concerning N losses during the biological process (Fig. 2(b)). Indeed, a first-order kinetic equation was found, characterizing the N degradation:

$$N \text{ loss} = A(1 - e^{-kt})$$

with $A = 35.965$ (2.14); $k = 0.028$ (0.0011); $R^2 = 0.9905$.

Thus, N loss was 19.45% during the first month and reached 37.43% at the end of composting. Although the same kinetic model was followed during composting, the kinetic constant of nitrogen decomposition was 1.4 times lower than that of OM. Similar findings were observed by Cayuela et al. [32], when composting two-phase olive mill waste with different bulking agents such as sheep litter and grape stalks. Indeed, during composting, nitrogen is mainly lost through ammonia volatilization at relatively high pH and temperatures. A suitable strategy for preventing N losses during the composting process could be the control of pH [33]. Further-

more, the initial C/N ratio has also been reported as a key factor in controlling ammonia loss within the composting process [34]. The results showed that increasing the initial C/N ratio of the compost mixture to 30 or more reduces ammonia loss. At the end of the process, N loss in the mixture was below that which is frequently observed during composting (50% N loss) [20].

The water-soluble carbon fraction including the easily biodegradable organic compounds was the most metabolised fraction by the composting microflora. Its composition provided the microorganisms with fats, carbohydrates and water-soluble polyphenols as well as unidentified easily biodegradable organic compounds. As a consequence, WSC decreased gradually to reach 67% at the end of the composting process. Compost could be considered as sufficiently mature (WSC = 1.12%) even though according to Parades et al. [20], the threshold value is below 0.7%. Indeed, the longer the addition period of effluents, the greater the water-soluble fraction available.

Water-soluble carbohydrates increased about 12% during the first month and then sharply decreased until the end of the composting process (Table 3). This increase can be attributed to the considerable amount of carbohydrates brought by watering the mixture with OMW and confectionary wastewater and met low windrow temperatures recorded, this substrate declined continuously until the 210th day.

While composting, the above results suggest that intense biological processes such as mineralization, microbial immobilisation and formation of non-water-soluble compounds prevailed during the release of organic compounds from the complex carbohydrates (cellulose and hemicellulose) [35].

A great decrease was also observed for the fats, present at a relatively high concentration, with a reduction of about 92%, exhibiting a satisfactory fat reduction. The quick and intense degradation of the lipid fraction has been previously reported when composting the solid by-product of olive oil extracted by the two-phase centrifugation system, mixed with other organic wastes [22].

The carbon/nitrogen ratio (C/N) affects biological activity during composting and initial ratio used is an acceptable value since it is in the range of 15–35 [24].

As shown in Table 3, the initial C/N ratio fell gradually during 7 months of composting. In spite of the periodical watering during the thermophilic period, the C/N ratio decreased continuously to stabilize at a relatively low value.

Although the C/N ratio in the solid phase cannot be used as an absolute indicator of compost maturation due to the large variation of the starting materials and the addition of effluents during the process, the value found in this experiment (below 20) is satisfying as it correlates with previous works [9,36,37]. This final C/N ratio is also comparable to that mentioned by Hachicha et al. [9], while composting poultry manure with olive mill wastes (C/N around 17 after 125 days). More recently, while co-composting OMW sludge mixed with bulking agents, Alburquerque et al. [35] found a comparable value of C/N (around 20 after around 300 days).

A substantial decrease in the C/N ratio (final value 16.5) was also reported by Ait Baddi et al. [29], while studying a composting mixture of olive mill wastes for one year.

3.1.2. Humification process

During composting, and simultaneously with the substantial total organic carbon decrease, the variation of extracted carbon (reflecting humic substances, fulvic acid and humic acid carbon), exhibited an enrichment of the composted materials with humic substances (Table 4). The humification ratio (HR), expressed as $(C_{EX}/TOC) \times 100$, and the humification index (HI), calculated as $(C_{HA}/TOC) \times 100$, did not show a clear tendency during the process. This agrees with the findings of Sánchez-Monedero et al.

Table 4

Changes in the humification parameters in OMW sludge compost during the composting process (dry weight basis)

Composting time (days)	C _{EX} (%)	C _{FA} (%)	C _{HA} (%)	HR (%)	HI (%)	P _{HA} (%)	C _{HA} /C _{FA}
0	7.10	4.97	2.13	21.12	6.34	29.81	0.43
30	6.35	4.45	1.90	22.92	6.86	29.90	0.43
60	5.46	4.01	1.45	22.20	5.89	26.53	0.36
90	5.90	3.98	1.90	24.53	7.95	32.23	0.48
210	4.50	2.11	2.48	22.40	12.12	53.98	1.18
lsd	1.64	0.44	1.81	3.14	4.99	13.34	0.42

C_{EX}: extractable carbon measured in a 0.1 M NaOH; C_{FA}: fulvic acid-like carbon; HR: humification ratio; C_{HA}: humic acid-like carbon; HI: humification index; P_{HA}: percentage of humic acid-like C; C_{HA}/C_{FA}: ratio of humic acid-like C/fulvic acid-like C.

[38], when composting different organic wastes. The humification process was better explained by the determination of the humic acid percentage (P_{HA}) as $P_{HA} = (C_{HA}/C_{EX}) \times 100$ and the evolution of C_{HA}/C_{FA} throughout the biological process. Although final values of P_{HA} and C_{HA}/C_{FA} ratio varied with the kind of wastes used, final values of 53.98 and 1.18, respectively, were very close to those proposed by Paredes et al. [20], as indicators of maturity when composting orange industrial waste, cotton waste and OMW. Furthermore, the increase of both the P_{HA} and C_{HA}/C_{FA} ratio, exhibiting the degree of polymerisation, reflected complex molecule (humic acids) formation from more simple molecules (fulvic acids), and reduction of the non-humic components of the fulvic acid fraction, which are the most easily degraded substrate by the composting flora.

3.1.3. Detoxification of composting substrates by polyphenols elimination during the composting process

3.1.3.1. Impact of composting on water-soluble phenols (WSPH). OMW is well known for its high phenolic compound concentration, is shown to be phytotoxic, and acts as a growth inhibitor against some microorganisms of the telluric flora [39].

The phenolic fraction in a composted matrix includes complex molecules, which are generated during the partial degradation of lignin and are more resistant to biodegradation because of their aromaticity. However, the water-soluble phenol fraction, which is characterized by simpler phenolic structures and smaller molecular sizes, is sensitive to biological transformations which occur during composting, and has been used as an indicator of the composting process [22,40].

The WSPH concentration varied during composting following a zero-order kinetic model (Fig. 3(a)). The curve fitting the experimental data gives the following equation:

$$WSPH = -0.00213t + 0.5581 \quad (R^2 = 0.842)$$

where t is the composting time. This fraction showed a gradual fall throughout composting. According to the established model, WSPH disappears after less than 8 months of composting.

After 210 days, a drop of more than 75% occurred. This result is in accordance with data from Filippi et al. [15] who found a similar decrease with reduction of toxicity during the initial phase of the composting process. Zenjari et al. [25] attributed the decrease of WSPH to the microbial bioconversion of polyphenolic compounds and their interaction with secondary metabolites contributing to the biosynthesis of humic substances. Indeed, compost stability and degree of maturity are closely related to polyphenols and lipids concentrations. Furthermore, a high concentration of these phenolic compounds has a phytotoxic effect which reduces plant growth [29]. Additionally, the short-chain aliphatic acids and polyphenolic compounds released during aerobic and anaerobic decomposi-

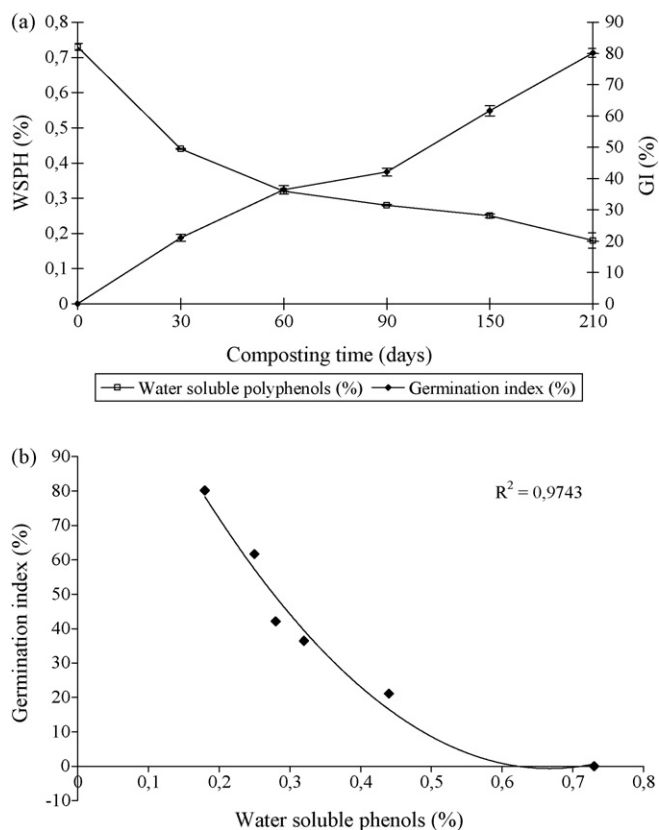


Fig. 3. Variation of water-soluble phenols during composting and their impact on germination index. (a) Evolution of water-soluble phenols and germination index during composting (vertical bars correspond to standard deviation). (b) Germination index as a function of water-soluble phenols.

tion reduce seed germination, root development and harvest yield. Moreover, it has been reported that the oxido-reducing enzymes included in OMW may remove phenols by oxidizing low-molecular weight phenolic compounds, which undergo a polymerisation to produce less soluble substances [22]. Alberquerque et al. claimed that several microbial groups can contribute to phenol degradation, and both microbial processes previously mentioned were described to detoxify the composting substrates [22].

In the present study, a strong correlation between the GI and WSPH contents was found (Fig. 3(b)) throughout the process. This result confirms the inhibitory effect of these compounds on seed germination, as previously mentioned.

3.1.3.2. Confirmation of phenols elimination by GC-MS. A marked reduction in phenolic compounds was confirmed by GC-MS analysis (Fig. 4). Indeed, the qualitative and quantitative compositions of the phenolic compounds were evaluated at the beginning of the process and in the final product (Table 5). Ethyl acetate, a very selective solvent for low and medium molecular weight phenolic compounds, was used as the suitable solvent for recovering simple phenolic compounds from olive mill residues [41].

Three phenolic compounds were identified (Fig. 4(a)). At the initial time of composting, Peak 1 was identified as 1-phenyl-2-diethyl methyl ethylene. The second peak (Peak 2) was the *m*-hydroxyphenyl ethanol. Peak 3 was identified as 4-hydroxyphenyl-ethanol, commonly known as tyrosol; this substance is described in the literature as one of the major compounds in olive mill residues [41].

The m/z of 282, 267, 193, 179 and 73 are specific to tyrosol TMS derivative ions. After electrospray ionization, the detected m/z ion

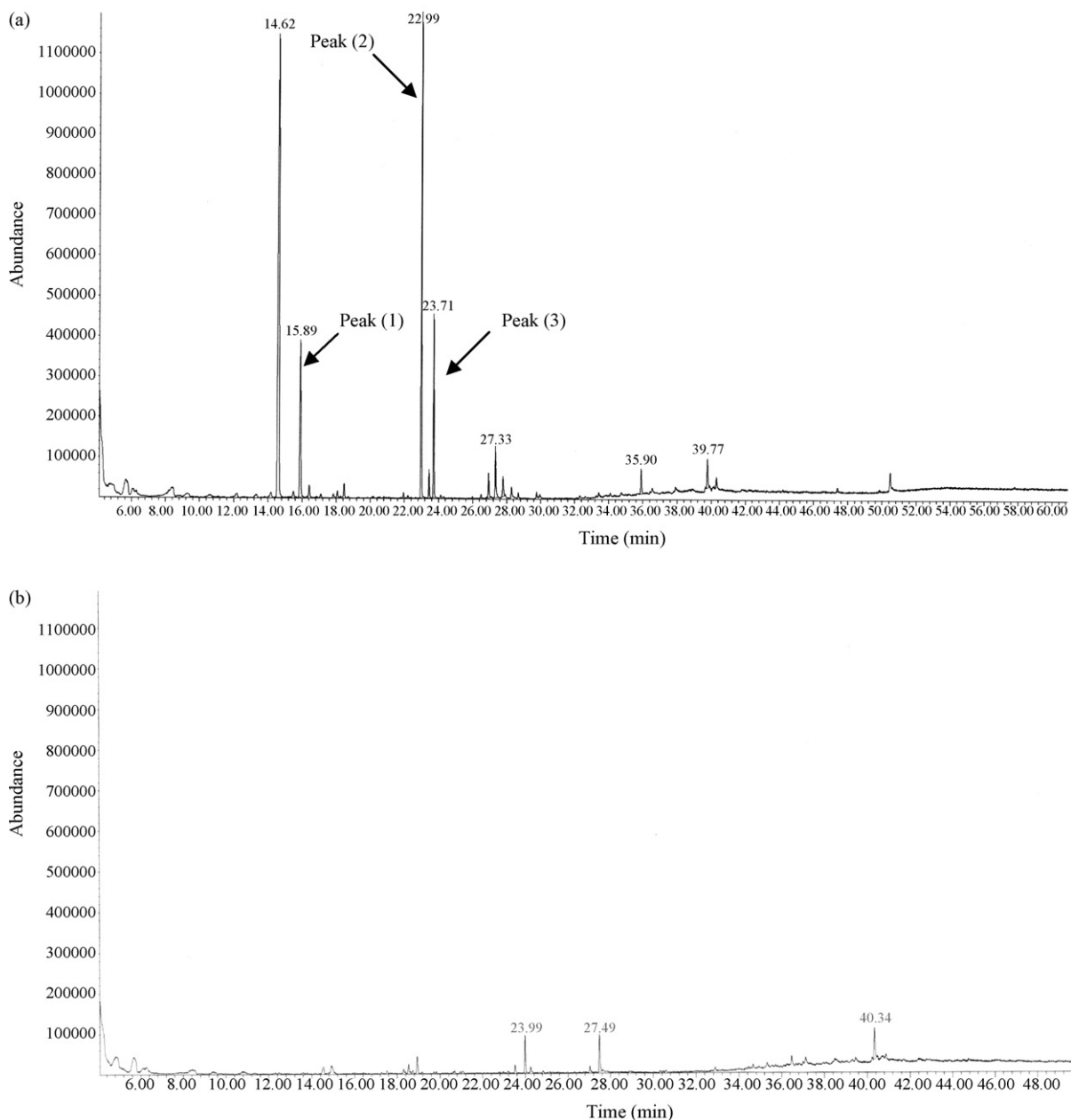


Fig. 4. GC-MS chromatograms on ethyl acetate extract from OMW sludge with sesame bark (a) initial time of composting and (b) final time of composting.

Table 5

Identification of the main compounds evidenced by GC-MS at initial and final times of composting

Assigned compound	Quality (%)	RT (min)	MW	Pic area (%)	TMS derivatives
Initial time					
Glycerol-tri-TMS ether	91	14.607	308.17	44.9	59; 73; 89; 103; 117; 133; 147; 163; 177; 191; 205; 218; 293
(E)-1-Phenyl-2-(diethylmethylsilyl) ethylene	50	15.887	204.13	12	59; 73; 89; 101; 117; 133; 147; 163; 175; 205
m-Hydroxyphenethanol	97	22.997	282.15	24	73; 103; 126; 147; 177; 193; 267; 282
Thiazolo (3,2- α) pyridinium	53	23.454	243.07	1.4	75; 103; 243
4-Hydroxyphenylethanol	97	23.711	282.15	8.3	73; 103; 179; 193; 267; 282
Cyclodecane	86	26.92	168.19	1.5	55; 69; 73; 79; 83; 97; 111; 127; 140; 168
2-Nitrodibenzothiophene	64	27.323	229.02	2.9	55; 75; 89; 101; 113; 127; 154; 171; 187; 199; 211; 229; 309
Monotrimethylsilyl palmitic acid	90	35.897	328.28	1.6	55; 75; 85; 97; 117; 132; 145; 269; 279; 294; 313; 328
Oleic acid, trimethylsilyl ester	93	39.766	354.30	1.86	55; 75; 85; 97; 117; 132; 145; 185; 199; 222; 279; 294; 309; 339
Final product: compost					
Thiazolo (3,2- α) pyridinium	64	23.988	243.07	31.01	75; 103; 243
Cyclodecane	91	27.497	168.19	34.37	55; 69; 83; 97; 111; 127; 140
Oleic acid, trimethylsilyl ester	89	40.340	354.30	34.62	55; 75; 86; 97; 117; 129; 145; 185; 199; 222; 279; 294; 309; 339

RT: retention time.

Table 6
Significant correlation between germination index and other composting parameters during the composting process

	Carbohydrates (%)	WSPH (%)	C/N	OM (%)	Fats (%)	TOC (%)	WSC (%)	GI (%)
Carbohydrates (%)	1.000							
WSPH (%)	NS	1.000						
C/N	0.909*	0.949**	1.000					
OM (%)	0.839*	0.984**	0.962**	1.000				
Fats (%)	0.880*	0.965**	0.995**	0.982**	1.000			
TOC (%)	0.868*	0.980**	0.985**	0.976**	0.986**	1.000		
WSC (%)	NS	0.829**	NS	NS	NS	0.827*	1.000	
GI (%)	-0.924**	-0.947**	-0.992**	-0.976**	-0.993**	-0.977**	NS	1.000

NS: not significant. *Significant at $p < 0.05$. **Significant at $p < 0.01$. OM: organic matter; TOC: total organic carbon; WSC: water-soluble carbon; WSPH: water soluble phenols; GI: germination index.

equal to 282 is characteristic of that of phenol and the m/z equal to 267 results from the loss of methyl group. α -Cleavage creates the base peak at m/z 179, which belongs to a cyclic trophylum-TMS ether cation. Signals at m/z 73 and 103 are created by the TMS group [42].

Fig. 4(b) presents the chromatogram of the final product. A comparison of both chromatograms suggests that composting had almost completely eliminated the phenolic compounds. Indeed, nearly all peaks related to phenols have disappeared in the final compost. Moreover, even upon magnification of this chromatogram, no traces of phenols can be observed. On the basis of these results, it is suggested that composting OMW is a promising process for the elimination of polyphenols and also for the detoxification of this waste.

3.2. Correlation between polyphenols, GI and other parameters during composting

Phytotoxicity or poor plant response can result from several factors; the most important is the lack of oxygen resulting from intense microbial activity, hence preventing their growth. Additionally, the accumulation of toxic compounds such as alcohols, phenolic compounds, low-molecular weight organic acids, ammonia and toxic nitrogen compounds, the presence of heavy metals and mineral interact on seed germination and it is very difficult to assess the significance of each factor separately [27].

The high phytotoxicity related to OMW is attributed to high polyphenolic content. The effect of this parameter on the germination index was widely studied during the last decade.

The changes occurring in the germination index and the WSPH are presented in Fig. 3(a).

During the process, low initial GI value was recorded during the first month, suggesting a severe phytotoxicity in the substrates at the early composting stage. Then, this parameter increased gradually to reach 80% at the end of the biological process.

A close relationship exists between the WSPH of the OMW sludge compost and the GI of the same mixture (Fig. 3(b)); the correlation coefficient between the two series of values reached 0.974. This result is in accordance with that of Zenjari et al. [25] who demonstrated a direct relationship between the polyphenol content of a wastewater-straw mixture and toxicity, determined as the concentration of WSPH giving 50% of the *Photobacterium phosphoreum* luminescence.

The results of the correlation test (Table 6) revealed strong correlations between polyphenols (WSPH), carbohydrates, fats, C/N, organic matter (OM), water-soluble carbon (WSC), total organic carbon (TOC) and GI. Indeed, evolutions of WSPH, carbohydrates and fats present strong negative correlation with GI ($p = -0.947$; -0.924 ; -0.993 , respectively). Correlations between the studied parameters were significant at a probability level of $p < 0.01$. These

results confirming the inhibitory effects of these components on cress germination were in accordance with those of Albuquerque et al. [22] who established relationships between both phytotoxicity and phenol concentration and phytotoxicity and lipidic content. In addition, the water extracts used to determine GI include lipidic compounds either emulsified or partially solubilised which may have negatively influenced the hydrological properties of the growing substrates and reduced biological activities such as the germination index. Regarding WSPH, the significant negative correlation was due to the gradual detoxification occurring during the biological process, when phytotoxic effects of phenols decreased with time.

Similarly, a significant negative correlation ($p < 0.01$) was established for C/N, OM and TOC correlated with GI ($p = -0.992$; -0.976 ; -0.977 , respectively). Indeed, at the initial stage of composting, OM decomposition leads to the production of phytotoxins, especially under deficient oxygenation conditions. During composting, these compounds are increasingly degraded by the intense aerobic microbial activity developed under appropriate temperature, oxygen concentration, water availability and nutrients [22]. In addition, a significant negative correlation was obtained between the GI and the OM ($p = -0.976$). This fact is related to the overall OM biodegradation that occurred during the process, leading to a reduction of the C/N ratio and a progressive decomposition of phytotoxic compounds such as polyphenols. It is well known that a germination index exceeding 60% indicates the disappearance of the compost's phytotoxicity [27]. In the present work, this value was reached by day 150 of composting.

These results confirmed the detoxification of the experimented mixture, OMW sludge with sesame bark, and are indicative for the production of adequate stabilized compost.

4. Conclusion

The non-controlled disposal of OMW in the Mediterranean area creates many environmental problems. The production of a high-end value product, which is the compost from by-products, is an important and promising solution providing an added income to the agro-industries and organic amendments useful for agriculture. Consequently, compost quality involving biological stability and raw materials detoxification must be assessed accurately.

In this study, the lack of toxicity of the final product was ascertained by criteria such as polyphenolic content and the germination index. Evolution of the physico-chemical, humification parameters and toxicity during the process ensured the occurrence of polymerisation mechanisms similar to those held during the humification process.

As regards to the results of the statistical analysis, the close correlation between polyphenols and the germination index ensures

the detoxification of OMW sludge and confirms that these polyphenols are involved in the initial toxic effect.

The final product has been proved to be a good soil fertilizer improving its agricultural use. It had a C/N ratio up to 18.75, while it was rich in humic substances and led to germination index of about 80% after 210 days of composting.

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