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Biological activity during co-composting of sludge issued from the OMW evaporation ponds with poultry manure—Physico-chemical characterization of the processed organic matter

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ABSTRACT

Olive mill sludge (OMS), a by-product resulting from natural evaporation of olive oil processing effluent, poses a major environmental threat. A current cost-effective practice of OMS management is composting. A mixture of OMS (60%) with poultry manure (PM) was successfully composted for 210 days. During the process, effluents of olive oil mill and confectionary were used to keep moisture at optimal level (40–60%). Biological indicators reflecting stability of the compost (microbial biota respiration and enumeration, and germination index) were analysed for the assessment of the product quality. The composted mixture showed a high microbial activity with a succession of microbial populations depending on the temperature reached during the biodegradation. The pathogen content from PM decreased with composting as did phytotoxic compounds. Phenols and lipids were reduced, respectively, by 40% and 84% while germination index increased with composting progress. Fourier transform infrared (FTIR) spectroscopic analysis revealed that the final compost improved the aromatic content compared to the starting materials, with a decrease in aliphatic groups and a reduction in the easily assimilated components by the microflora acting during the biological process. The final compost was characterized by relatively high organic matter content (26.21%), a low C/N ratio (16.21), an alkaline pH (8.32), a relatively high electrical conductivity (9.21 mS/cm) and a high level of nutrients. The germination index for *Lepidium sativum* L. was 87.71% after 210 days of composting, showing that the final compost was not phytotoxic.

1. Introduction

Olive mill wastewater (OMW) is a notorious pollutant of both terrestrial and aquatic ecosystems in the Mediterranean region [1–3]. OMW is a dark, turbid and acidic effluent (pH 4.5–5.5), with an excessive high organic load. It also includes high levels of phytotoxic and microbial inhibitor compounds, such as phenolic and long-chain fatty acids. This effluent displays antibacterial properties, inhibits seed germination and is phytotoxic [1,4,5].

In Tunisia, the main OMW management practice regulated and adopted by the majority of olive oil industries is its storage in evaporation ponds. This disposal way could be considered as a biological–natural treatment, where high evaporation rates prevailing during the summer period lead to sludge. Nevertheless, this method does not alleviate the high COD or toxicity of OMW since large quantities of unstabilised sludge are produced [6]. The treatment of olive mill sludge (OMS) is becoming a relevant problem in olive oil producing countries [7]. Indeed, due to evaporation, polyphenols and dissolved minerals are concentrated in OMS and can have a negative impact on the performance of the sludge treatment [8]. Paredes et al. and Sellami et al. [9,10] studied the composting process of OMW sludge with agrofood wastes and concluded a complete phytotoxicity removal in the compost/stabilised product. Vitolo et al. [8] proposed the preparation of fuel by mixing the solid residue of OMW with olive husk. The other way of recycling this waste by its incorporation in building materials was investigated by Hytiris et al. and Mekki et al. [6,11]. The global

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Abbreviations: OMW, olive mill wastewater; COD, chemical oxygen demand; OMS, olive mill sludge; PM, poultry manure; CFU, colony forming units; EC, electrical conductivity; C/N, carbon to nitrogen ratio; OM, organic matter; NT, total nitrogen; C_{org}, organic carbon; COT, total organic carbon; GI, germination index; WSPH, water-soluble phenols; FTIR, Fourier transform infrared.

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synthesis of these studied practices exhibited that for such solid wastes, composting is the recommended beneficial method, and co-composting of such waste mixed with other agricultural wastes seems to be a reasonable approach before ultimate disposal.

Poultry manure (PM) contains microorganisms originating from faeces, bedding material and is a potential feed residue. In Tunisia, this material is an abundant waste and its inappropriate disposal can generate environmental negative impacts, including bad odours, pathogens dissemination, nitrate leachate and groundwater-pollutant contamination [12,13]. Furthermore, excessive manure application is plant toxic due to the high salt content and accumulation of trace heavy metals in plants [14,15]. The co-composting technique is an efficient way contributing to the resolution of PM problems.

Composting process has been usually characterized by physico-chemical parameters [16–20]. However, few studies have considered parameters indicating the changes in microbial activity and spectroscopic-structural information concerning the resulting transformation of organic matter during the biological process. Indeed, composting is a process where the activity of indigenous microorganisms is enhanced by the physico-chemical environmental parameters of the composted solid matrix. Typically, moisture content, nutrient balance (C and N), temperature, particle size distribution, and aeration are the most important parameters acting on microbial activity [21]. When environmental conditions are optimum for microbial activity, mesophilic and thermophilic microorganisms rapidly decompose the substrate, increasing temperature through metabolic heat release. Subsequently, microbial community shifts from mesophilic to thermophilic flora, switching the process to thermophilic phase [13,22,23]. The process end-product is a stable compost. Compost stability refers to the resistance of composted organic matter to further rapid degradation, and can be directly measured by respiration rates [24–26].

Based on the literature, co-composting olive mill sludge with PM provides adequate C/N ratio and physical structure, and is hence expected to be a suitable environmental friendly treatment for both by-product detoxification and organic matter stabilisation. Therefore, the aim of this study was to evaluate composting efficiency as a low cost technique for PM sanitation and OMS stabilisation using (i) microbial activity monitoring during co-composting of OMS and PM, (ii) Fourier transform infrared (FTIR) spectroscopy to assess the maturity progress of the mixture during composting and (iii) physico-chemical analyses to characterize the end-product.

2. Materials and methods

2.1. Composting procedure

A windrow of 60 tons – having trapezoidal heap shape with an initial maximum height of 2.0 m and a about 2.0 m base by 8.0 m length – was prepared by mixing OMS with PM. The OMS was collected from the evaporation basins located in the city of Sfax (Tunisia), and the poultry manure (PM) from an industrialized farm in the same area. The study was held just at the end of olive harvesting (May 2005). The composition based on a fresh weight was as follows (dry weight in brackets):

$$60\%\mathrm{OMS} + 40\%\mathrm{PM} \quad (66:34).$$

The physico-chemical characteristics of the solid wastes used are presented in Table 1

Table 1
Physico-chemical characterization of raw materials: olive mill sludge (OMS) and poultry manure (PM) (dry weight basis)

Parameters	OMS	PM
pH	9.69 ± 0.03	8.27 ± 0.07
EC (mS/cm)	4.84 ± 0.04	4.07 ± 0.25
TOC (%)	23.120 ± 0.310	6.56 ± 0.25
N_T (%)	0.780 ± 0.011	0.56 ± 0.06
C/N	30.35	12.17
Polyphenols (%)	0.920 ± 0.014	0.14 ± 0.001
Carbohydrates (%)	0.520 ± 0.009	0.12 ± 0.004
Potassium (ppm)	23.20 ± 0.01	10.55 ± 0.01
Magnesium (ppm)	5.000 ± 0.015	3.745 ± 0.013
Sodium (ppm)	4.000 ± 0.005	2.01 ± 0.002
Calcium (ppm)	12.800 ± 0.017	50.40 ± 0.014
Phosphorus (ppm)	15.200 ± 0.028	75.76 ± 0.024
Copper (ppm)	0.020 ± 0.012	0.034 ± 0.015
Nickel (ppm)	<0.088	<0.088
Zinc (ppm)	0.050 ± 0.010	0.075 ± 0.010
Lead (ppm)	<0.041	<0.041
Iron (ppm)	0.870 ± 0.042	16.770 ± 0.025
Cadmium (ppm)	<0.004	<0.004
Manganese (ppm)	0.100 ± 0.034	0.210 ± 0.021
Chromium (ppm)	<0.015	<0.015

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

During composting process, moisture content was adjusted to around 45–60%. The windrow was watered by a mixture of OMW collected from the storage lagoon and an effluent from a confectionary (Table 2). This humidification process was carried out while turning the windrow by a bulldozer. The total volume of both equally mixed effluents used during all the process was 2.1 m^3 per tons of composted solid materials.

The windrow was turned mechanically every 3 or 4 days during the most active bio oxidative phase, and once a week all the way through the maturation period. Temperatures were measured on a daily basis by a mercuric thermometer fixed on a long rod, at 0.5 m depth at different locations spanning the whole windrow, the measurements were recorded after the stabilisation of the measured temperature (approximately 10 min), and the average value was used to represent the temperature profile during composting.

Samples were collected during the mechanical turning. These were taken by mixing six sub-samples from six sites of the pile, spanning the whole profile (from the top to the bottom), according to ISO 8633 [27]. Each sample was divided into two parts: one was immediately cooled and kept for microbiological analysis; the other was air-dried and ground to 0.5 mm for physico-chemical analysis.

Table 2
Physico-chemical characterization of OMW and confectionery wastewater used for watering raw materials during the composting process

Parameters	OMW	Confectionery effluent
Dry matter (%)	10.00 ± 0.10	13.50 ± 0.15
pH	5.30 ± 0.21	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)	280.40 ± 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.

2.2. Microbiological analysis

Microbiological analysis was performed during the biological process to characterize the organic matter stabilisation and to ensure the compost hygienic aspect. A 10 g sample was suspended in 90 ml of a sterile peptone water solution and stirred at 150 rpm for 10 min at 28 °C. The suspension was used for microbial count by cell enumeration assessed by the determination of the number of colony forming units (CFU), according to ISO 7218 [28]. Serial decimal dilutions of each suspension (10^{-1} to 10^{-8}) were plated in triplicate on different agar media: Plate Count Agar (PCA, Pronadisa, Madrid, Spain), for the total aerobic mesophilic and thermophilic bacteria incubated, respectively, at 30 °C and 55 °C for 72 h, desoxycholate lactose agar (Biomerieux, France) for total coliform and fecal coliform flora after incubation for 24 h at 37 °C and 44 °C, respectively, and potato dextrose agar (PDA) (Pronadisa, Madrid, Spain) for thermophilic and mesophilic yeasts and fungi enumeration, incubated, respectively, at 30 °C and 55 °C for 72 h. The sporulating aerobic bacteria (bacilli) were quantified according to Jouraiphy et al. [29]; the suspension was heated at 80 °C for 10 min then plated on PCA at 30 °C for 72 h.

Basal respiration was evaluated after defrosting and homogenizing the frozen sub-samples. Fresh samples, equivalent to 20 g dry weight, were incubated in 1.0 l glass jars, at 25 °C for 10 days. A vial containing 10 ml of NaOH (2 M) was placed in each jar to remove the CO_2 released by the microbial biota in the sample. After the incubation period, the NaOH-containing vial was removed and titrated with HCl (2 M).

Basal respiration rate was expressed as mg $C\text{-}CO_2$ evolved per gram of dry material per day.

2.3. Physico-chemical analysis

Physico-chemical characterization of the two effluents was made according to French standards [30]. 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 [31].

Total nitrogen (N_T) and total organic carbon (TOC) were determined by automatic microanalysis [32].

Macro- and micro-elements and heavy metals were brought into solution by acidic digestion (2 g of compost digested with HNO_3 and HCl), then analysed by an atomic absorption spectrophotometer. Phosphorus was determined colorimetrically at 430 nm as a molybdo-vanadate phosphoric acid [30]. Total fat content was determined by extraction with diethyl ether and later weighing. Water-soluble phenolic substances (WSPH) were determined by the modified Folin method [33]. Carbohydrates were analysed in the water extract (1:10, w/v) by the anthrone method [34].

Toxicity was assessed by germination index (GI) determination. This was performed with cress seed (*Lepidium sativum* L.) according to Alburquerque et al. [18].

2.4. Fourier transform infrared analysis

The Fourier transform infrared (FTIR) spectra of each composting step was recorded between 4000 and 400 cm^{-1} wave-numbers using a PerkinElmer 1600 FTIR. Pellets were prepared by mixing 2 mg ground sample with 300 mg of potassium bromide. The mixture was compressed under vacuum for 10 min. In order to limit moisture interference, both composted samples and KBr were dried at 105 °C for 72 h before pellets preparation.

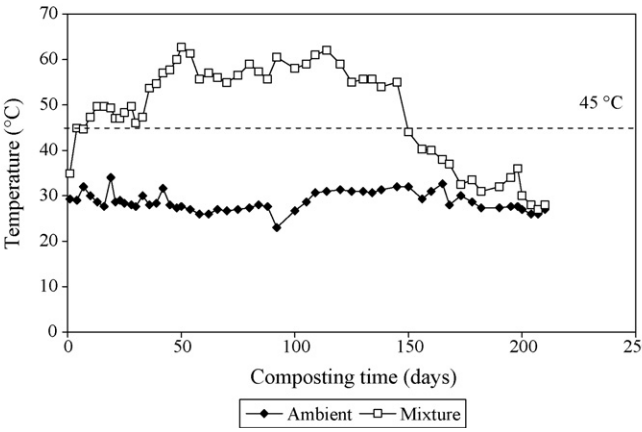

Fig. 1. Changes in temperature during co-composting of OMW sludge and poultry manure.

2.5. Statistical analysis

The mean was reported with the statistical analysis expressing the incertitude using ANOVA software. During composting, significant differences among the values of each studied parameter were calculated by the least significant difference (LSD) test at $p < 0.05$.

All analyses were performed in duplicate.

3. Results and discussions

3.1. Temperature evolution

After windrow establishment, the composting mixture achieved rapidly thermophilic temperatures (>45 °C) (Fig. 1). The maximum temperature reached during composting was 62 °C (thermophilic phase). During the process, temperatures exceeding 60 °C maintained for several days ensured the organic matter stabilisation and the pathogenic microorganism suppression. Initially, the temperature of the composting windrow was 35 °C (mesophilic phase). It rose above 45 °C after 4 days (thermophilic phase) to reach temperatures exceeding 50 °C after 13 days. Thereafter, the temperature remained constant in the average of 60 °C for at least 3 months, before dropping to ambient value. This phenomenon seems to be specific to OMS composting since the use of PM allows a thermophilic phase handle during few weeks [19]. Indeed, the long thermophilic phase reflects the availability of degradable substances as well as the self-insulating capacity of the composted OMS material. Furthermore, watering with a mixture of effluents (OMW and confectionary's effluent) provides organic compounds, especially carbohydrates, easily biodegradable. Such excessive thermophilic phase lasting (more than 100 days) indicates an abnormally extended decomposition and a delayed transition to the stabilisation stage [24].

Temperature fluctuations during composting followed a pattern similar to that previously noted in composting systems [2,18–20,35,36]. Initially, the temperature of the composting mixture rose as a consequence of the rapid breakdown of the available organic matter and nitrogenous compounds by microorganisms (thermophilic phase). As the organic matter became more stabilised, the microbial activities and the organic matter decomposition rate slowed down. The temperature gradually decreased to reach ambient value, indicating the end of the thermophilic phase.

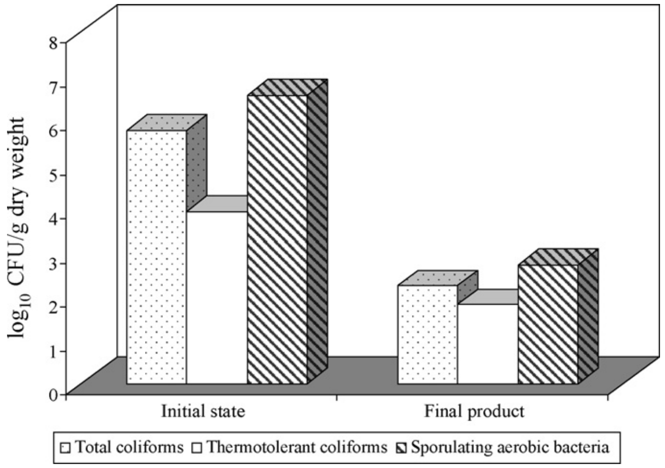

Fig. 2. Concentrations of indicator microorganisms (expressed as colony forming units/g fresh material) in the initial composted mixture and the final compost.

3.2. *Sanitation indicator bacteria opportunist: pathogenic microorganisms*

One of the problems posed by the direct use of poultry manure in agriculture is the risk of plant and human contamination by pathogens. Initially, the mixture of by-products showed a high density of sporulating aerobic bacteria, total coliforms and thermotolerant coliforms. They were significantly log reduced by 2.4, 2.5 and 1.5, respectively, at the end of the process (Fig. 2), in respect to the initial count.

During composting, the average temperature was of 57.17 ± 3.19 °C, held for more than 4 months caused a significant elimination of the pathogenic microorganisms. At this temperature, only a few days are required to eliminate almost all pathogens and nematodes according to Jouraiphy et al. [29]. Similar results were found by Lemunier et al. [37], when composting bio-waste using an "in-vessel aerated forced system". Moreover, Ros et al. [13] explained that at high temperatures reached during composting, pathogens are destroyed making the pig slurry-derived compost safe for agricultural use. Recently, Heinonen-Tanski et al. [12] confirmed hygienization of composted manure by heat produced during organic matter decomposition. Simultaneously, proteins degradation leads to ammonification increasing pH and ammonia concentration. The alcalinization of the substrate may inhibit the survival of many pH sensitive microorganisms contributing to hygienization.

While processing, thermotolerant coliforms concentration used as environmental sanitation indicator [37], was reduced confirming the composting efficiency. Tiquia et al. [38] and Larney et al. [39] found that the coliform concentrations reduced from 10^7–10^8/g to 10^2–10^3/g and the enteric pathogens concentration decreased below the detection limits during a successful composting process. Ros et al. [13] recommended a density of fecal coliforms for compost sanitation of 10^3 /g of solid and considered faecal streptococci to be more useful indicators of the disinfection processes in sewage sludge composts. Furthermore, Lasaridi et al. [40] proposed a fecal coliforms limit value of 5×10^2 CFU/g of dry weight for sanitized compost. In our experiment, thermotolerant coliforms density found in the final compost was below the recommended limit. Moreover, during the experiment, long period of composting temperature ranged from 55 to 63 °C. These values can destroy harmful species of fecal coliforms according to Déportes et al. [41].

Sporulating aerobic bacteria concentration was similar to that found by Jouraiphy et al. [29]. Tuomela et al. [23] reported that *Bacillus* is a typical bacterium of the thermophilic phase and it is among the thermophilic genus that has been isolated from compost at high temperatures. These bacteria produce thick-walled endospores very resistant to heat, radiation and chemical disinfectant. Although sporulating aerobic bacteria (bacilli) are active between 60 and 65 °C and destroyed at 75 °C, in compost where temperature do not exceed 65 °C, bacterial enzymes would be irreversibly denaturized [29].

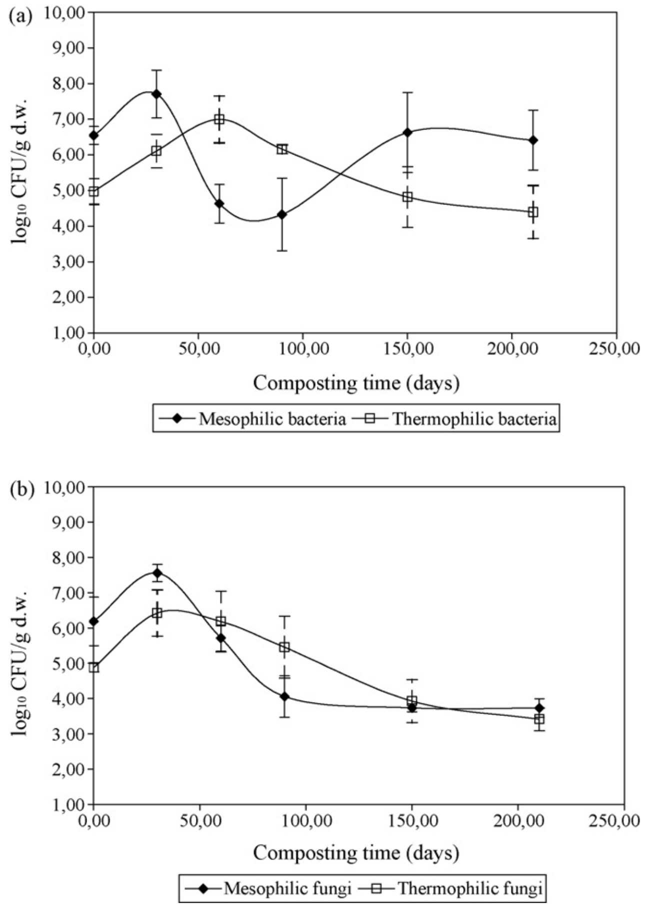

Fig. 3. Changes in total populations (expressed as $\log_{10}$ CFU/g d.w.) of (a) mesophilic and thermophilic bacteria; (b) mesophilic and thermophilic fungi. The error bars indicate standard errors.

3.3. *Biological parameters*

3.3.1. *Microbiological changes*

During composting, diverse microbial activities conduct the process [23]. Bacterial and fungi populations of mesophilic and thermophilic biota at various composting process stages were evidenced (Fig. 3).

Mesophilic bacteria were predominant at 30 days of composting. Then, their concentration declined with time to reach log 4 N/g d.w. during the thermophilic phase, and finally it rose during the cooling stage. Besides, after 210 days of composting, thermophilic bacteria were at the same concentration as mesophilic bacteria initially present in the composted mixture. An increase of about one order of magnitude was observed just at the beginning of the thermophilic phase, after 30 days of the composting process. At 60 days, thermophilic bacteria were at their maximum rate and then declined until the end of the process to stabilise at almost the same initial concentration (Fig. 3a). It is important to notice that during the

Table 3
Daily CO2 release during composting of OMW watered by confectionery wastewater.

	Composting time (days)					
	0	30	60	90	150	210
mg CO_2 released g^{-1} d.w. day^{-1}	5.80 ± 0.26	6.85 ± 0.97	7.80 ± 0.36	3.38 ± 0.75	1.37 ± 0.21	0.48 ± 0.09

cooling phase, there was a resurgent growth of mesophilic bacteria which reached approximately the same value at starting time (2.6×10^6 CFU/g d.w.).

Similarly to bacterial evolution, mesophilic fungal population has the same trend as the total mesophilic bacteria. The maximum concentration was observed at 30 days of composting and then declined to a level of log 4 N/g d.w. at 90 days of the biological process and stabilised at this concentration throughout the composting process (Fig. 3b). The number of thermophilic fungi increased slightly during the first 30 days of composting and then a continuous decrease was observed until the end of the biological cycle reaching the same concentration as that of mesophilic fungi. As a result, mesophilic and thermophilic microorganisms' growth, including bacteria and fungi, are related to the mesophilic and thermophilic stages of composting. This observation is in line with the findings of Goyal et al. [42] studying composting different organic wastes At laboratory scale, Tuomela et al. [23] described the succession of microflora related to temperature evolution during composting and reported the active development of bacteria and actinomycetes on thermophilic fungi mycelium.

Furthermore, no inhibitory effect on microbiota growth was noticed during the process in spite of watering with OMW. This result is in complete disagreement with the findings of Abid and Sayadi [43] stipulating that phenols accumulation brought by moistening with OMW caused a decrease in viable counts of thermophilic bacteria.

Microbial biota succession plays a key role in composting process, and the appearance of some microorganisms reflects the quality of compost maturity [44]. The results showed that at the early stage of composting (temperatures of 20–40 °C) mesophilic populations were the dominant biodegraders of fresh organic materials and were partially killed or inactivated during the thermogenic stage (temperatures of 40–60 °C). Finally, the thermophilic microorganisms emerged; their concentration was reduced because of physico-chemical conditions.

3.3.2. *Microbial respiration*

The evolution of microbial respiration of the composted mixture is presented in Table 3. This parameter is widely used to monitor microbial activity and organic matter breakdown [13].

The levels of CO_2 emission increased during the first 3 months then a gradual decrease was observed until the end of the biological process. This behaviour indicates two different kinetic phases in C mineralization: a first phase when a rapid decomposition of the most easily biodegradable substrates occurred leading to the proliferation of various microflora with different enzymatic activities (Fig. 3). A second phase is revealed, when microbial respiration decreased with composting, reflecting the occurrence of organic matter stabilisation and composted mass becomes concentrated in recalcitrant compounds. Indeed, during composting progress, microorganisms metabolized the soluble organic matter provided by easily biodegradable substances and especially nitrogen, mainly supplied by poultry manure. As a result, this aerobic degradation principally generated heat, biomass and carbon dioxide.

During the first 3 months of composting, microbial respiration increase can be explained by the windrow's aeration which improved the organic compounds degradation; those were supplied by watering the mixture with both effluents. Watering with such wastewaters had not inhibited the microbial activity since temperature in the mixture was relatively high (Fig. 1), with an important CO_2 emission (Table 3). These findings are different from those of Abid and Sayadi [43], who noticed a decline in microbial activity when watering with OMW attributed to high phenol and organic acid content of the effluent. In our result, the use of OMW did not have any negative impact on microbial activity. Indeed, the developed microbial communities nesting the windrow were adjusted to grow on OMS in which the toxic phenolic compounds were concentrated. While watering, microorganisms would be stimulated by the organic compounds supplied.

Goyal et al. [42] mentioned that in mature municipal compost, the amounts of CO_2-C evolved should be less than 500 mg CO_2-C g in the compost. More CO_2 exhausted indicates that compost has not been stabilised and needs further decomposition. In the present investigation, the CO_2-C evolved, expressed as mg CO_2-C g^{-1} d.w. day^{-1}, indicated a stabilised compost.

3.4. *Characteristics of the final composts*

3.4.1. *Organic matter, EC and pH*

Stabilisation of the end-product was assessed by characterizing compost's physico-chemical properties and comparing them to those of the conventional cow manure (Table 4) and to International Standards. Overall, the prepared compost exhibited an adequate agronomic quality with high content of organic matter and low C/N ratio reflecting a stabilisation and maturity of the compost. To get the EU eco-label, composted material should contain no less than 20% OM, which was met at the end-product [40]. Furthermore, a C/N ratio of about 20 is enhanced by high mineral nutrients concentrations, indicative of various organic fertilisers like manure and composted vegetables [45].

According to Lasaridi et al. [40], compost should have a pH value within the range of 6.0–8.5 to ensure compatibility with most plants. Because of the irrigation with OMW, the compost EC was 9.21 (S m^{-1}), a relatively high level exceeding the Greek standards' upper limits (4.0 S m^{-1}), and hence indicating a possible toxicity for most plants due to salts if used undiluted in potting mixture [40,46]. However, the prepared compost was slightly alkaline as required.

3.4.2. *Mineral content and heavy metals*

As composting leads to production of minerals [20], the concentrations of P, K, Ca and Mg were determined. According to Table 4,

Table 4
Physico-chemical characterization of the final compost and the farm manure (dry weight basis)

Parameters	Compost at 210 days of composting	Cow farm manure
pH	8.32 ± 0.01	8.87 ± 0.03
EC (S m^{-1})	9.21 ± 0.08	2.05 ± 0.06
C_{org} (%)	18.47 ± 0.01	33.80 ± 0.34
N_{org} (%)	1.19 ± 0.11	2.00 ± 0.01
C/N	16.21 ± 1.42	16.90 ± 0.28
OM (%)	26.21 ± 0.03	69.560 ± 0.010
P (g kg^{-1})	61.700 ± 0.020	4.600 ± 0.017
K (g kg^{-1})	24.100 ± 0.025	10.376 ± 0.340
Ca (g kg^{-1})	42.300 ± 0.015	16.400 ± 0.010
Mg (g kg^{-1})	6.300 ± 0.017	3.800 ± 0.030
Fe (g kg^{-1})	11.40 ± 0.10	Non determined

Table 5
Heavy metal concentrations in the examined compost and standard limit values [36] (dry weight basis)

Heavy metal	Cu (mg kg^{-1})	Ni (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Mn (mg kg^{-1})	Cr (mg kg^{-1})
Raw materials mixture	27.71 ± 0.01	<0.008	141.32 ± 0.01	<0.041	<0.004	152.40 ± 0.04	27.71 ± 0.01
Compost	31.83 ± 0.01	<0.008	184.63 ± 0.06	<0.041	<0.004	101.86 ± 0.01	35.01 ± 0.00
Eco-label limits	100.00	50.00	300.00	100.00	1.00	Non defined	50.00

the prepared compost contained high amounts of P, K, Ca and Mg. This mineral composition confirmed the beneficial effect of this organic fertilizer.

When applying municipal solid waste compost to soil, Weber et al. [47] observed a large increase of plant-available P, K and Mg. Zorpas et al. [45] obtained a final compost with high organic matter and nutrients (N, P, K and cation-exchange capacity) when composting vinasse with olive stone residue.

Heavy metals concentrations indicated the extent of contamination in prepared compost. Indeed, when applied as a soil amendment or fertilizer, the final product must be free of hazardous pollutants such as heavy metals. After composting OMS with poultry manure, the total metal concentrations in the final composts showed a wide variation (Table 5). The most abundant heavy metal was Zn but all the values were below the eco-label standards required for Cu, Ni, Zn and Pb. Compared to the respective values of heavy metals in the initial mixture, the concentrations in the final compost globally increased (Table 5). Similar increase in the total metal concentrations was found in many relevant studies [48,49]. This increase was mainly due to the weight loss of materials following organic matter decomposition, watering with OMW and mineralization processes during the composting period.

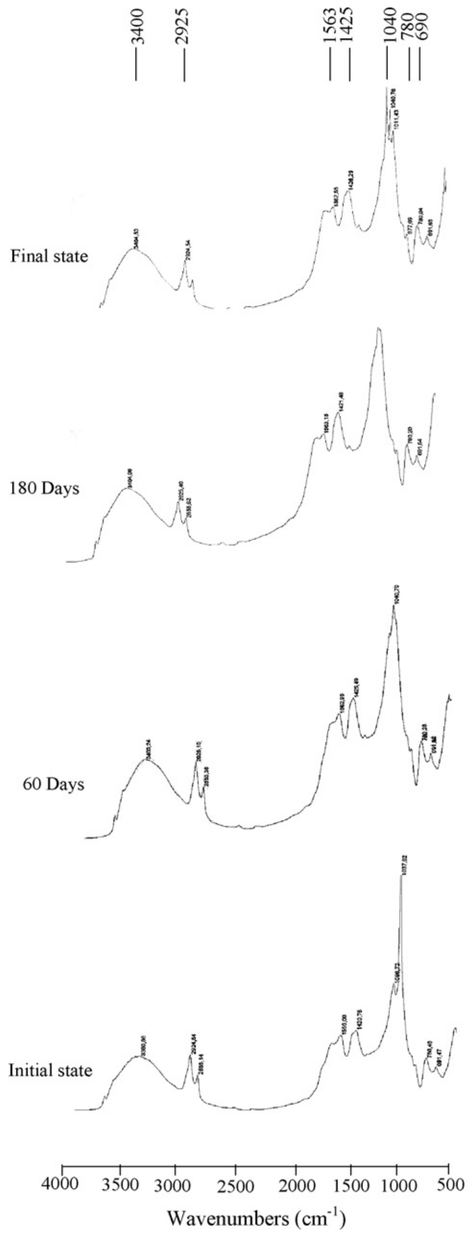

Fig. 4. FTIR spectra of co-composted OMS and PM at different process stages.

3.4.3. *Organic matter (FTIR spectroscopy)*

The FTIR spectra of OMS composted at various stages (initial time, 60, 180 and 210 days) exhibited a great resemblance in some absorption bands, but with different intensities (Fig. 4). The principal absorption bands in the FTIR spectra and their corresponding assignments based on the literature [10,50,51] are summarized in Table 6.

The main features of the FTIR spectra are the following:

(a) an intense broad band centered between 3700 and 3300 cm^{-1} usually attributed to O–H stretching in carboxylic functions, and –H bonded O–H groups of phenols and N–H stretching in amide functions. These specified functions and compounds are abundant at initial time in the raw materials. Their intensity decreased with composting time;
(b) a band at about 2925 and 2855 cm^{-1} reflecting aliphatic C–H group stretching;
(c) a band at about 1650 cm^{-1} generally attributed to absorption of several groups including aromatic C=C, C=O stretching of amide groups, ketones and/or quinones;

Table 6
Major absorption bands and assignments in FTIR spectra, for OMW sludge compost at different stages of composting

Wave-number (cm^{-1})	Assignment
3450–3300	- O–H stretching in alcohols and carboxyl functions; - bonded OH groups of phenols; - N–H stretching in amides and amines second.
2925	- Symmetric C–H stretching of aliphatic chains (fatty acids, waxes and aliphatic components).
2855	- C–H stretching of aliphatic methylene.
1660–1640	- Aromatic C=C, C=O stretching of amide groups; - C=O of quinone and/or H-bonded conjugated ketones in acids and primary amides.
1565	- N–H deformation and C–N stretching of amides II in proteineous chains; - Aromatic C=C stretching and/or N–H deformation.
1425	- C–H deformation in lignin and carbohydrates; - phenolic OH; - COO$^-$ asymmetric stretching and stretching of O$^-$ distributed aromatic rings.
1100–1000	- C–O–C stretching of polysaccharides; - Si–O stretching.

Table 7
Changes in water-soluble phenols, fats and germination index during composting OMW sludge with poultry manure (dry weight basis)

Composting time (days)	WSPH (%)	Fats (%)	GI (%)
0	0.650 ± 0.001	10.38	0.00 ± 0.00
30	0.500 ± 0.004	8.80	2.26 ± 1.86
90	0.380 ± 0.003	3.23	14.15 ± 1.12
210	0.390 ± 0.004	1.70	87.71 ± 1.27

WSPH: water-soluble phenols; GI: germination index; ±S.D.

(d) a peak at about 1560 cm^{-1} preferentially ascribed to N–H deformation and C═N stretching of amides;
(e) an absorption at about 1420 cm^{-1} attributed to several structures of carbohydrates and lignin, and also phenolic OH as well as O^{-} distributed on aromatic rings;
(f) a band at about 1040 cm^{-1} generally attributed to C–O–C stretching of polysaccharides or polysaccharide-like substances and Si–O of silicate impurities, reflecting sand contamination. The last band intensity tends to decrease with composting time.

All spectra showed a high intensity for three bands: 3400, 1425 and 1040 cm^{-1}. These characteristic bands are common to FTIR of composted olive oil mill by-products and confirm the presence of highly oxidized polymerised humic substances structures in the OMS compost [52].

During composting, the structures absorbing at around 2925, 2850 and 1540 cm^{-1} decreased and those absorbing at around 1425 cm^{-1} rose. Peaks at 2925 and 2850 cm^{-1} decreased reflecting a preferential aliphatic structures biodegradation. In addition, the decrease of 1563 cm^{-1} explained the degradation of peptide initially included in poultry manure. In contrast, at the final stage of the process the peak at 1425 cm^{-1}, attributed to the phenolic structures, increased indicating the occurrence of oxidation reactions. The results provided by FTIR spectroscopy supported previous studies [10,29,50,53] and confirmed that with increasing composting time, aliphatic materials and carbohydrates decreased whereas aromatic and N-containing groups reflecting a biosynthesis of humic substances (humic and fulvic acids), and probably stabilised proteinaceous materials, increased. These changes, indicating the humification degree increase, confirm the efficiency of OMW composting.

3.4.4. Germination index

Compost stability was also estimated by the germination index (GI) (Table 7). In the first 3 months of the composting process, the GI was too low, indicating phytotoxicity of the composted substrate. However, the decrease of fats and WSPH occurred during the biological process contributed to the detoxification of the materials proceeded. Similar decrease was described to be responsible for toxicity reduction during composting progress [18,53,54]. Our results support these previous findings. After 210 days of composting, GI exceeded 80%; according to Paredes et al. [9], GI value above 50% indicates that the soil amendment used would not hurt plants while Lasaridi et al. [40] considered that compost experimented with GI value below 80% is phytotoxic. Nevertheless, other authors characterized a non-phytotoxic and stable substrate with GI in the range of 66–100% [45]. The EC decision on the eco-label does not specify any limit value for GI.

4. Conclusion

It can be concluded that monitoring both chemical and microbiological parameters provide sustainable information on the state of compost and its evolution during the composting process.

Biological changes during composting indicated a succession of microbial populations depending on the temperature reached in the windrow and microbial respiration measurement has been shown to be useful tools for monitoring the organic matter mineralization, evaluating compost maturity. These microbial transformations were also confirmed by FTIR analysis which revealed the biodegradation of the easily assimilated components by microorganisms and an enrichment of aromatic structures suggesting humification during the process. Important decrease in phenol and lipid was observed with a notable germination index increase indicating compost stability and maturity. Final compost characterization confirmed its good notable quality with the presence of macronutrients and the absence of trace heavy metal contents; as required by the eco-label standards.

As a consequence, composting has been demonstrated to be a suitable process for PM sanitation and OMS detoxification leading to compost exhibiting a substantial richness of stabilised organic matter and absence of phytotoxicity suitable for soil amendment as organic fertilizer. Such biological treatment could be considered as a sustainable method of waste valorization by recycling.

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References

[1] C.I. Piperidou, C.I. Chaidou, C.D. Stalikas, K. Soulti, G.A. Pilidis, C. Balis, Bioremediation of olive oil mill wastewater: Chemical alterations induced by *Azotobacter vinelandii*, J. Agric. Food Chem. 48 (2000) 1941–1984.
[2] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Agrochemical characterisation of “alperujo”, a solid by-product of the two-phase centrifugation method for olive oil extraction, Biores. Technol. 91 (2004) 195–200.
[3] M. Kotsou, I. Mari, K. Lasaridi, I. Chatzipavlidis, C. Balis, A. Kyriacou, The effect of olive mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*, Appl. Soil Ecol. 26 (2004) 113–121.
[4] E. Ammar, M. Nasri, K. Medhioub, Isolation of Enterobacteria able to degrade simple aromatic compounds from wastewater from olive oil extraction, Word J. Microbiol. Biotechnol. 21 (2004) 251–259.
[5] M. Chtourou, E. Ammar, M. Nasri, K. Medhioub, Isolation of a yeast *Trichosporon cutaneum*, able to use low molecular weight phenolic compounds: application to olive mill wastewater treatment, J. Chem. Technol. Biotechnol. 79 (2004) 869–878.
[6] N. Hytiris, I.E. Kapellakis, R. La Roij de, K.P. Tsagarakis, The potential use of olive mill sludge in solidification process, Res. Conserv. Recycling 40 (2004) 129–139.
[7] R. Lanciotti, A. Gianotti, D. Baldi, R. Angrisani, G. Suzzi, D. Mastrocola, M.E. Guerzoni, Use of *Yarrowia lipolytica* strains for the treatment of olive mill wastewater, Biores. Technol. 96 (2005) 317–322.
[8] S. Vitolo, L. Petarca, B. Bresci, Treatment of olive oil industry wastes, Biores. Technol. 67 (1999) 129–137.
[9] C. Paredes, M.P. Bernal, J. Cegarra, A. Roig, Bio-degradation of olive mill wastewater sludge by its co-composting with agricultural wastes, Biores. Technol. 85 (2002) 1–8.
[10] F. Sellami, S. Hachicha, M. Chtourou, K. Medhioub, E. Ammar, Bioconversion of wastes from the olive oil and confectionary industries: spectroscopic study of humic acids, Environ. Technol. 28 (2007) 1285–1298.
[11] H. Mekki, M. Anderson, E. Ammar, G. Skerratt, M. Ben Zina, Olive oil mill wastewater as a replacement for first water in the manufacture of fired clay bricks, J. Chem. Technol. Biotechnol. 81 (2006) 1419–1425.
[12] H. Heinonen-Tanski, M. Mohaibes, P. Karinen, J. Koivunen, Methods to reduce pathogen microorganisms in manure, Livestock Sci. 102 (2006) 248–255.
[13] M. Ros, C. Garcia, T. Hernandez, A full-scale study of treatment of pig slurry by composting: kinetic changes in chemical and microbiological properties, Waste Manage. 26 (2006) 1108–1118.
[14] F.A. Nicholson, B.J. Chambers, K.A. Smith, Nutrient composition of poultry manures in England and Wales, Biores. Technol. 58 (1996) 279–284.

[15] J.W.C. Wong, Kh. Ma, K.M. Fang, C. Cheung, Utilisation of a manure compost for organic farming in Hong Kong, Biores. Technol. 67 (1999) 43–46.
[16] E. Madejón, E. Galli, U. Tomati, Composting of wastes produced by low water consuming olive mill technology, Agrochimica 42 (1998) 135–146.
[17] J. Cegarra, J.B. Amor, J. Gonzalvez, M.P. Bernal, A. Roig, Characteristics of a new solid olive-mill by-product ("alperujo") and its suitability for composting, in: P.R. Warman, B.R. Taylor, Halifax (Eds.), Proceedings of the International Composting Symposium (ICS' 99), Canada, Part I, 2000, pp. 124–140.
[18] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Measuring detoxification and maturity in compost made from "alperujo", the solid by-product of extracting olive oil by the two-phase centrifugation system, Chemosphere 64 (2006) 470–477.
[19] S. Hachicha, M. Chtourou, K. Medhioub, E. Ammar, Compost of poultry manure and olive mill wastes as an alternative fertilizer, Agron. Sustain. Dev. 26 (2006) 135–142.
[20] F. Sellami, R. Jarboui, S. Hachicha, K. Medhioub, E. Ammar, Co-composting of oil exhausted olive-cake, poultry manure and industrial residues of agro-food activity for soil amendment, Biores. Technol. 99 (2008) 1177–1188.
[21] K.C. Das, Co-composting of alkaline tissue digester effluent with yard trimmings, Waste Manage. 28 (2008) 1785–1790.
[22] M. Mustin, in: F. Dubusc (Ed.), Le compost: gestion de la matière organique, F. Dubusc, Paris, 1987, pp. 117–263.
[23] M. Tuomela, A. Vikman, M. Hatakka, M. Itavaara, Biodegradation of lignin in a compost environment: a review, Biores. Technol. 72 (2000) 169–183.
[24] C. Tognetti, M.J. Mazzarino, F. Laos, Imroving the quality of municipal organic waste compost, Biores. Technol. 98 (2007) 1067–1076.
[25] N.V. Hue, J. Liu, Predicting compost stability, Comp. Sci. Util. 3 (1995) 8–15.
[26] T. Eggen, O. Vethe, Stability indices for different composts, Comp. Sci. Util. 9 (2001) 27–37.
[27] International Standardization Organization ISO 8633 Solid fertilizers—sampling method for small lots, 1992, 4 pp.
[28] International Standardisation Organisation, ISO 7218—Règles générales pour les examens microbiologiques, 1996, 47 pp.
[29] A. Jouraiphy, S. Amir, M. El Gharous, J.C. Revel, M. Hafidi, Chemical and spectroscopic analysis of organic matter transformation during composting of sewage sludge and green plant waste, Int. Biodeter. Biodegrad. 56 (2005) 101–108.
[30] Association française de normalisation. Recueil des normes AFNOR, Qualité de l'eau, Méthodes d'analyse (4ème tome), 2ème Ed., Eds Afnor, 1997.
[31] A.F. Navarro, J. Cegarra, A. Roig, D. Garcia, Relationships between organic matter and carbon contents of organic wastes, Biores. Technol. 44 (1993) 203–207.
[32] A.F. Navarro, J. Cegarra, A. Roig, M.P. Bernal, An automatic microanalysis method for the determination of organic carbon in wastes, Commun. Soil Sci. Plant Anal. 22 (1991) 2137–2144.
[33] R. Maestro Durán, R. Borja Padilla, A. Martín Martín, J.A. Fiestas Ros de Ursinos, J. Alba Mendoza, Biodegradación de compuestos fenolicos presentes en el alpechin, Grasas y Aceites 42 (1991) 271–276.
[34] C. Paredes, J. Cegarra, A. Roig, M.A. Sánchez-Monedero, M.P. Bernal, Characterization of olive mill wastewater (alpechin) and its sludge for agriculture purposes, Biores. Technol. 67 (1999) 111–115.
[35] M.P. Bernal, C. Paredes, M.A. Sanchez-Monedero, J. Cegarra, Maturity and stability parameters of compost prepared with a wide range of organic wastes, Biores. Technol. 63 (1998) 91–99.
[36] I. Mari, C. Balis, D. Georgakakis, C. Ehaliotis, M. Kotsou, Respiration profile in monitoring the composting of by-products from olive oil agro-industry, Biores. Technol. 87 (2003) 331–336.
[37] M. Lemunier, C. Francou, S. Rousseaux, S. Houot, P. Dantigny, P. Piveteau, J. Guzzo, Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts, Appl. Environ. Microbiol. 71 (2005) 5779–5786.
[38] S.M. Tiquia, N.F.T. Tam, I.J. Hodgkiss, *Salmonella* elimination during composting of spent pig litter, Biores. Technol. 63 (1998) 193–196.
[39] F.J. Larney, L.J. Yanke, J.J. Miller, T.A. McAllister, Fate of coliform bacteria in composted beef cattle feedlot manure, J. Environ. Qual. 32 (2003) 1508–1515.
[40] K. Lasaridi, I. Protopapa, M. Kotsou, G. Pilidis, T. Manios, A. Kyriacou, Quality assessment of composts in the Greek market: the need for standards and quality assurance, J. Environ. Manage. 80 (2006) 58–65.
[41] I. Déportes, J.L. Benoit-Guyod, D. Zmirou, Hazard to man and environment posed by the use of urban waste compost: a review, Sci. Total Environ. 172 (1995) 197–222.
[42] S. Goyal, S.K. Dhull, K.K. Kapoor, Chemical and biological changes during composting of different organic wastes and assessment of compost maturity, Biores. Technol. 96 (2005) 1584–1591.
[43] N. Abid, S. Sayadi, Detrimental effect of olive mill wastewater on the composting process of agricultural wastes, Waste Mange. 26 (2005) 1099–1107.
[44] J. Ryckeboer, J. Mergaert, J. Coosemans, K. Deprins, J. Swings, Microbiological aspects of biowaste during composting in a monitored compost bin, J. Appl. Microbiol. 94 (2003) 127–137.
[45] S. Hachicha, J. Cegarra, F. Sellami, R. Hachicha, N. Drira, K. Medhioub, E. Ammar, Elimination of polyphenols toxicity from olive mill wastewater sludge by its co-composting with sesame bark, J. Hazard. Mater. 161 (2009) 1131–1139.
[46] I.S. Arvanitoyannis, A. Kassaveti, Current and potential uses of composted olive oil waste, Int. J. Food Sci. Technol. 42 (2007) 281–295.
[47] J. Weber, A. Karczewska, J. Drozd, M. Licznar, S. Licznar, E. Jamroz, A. Kocowicz, Agricultural and ecological aspects of a sandy soil affected by the application of municipal solid waste composts, Soil Biol. Biochem. 39 (2007) 1294–1302.
[48] G.D. Zheng, D. Gao, T.B. Chen, W. Luo, Stabilisation of nickel and chromium in sewage sludge during aerobic composting, J. Hazard. Mater. 142 (2007) 216–221.
[49] Q.Y. Cai, C.H. Mo, Q.T. Wu, Q.Y. Zeng, A. Katsoyiannis, Concentration and speciation of heavy metals in six different sewage sludge-composts, J. Hazard. Mater. 147 (2007) 1063–1072.
[50] G. Gigliotti, P.L. Guisquiani, D. Businelli, A long chain and infrared spectroscopy study on a soil amended with municipal sludge, Agronomie 21 (2001) 169–178.
[51] E. Smidt, K. Meissl, The applicability of Fourier transform infrared (FT-IR) spectroscopy in waste management, Waste Manage. 27 (2007) 268–276.
[52] M. Fuentes, R. Baigorri, G. González-Gaitano, J.M. García-Mina, The complementary use of ^{1}H NMR, ^{13}C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin, Org. Geochem. 38 (2007) 2012–2023.
[53] G. Ait Baddi, J.A. Alburquerque, J. Gonzalvez, J. Cegarra, M. Hafidi, Chemical and spectroscopic analyses of organic matter transformations during composting of olive mill wastes, Int. Biodeter. Biodegrad. 54 (2004) 39–44.
[54] B. Zenjari, H. El Hajjouji, G. Ait Baddi, J.R. Bailly, J.C. Revel, A. Nejmeddine, M. Hafidi, Eliminating toxic compounds by composting olive mill wastewater–straw mixtures, J. Hazard. Mater. 138 (2006) 433–437.