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Eliminating toxic compounds by composting olive mill wastewater–straw mixtures

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Abstract

The present work studies the changes occurring in organic matter, phenols and biotoxicity on composting olive mill wastewater with barley straw. The total organic matter decreased, a drop of 25% was reached after the stabilization phase and 52% at the end of the maturation phase. Degradation of the phenols reached 54% and 95%, respectively, after these periods. The toxicity of the water extract, evaluated by the *Photobacterium phosphoreum* fluorescence, decreased to disappear after only 2 months of composting. This trend was confirmed by the tight correlation between the physico-chemical and toxicity parameters, indicating that the degradation of organic matter leads to a strong reduction of the C/N ratio and of toxicity. The results obtained indicate a normal process of humification occurring during the composting of the formerly highly toxic olive mill wastewater–straw mixture and resulting in a product, which has good agricultural properties as organic fertilizer.

Keywords: Olive mill wastewater; Composting; Polyphenols; *Photobacterium phosphoreum*; Organic fertilizer

1. Introduction

When using either the press system or three-phase centrifugation, the olive oil processing industry has two by-products. The first is a solid resulting from the squeezed residue termed "pomace or husk". The other is a liquid effluent called "olive mill wastewater". It is made up of the water contained in the fruit plus that involved in the different steps of oil extraction. The estimated volume of wastewater generated in the Mediterranean olive oil producing countries is about 30 million m^3/year [1–3]. Despite current legislation, this wastewater is frequently discharged into the environment without prior treatment leading to serious problems of pollution. Olive water is in fact both pollutant and toxic due to its high content of organic matter and of noxious phenol compounds, respectively [4,5].

Several treatment systems based on lagooning and other biological processes have been examined to reduce the pollution caused by this type of wastewater. However, few techniques have been applied on an industrial scale due to the high cost of the treatment plant [6,7].

At the same time, recycling studies have been performed to investigate the utilization potential of the mineral constituents, plant nutrients, and the organic components of the wastewater.

In Morocco, management of the olive mill wastewater produced by factories poses problems to both producers and the authorities because of the absence of detoxification systems, the wastewater often being illegally spread on the soil, poured directly into the sewage system or evaporated in lagoons to reduce its volume [8]. This leads to various environmental problems, for instance the overflow of lagoons as the oil-pressing season coincides with the annual wet season (December to March).

A practical approach is to compost this liquid effluent, which reduces the volume and generates a recyclable prod-

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uct, but so far this has provoked little scientific investigation [9–13].

The present work was carried out to test the feasibility of including olive mill wastewater in a composting process and to evaluate the efficiency of the treatment in degrading the phenolic components, thereby reducing the toxicity of the effluent. The olive mill wastewater was mixed with a cellulose-rich waste product and the resulting damp organic matter composted. The changes in the phenolics content and the resulting toxicity were monitored.

2. Materials and methods

2.1. Composting trial

Olive mill wastewater (Table 1) was taken from the lagoons of a semi-industrial olive oil plant in the Marrakech region (Morocco).

Dried, coarsely shredded barley straw (Table 1) was soaked for a few hours in the olive mill wastewater to reach saturation. One kilogram of barley straw absorbed 1174 ml of wastewater containing 229 g of dry residue. The proportions were determined from various previous works as reported in the literature [14,15].

Nitrogen and water supplements were added as $(NH_4)_2SO_4$ solution to bring the C/N ratio near to 30 and humidity to between 50% and 60%: optimal values for microbial development. The actual values obtained were C/N ratio: 28.6, and humidity: 53%.

An inoculum of mature compost was incorporated into the crude mixture (8.33 g/kg).

The final mixture was then introduced (1.2 kg/load) into a PVC reactor of 2.7 l capacity (length 35 cm, diameter 10 cm) for composting under aerobic conditions [16]. Within the first period (reactor incubation period) the measurement of CO_2 release and temperature revealed an initially strong increase in biological activity followed by a decrease. The product obtained was then stored for 3 months at ambient temperature in perforated plastic bags and shaken each week for 3 months.

Sampling periods for monitoring the evolution of the mixture over time were at T0 = initial stage, before filling the reactor; at T1 = end of the reactor incubation period, and at T2 = after 3 months of "bag incubation period".

Table 1
Physico-chemical composition of the olive mill wastewater and straw before composting

	Organic matter (fresh weight)	Straw
COD (g/l)	180.00	–
pH	4.68	7.30
EC (ms/cm)	41.00	–
Total polyphenols (mg/l)	5500.00	458.00
Pt (mg/l)	346.00	–
TKN (mg/l)	2100.00	–
Ca^{++} (mg/l)	560.00	–
Mg^{++} (mg/l)	280.00	–
Na^{+} (mg/l)	4430.00	–
K^{+} (mg/l)	3930.00	–
TOC (%)	–	46.60
TKN (mg/g DW)	–	4.90
C/N	–	95.50

COD: chemical oxygen demand; EC: electrical conductivity; TKN: total Kjeldahl nitrogen; TOC: total organic carbon; C/N: carbon/nitrogen ratio; Pt: total phosphorus; DW: dry weight.

2.2. Analytical methods

pH was measured on a mixture of the fresh matter and water (1/10, w/v) [2].

Total organic carbon (TOC) was determined using Anne's method [17]. Organic carbon was oxidized by potassium dichromate ($K_2Cr_2O_7$) in the presence of the sulfuric acid. The excess dichromate was titrated against a solution of iron sulphate ($FeSO_4$). The organic matter loss was calculated from the Ci (percentage ash content of the initial mixture) and Cf (percentage of ash in the product) at the time of sampling according to the following equation [2]:

$$\text{Percentage decomposition (\%)} = [100\,(Cf - Ci)/(100 - Ci)Cf] \times 100$$

The polyphenols were extracted using the method of Macheix et al. [18]. The extraction was carried out with methanol (80%) in the presence of phosphoric acid and ammonium sulphate. The polyphenols were assayed using Folin-Ciocalteu reagent [19]. The purified extracts were analyzed qualitatively by high performance liquid chromatography (HPLC) using a WATERS 600 system fitted with a WATERS 990 diode array detector.

Sample toxicity was determined from the reduction of luminescence in the marine bacterium *Photobacterium phosphoreum* based on the method described by Monteoliva Sanchez et al. [1]. Ten-gram samples were put into suspension in 100 ml of distilled water then filtered through Whatman filter paper. Dilutions ranging from 0.1% to 0.9% were then prepared from this filtrate in physiological saline containing 9‰ NaCl. Toxicity was determined using a LUMISTOX apparatus [20], by determination of the concentration giving 50% reduction in the luminescence of *Photobacterium phosphoreum* (EC 50%) within 15 min at 15 °C. The results, expressed in toxicity units TU (TU = 100/EC 50%), were then subjected to statistical analysis using the LUMISSOFT program [20].

A correlation test was performed by SPSS 11.5 for Windows between the different variables monitored (pH, C/N, decomposition rate, TU).

3. Results and discussion

3.1. Decomposition of the olive mill wastewater–straw mixture during composting

Table 1 shows the physico-chemical composition of the materials to be mixed and Table 2 shows the changes occurring in the physico-chemical characteristics of the olive mill wastewater–straw mixture during composting. The percentage loss rose from 25% at the end of the reactor incubation period

Table 2
Physico-chemical parameters of the olive mill wastewater–straw mixture at the three test times

	Before composting (T0)	End of "reactor incubation period" (T1)	Three months of "bag incubation period" (T2)
pH	5.50 ± 0.12	5.83 ± 0.31	7.08 ± 0.04
TOC (%)	63.16 ± 2.68	42.44 ± 3.20	27.74 ± 2.81
TKN (%)	2.21 ± 1.43	2.75 ± 1.20	3.31 ± 1.48
C/N	28.58 ± 1.23	15.40 ± 0.47	8.39 ± 0.61
Reduction (%)	–	25.36 ± 1.24	52.07 ± 1.80

to 52% after the 3 months "bag incubation period". Significant and quick decomposition of the organic matter occurred during the first period, owing to the controlled conditions in the reactor. Decomposition continued during maturation in the bags [5]. At the same time, the initial C/N ratio of 28.6 fell to 15.4 and then to 8.4. Decomposition of the organic matter therefore released a considerable amount of CO_2 and possibly small quantities of other volatile carbon compounds [21].

The initial acid pH of 5.5 is due to the constituents of the wastewater. However, the changes in pH during composting corresponded to the metabolism of organic acids and to ammonia production during the hydrolysis of the organic and especially protein nitrogen by the bacteria present [22]. The final pH of 7.08 indicates a classic evolution of compost under aerobic conditions. Moreover, this value also suggests the formation of humic substances, which act as pH buffers [23].

3.2. Evolution of polyphenols during composting

The way the levels of total polyphenol compounds varied during composting are presented in Fig. 1. The total polyphenol content fell from 1172 μg/g fresh weight (FW) in the initial mixture to 68 μg/g FW at T2, representing a drop of more than 94%. This result is in accordance with earlier data of Zenjari et al [24], who demonstrated a very clear correlation between organic matter degradation and the drop in the level of polyphenols during the aerobic treatment of olive mill wastewater by soil microorganisms. The same result was reported by Ait Baddi et al. [25], during the composting of olive mill waste–straw mixtures.

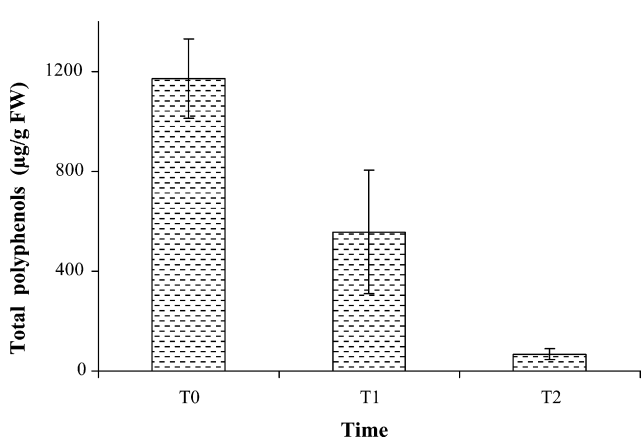

Fig. 1. Total phenol content of the olive mill wastewater/straw mixture at the different phases of composting. FW, fresh weight; T0, initial time; T1, after the "reactor incubation period"; T2, at the end of "bag incubation period".

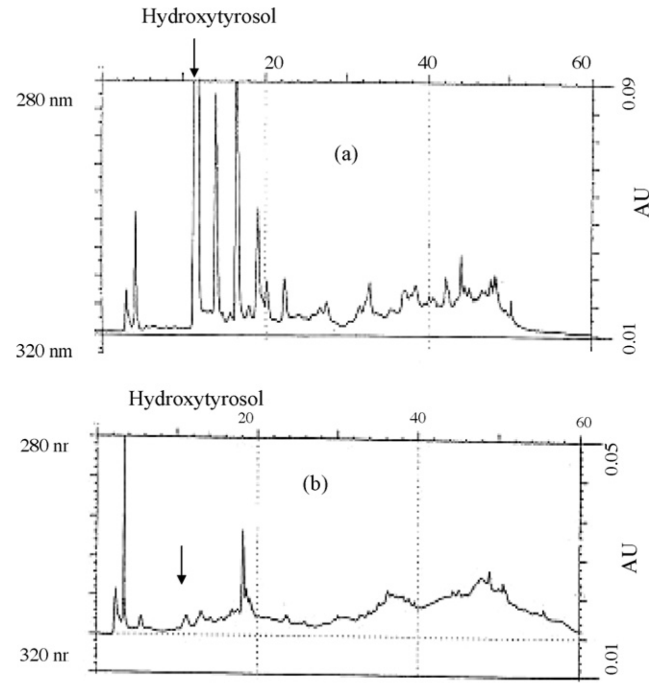

Fig. 2. HPLC chromatograms of polyphenols extracted from the olive mill wastewater–straw mixture at T0 (a) and after 3 months of "bag incubation" T2 (b).

It may be noticed that the final content of 68 μg polyphenols/g is less than the 458 μg/g dry weight (DW) of the straw alone indicating that the polyphenolic components of the straw were also decomposed. In this respect, Ait Baddi et al. [25], highlighted the role of the straw in the adsorption of some phenolic compounds which are reduced during the composting of olive mill waste–straw mixtures.

This very marked reduction was confirmed in the analysis of the simple polyphenols by HPLC (Fig. 2). The polyphenol extract of the mixture of olive mill wastewater and straw at T0 contained six major polyphenol compounds, one of them identified as 2(3,4-dihydroxyphenyl)ethyl alcohol, commonly known as hydroxytyrosol, and a well-known component of olive mill wastewater. After 3 months of maturation, no traces of these compounds were detectable.

This could be attributed to the oxidation of polyphenolic compounds with secondary metabolites to form humic substances

Table 3
Toxicity of olive mill wastewater–straw mixture on *Photobacterium phosphoreum* during different phases of composting

Composting time (days)	Concentration (EC 50%)	Toxicity units (TU)
0	0.396	255.52
15 (end of "reactor incubation period)	0.490	204.08
45 (1 month of "bag incubation period")	0.904	110.61
75 (2 months of "bag incubation period")	[a]	[a]
105 (3 months of bag incubation period")	[a]	[a]

[a] Toxicity not detected.

Table 4
Correlation between the various physical–chemical and toxicity parameters as a function of the composting time

	Time	pH	TOC (%)	TKN (%)	C/N	DEC	PPH	TU
Proximity matrix								
Time	1.000							
pH	0.948	1.000						
TOC (%)	−0.995	−0.912	1.000					
TKN (%)	1.000	0.947	−0.995	1.000				
C/N	−0.985	−0.878	0.997	−0.985	1.000			
DEC	1.000	0.953	−0.994	1.000	−0.982	1.000		
PPH	−0.998	−0.925	0.999	−0.998	0.994	−0.997	1.000	
TU	−0.945	−1.000	0.909	−0.945	0.874	−0.950	0.922	1.000

TKN: total Kjeldahl nitrogen; TOC: total organic carbon; C/N: carbon/nitrogen ratio; DEC: decomposition rate; PPH: polyphenols; TU: toxicity units.

[21]. This point supports the hypothesis of the contribution of polyphenols to the synthesis of humic substances as reported by Ait Baddi [7] and Sanchez Monedero et al. [26].

3.3. *Toxicity decrease of the olive mill wastewater–straw mixture*

The changes occurring in the toxicity of the olive mill wastewater–straw mixture during composting are presented in Table 3.

The toxicity of the extract fell by 20% during the stabilization phase, from 255 to 204 toxicity units (TU), and could no longer be detected after 2 months of maturation. This is in accordance with the results of Monteoliva Sanchez et al. [1], who reported the disappearance of toxicity within 49 days, after the composting of olive mill wastewater with cotton waste.

A close relationship exists between the polyphenol content of the wastewater–straw mixture and the toxicity of the extract of the same mixture (Fig. 3), the correlation coefficient between the two series of values attaining 99.7%. This result is consistent with that of Borja et al. [27] who demonstrated a direct relationship between the polyphenol content and toxicity of olive mill wastewater.

The results of the correlation test (Table 4) revealed the strong correlation between pH, total organic carbon (TOC), C/N ratio, decomposition rate, polyphenol content and toxic units as a function of time. Indeed, the variation of TOC, C/N ratio, polyphenol content and toxicity units with time presents a clear negative correlation ($p = -0.995$, -0.985, -0.998 and -0.945, respectively). Likewise, there is also a negative correlation as a function of time between pH, C/N ratio, TOC, polyphenol content and the TU ($p = -0.878$, -0.912, -0.925, -1.000, respectively). Similarly, a negative correlation was established for the TOC with the total Kjeldahl nitrogen (TKN) and the decomposition rate ($p = -0.995$ and -0.945, respectively) and also for TKN with the C/N ratio, polyphenol content and TU ($p = -0.985$, -0.998, -0.945). Furthermore, the C/N ratio was negatively correlated to the decomposition rate ($p = -0.982$), the latter parameter being negatively correlated to polyphenols and TU ($p = -0.997$ and -0.950, respectively). Above all, these results confirm that the degradation of organic matter leads to a strong reduction of the C/N ratio and of toxicity during composting in our experimental conditions.

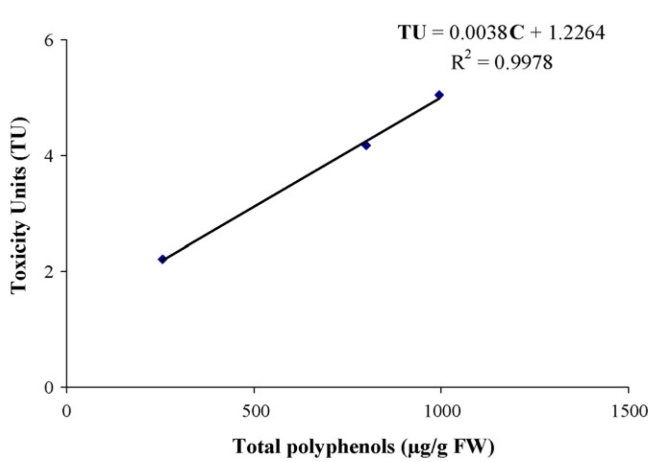

Fig. 3. Correlation between total phenol concentration and toxicity of the aqueous extract of the olive mill wastewater/straw mixture. FW, fresh weight.

4. Conclusion

Composting of straw saturated with olive mill wastewater leads to the production of a compost with satisfactory analytical qualities. The lack of toxicity of the final product was judged by criteria such as polyphenolic content and bacterial toxicity. The evolution of the physico-chemical and toxicity parameters supports the occurrence of polymerisation mechanisms similar to those occurring during the humification process.

Using such a process to eliminate the pollution resulting from olive mill wastewater is then feasible, yielding a good product suitable for use in agriculture.

In addition, the close correlation between the disappearance of the phenolic compounds and toxicity, in accordance with the literature, strongly suggests that these polyphenols are involved in the initial toxic effect. Furthermore, our results confirmed the hypothesis of the contribution of polyphenols to the synthesis of humic substances.

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