



COMPOSTAJE DE RESIDUOS DE ALMAZARA

ELABORACIÓN Y USO AGRÍCOLA DE ABONOS OBTENIDOS A PARTIR DE RESIDUOS DE ALMAZARA

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1. Introducción.

La producción de residuos y subproductos de la industria extractiva del aceite de oliva en España en la campaña 1997/98 puede estimarse en mas de 3 millones de Tm. , lo que si se compara con la producción anual de basuras de origen doméstico de toda la población española, se aproximaría al contenido de materia orgánica, directamente aprovechable como abono orgánico del suelo, existente en el 50 % de esa producción total de basuras domésticas.

Como subproducto más importante dentro de ésta enorme cantidad de biomasa residual, es ciertamente el alpeorujo el residuo de la industria oleícola del olivar cuya problemática atrae la mayor atención , ya que se genera por el sistema de centrifugación en dos fases, tecnología que comenzó a introducirse en la campaña de 1992-93 con un ritmo creciente y con la que se alcanza ya a procesar el 80 % de toda la producción olivarera de España. La ventaja principal de este sistema sobre el de tres fases (aceite, orujo y alpechín) radica sobre todo en la importante economía de agua que conlleva, si bien la excesiva humedad del alpeorujo obliga a secarlo como paso previo a la extracción con hexano de su notable contenido de aceite residual. Los costes asociados al secado y la caramelización de los azúcares que dificulta la extracción química son dos claras dificultades que están conduciendo al rechazo creciente por parte de las orujeras de este subproducto. Si a lo anterior se añade la práctica cada vez mas habitual en numerosas almazaras de someter el alpeorujo a una última centrifugación (proceso de repasado) para la extracción de casi un 50 % de su aceite residual, no es aventurado afirmar que este subproducto, a mayor parte deshuesado, se está transformando rápidamente en un residuo incómodo de manejar y costoso de almacenar al que urge encontrar métodos alternativos de tratamiento que, como mínimo, limiten su impacto ambiental.

A parte de la cogeneración de energía eléctrica, a la que se dedica una relativamente pequeña proporción de la importante producción nacional de alpeorujo, poco es lo que todavía se ha investigado sobre otras alternativas de utilización y tratamiento entre las cuales cabe sugerir el compostaje como método para su transformación en abonos orgánicos. En la presente comunicación se apunta tal solución para el problema del alpeorujo, considerando su contenido de nutrientes y otras características químicas y físicas del mismo y tomando en consideración los resultados obtenidos por el autor en anteriores experiencias de compostaje de alpechín y en ensayos agrícolas dirigidos a evaluar la potencialidad de éste método para el tratamiento y aprovechamiento de este residuo líquido de elevado poder contaminante (Parcés y col., 1996 ; Cegarra y col., 1996).

2. Características del alpeorujo.

Los resultados del análisis de alpeorujo realizados sobre 21 muestras de este subproducto procedentes de las provincias de Jaén, Córdoba, Granada y Murcia, tomadas directamente sin transformar en almazaras diferentes y también en orujeras después del repasado y deshuesado del mismo, permite afirmar que este material de naturaleza pastosa y muy húmedo (en torno al 64% de

humedad) posee un contenido de materia orgánica sobre materia seca (s.m.s) muy elevado (hasta el 95%) y también de carbono orgánico (52% como valor medio). Su contenido de nitrógeno no es demasiado alto (1,4 % de media s.m.s.), aunque se han encontrado muestras de alpeorujo con una riqueza en nitrógeno total de hasta el 2,3% y lo mismo puede decirse de los niveles de potasio (1,5 % de media y un intervalo de variación comprendido entre 0,7 y 2,8 %) y más aún para los de fósforo total que nunca superaron el 0,2 %. Entre los micronutrientes se encontró como más abundante el hierro (875 y 1706 ppm, respectivamente, como valores medio y máximo), y en mucha menos concentración cobre, manganeso y zinc que mostraron valores medios de 22, 22 y 30 ppm, respectivamente.

Otros parámetros también determinados fueron el contenido de lignina (45,3 % de media); el de grasas (valor medio 12,7% s.m.s.) pero con valores muy variables dependientes de si las muestras de alpeorujo habían sido o no repasadas para extraer aceite residual o si habían o no sido deshuesadas (intervalo de variación comprendido entre 19,8 y 7,2%); el pH, con un valor medio estimado en 5,5 y un intervalo de variación comprendido entre 4,7 y 6,5 y finalmente, la conductividad eléctrica cuyo valor medio fue 3,86 mS/cm y que mostró un coeficiente de variabilidad notablemente mayor que el pH.

3. Compostaje del alpeorujo.

Este material puede considerarse, en principio, apropiado para la elaboración de abonos orgánicos mediante compostaje ya que, como se ha indicado, posee un aceptable contenido de nutrientes, es muy rico en materia orgánica, tiene un pH moderadamente ácido, posee un contenido salino relativamente bajo y muestra valores de la relación C/N ni demasiado bajos ni tampoco altos (intervalo comprendido entre 22,8 y 51,7), con un valor medio en torno a 38.

Sin embargo esta notable variabilidad de su relación C/N, junto con el hecho de tratarse de un material con un gran contenido de agua, muy triturado y, por tanto de pequeño tamaño de partícula, determina la necesidad de mezclarlo para su compostaje con otros materiales residuales con valores de C/N diferentes y mayor tamaño de partícula, de tal modo que pueda equilibrarse dicha relación, en torno a 30 por ejemplo, a la vez que facilitar la aireación durante el proceso aeróbico de transformación.

Las primeras experiencias desarrolladas en la planta-piloto de compostaje del CEBAS, mediante el sistema Rutgers utilizando como componente complementario de la mezcla con alpeorujo un residuo muy común procedente de plantas desmotadoras de algodón, han permitido obtener resultados muy prometedores. Así, la mezcla inicial que mostró un valor de C/N en torno a 28, se mineralizó moderadamente a través del proceso de compostaje obteniéndose un valor final de ésta relación del orden de 18 en el compost maduro. Esta evolución fue consecuencia de la degradación de la materia orgánica, habiéndose apreciado una caída del porcentaje de carbono desde 51 al principio del proceso hasta 45 en el producto maduro y un escaso incremento del nitrógeno desde 1,9 hasta 2,5 %. Como un efecto del compostaje, también se observó una importante elevación del pH desde 6,3 hasta 9,4, una casi constancia de los valores de conductividad eléctrica que a penas varió de 4 mS/cm y una clara disminución del contenido graso, cuyo valor inicial de 8 % decreció hasta el 1,3% al final del proceso.

4. Características del compost de alpeorujo.

El compost obtenido tras una fase biooxidativa con ventilación forzada de 12 semanas, se dejó madurar durante 3 meses, tras lo cual se molió y homogeneizó, procediendo después a su análisis. El producto maduro mostró un elevado porcentaje de materia orgánica (82 %) y contenidos notables de nitrógeno y potasio (2,5 y 2,7 %, respectivamente), pero sólo se encontraron pequeñas concentraciones de nitrógeno en forma amónica y nítrica (152 y 23 ppm, respectivamente).

El valor de pH fue francamente elevado pero no, en cambio, el de conductividad eléctrica, mientras que su índice de germinación (57 %) debe considerarse en el límite de lo permitido para su empleo como abono orgánico. El extracto húmico total, medido como carbono extraíble en medio alcalino, fue del orden del 14 % y la relación entre los contenidos de ácidos húmicos y fúlvicos (AH/AF) estuvo en torno a 2. Por último, su capacidad de cambio catiónico alcanzó los 123 me/100 g.

La consideración global de estas características permite definir este primer compost obtenido a partir de alpeorujo, como un material susceptible de ser utilizado como abono orgánico, con algunas cautelas derivadas del moderado valor encontrado para su índice de germinación, el cual aun dentro de la normalidad no fue demasiado elevado. Esto último podría relacionarse con una posible insuficiente degradación de los polifenoles existentes en el alpeorujo a lo largo del proceso de compostaje.

En la actualidad, se está diseñando una experiencia que implicará ensayar este compost como abono para la producción de plantas hortícolas, para lo que se comparará su eficacia con la del abonado químico convencional y con la efectividad de un estiércol elaborado industrialmente y que se comercializa con notable aceptación en amplios sectores agrícolas del Levante español.

Los resultados del ensayo agrícola junto con el estudio del conjunto de todos los datos obtenidos en la experiencia de compostaje, deberán servir de base para desarrollar nuevas experiencias en la planta-piloto que permitan optimizar la metodología mas adecuada para transformar el alpeorujo, vía compostaje, en abonos orgánicos de calidad, adaptados a las necesidades de la demanda comercial de los sectores mas dinámicos de la producción agraria.

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Agrochemical characterisation of “alperujo”, a solid by-product of the two-phase centrifugation method for olive oil extraction

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Abstract

Introduction of the two-phase centrifugation system for olive oil extraction during the early nineties in Spain has led to the generation of approximately four million tons per year of a solid olive-mill by-product called “alperujo” (AL). Agrochemical characterisation showed that AL has a high moisture content, slightly acidic pH values and a very high content of organic matter, mainly composed by lignin, hemicellulose and cellulose. It also has a considerable proportion of fats, proteins, water-soluble carbohydrates and a small but active fraction of hydrosoluble phenolic substances. Amongst plant nutrients, AL is rich in potassium, less so in (mainly organic) nitrogen and poor in phosphorus and micronutrients. Some of those properties are not compatible with agricultural requirements, for which reason composting may be considered a suitable alternative for its disposal. AL needs to be characterised before composting, and so an attempt was made to correlate the easily determined ash content with other parameters; the regression models thus obtained are discussed.

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1. Introduction

Most of the world's production of olive oil, 2,500,000 tons per year (Food and Agriculture Organisation, 2003), is based in the countries of southern Europe, the Near East and North Africa, where olive (*Olea europaea* L.) cultivation is a centuries old tradition, especially for oil extraction. Spain is the greatest producer of olive oil (a mean production of 990,400 tons per year for the 1997–2002 period, Spanish Agency for Olive Oil database, 2003), and production is likely to continue increasing because of the substantial increment recorded in olive tree cultivation. The olive fruit consists of pulp (70–90%), stone (9–27%) and seed (2–3%) on a total weight basis (Hermoso et al., 1998), and the two main constituents (water and oil) are mainly concentrated in the pulp and seed.

The technology for olive oil extraction in Spain has progressed significantly since the beginning of the sev-

enties, when the three-phase centrifugation system appeared. By means of this system, the oil, vegetation water and solid phase of the olive can be separated in a continuous process, with the result that the traditional discontinuous press system is almost non-existent at present. The main inconvenience of the three-phase system is the generation during a short period of the year (November–February) of large quantities of olive-mill wastewater (OMW), which is a very polluting liquid made of the olive vegetation water plus the water added in the different steps of oil production. In an attempt to lessen its environmental impact, different methods have been tried to make the best use of OMW; these include storage in evaporation ponds and its direct application to agricultural soils as fertilizers. However, these methods have gradually become less viable for OMW disposal, and so a new two-phase centrifugation system for oil extraction was developed during the early nineties. Although this is called the ecological system because it greatly reduces wastewater generation and its contaminant load, it still produces a solid and very humid by-product called “alperujo” or “alpeorujo” (AL). The production of olive oil in Spain with this new

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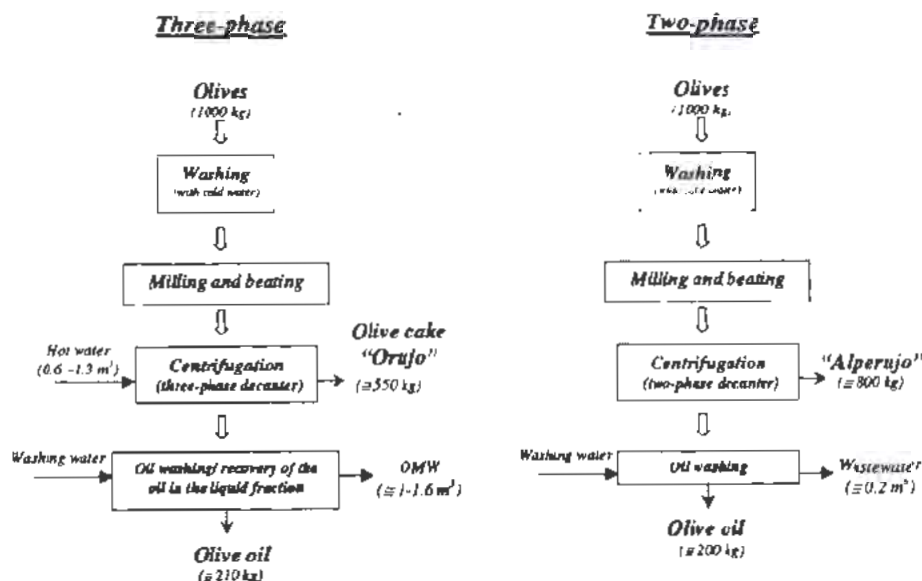


Fig. 1. Comparison of the three and two-phase centrifugation systems for olive oil extraction.

centrifugation system, which saves both water and energy compared with the three-phase system, is estimated to represent about 75% of the total and the system is used roughly by 90% of olive-mills. As can be seen in Fig. 1, which schematically compares the three and two-phase centrifugation systems, approximately 800 kg of AL per ton of processed olives result from using the two-phase system, meaning that the yearly production of this by-product from the whole Spanish olive oil industry may approach four million tons.

AL is usually treated with a second centrifugation to extract the residual oil. The resulting by-product of this second extraction is dried, then subjected to chemical extraction with hexane in order to produce an extra yield of oil. Nevertheless, the recently discovered problems concerning the detection of PAHs in this oil as a result of drying the AL before oil chemical extraction has obliged manufacturers to perform a further purification step, which greatly increases production costs. Finally, the new resulting waste of the chemical extraction ("orujillo") can be used for the co-generation of electrical power, although this method needs to be subsidised by public authorities.

The problem of AL disposal, then, has not been fully resolved and research into new technological procedures that permit its profitable use is needed. One possibility is to use composting as a method for the preparation of soil organic fertilizers and amendments, since the direct application of AL to the soil has been seen to have a detrimental effect on the soil structural stability (Tejada et al., 1997). It may also negatively affect seed germination, plant growth and microbial activity. In fact several studies have reported the phytotoxic and antimicrobial effects of both olive-mill wastes

and by-products due to the phenol, organic and fatty acid contents (González et al., 1990; Riffaldi et al., 1993; Linares et al., 2001).

In this study the composition of AL has been determined by analysing samples from different mills in southern and eastern Spain and an attempt has been made to correlate its characteristics in order to ascertain whether the composition of a given sample can be estimated by reference to one or more easily measured analytical parameters.

2. Methods

2.1. Sampling of AL

In order to characterise the AL, twenty samples were taken from eleven olive-mills located in different provinces of Spain (Albacete, Córdoba, Cuenca, Granada, Jaén, Málaga, Murcia and Tarragona) during the three successive olive campaigns included in the 1997–2000 period. Fresh samples were collected and kept in a cool environment during the journey to the laboratory, where they were subdivided into two sub-samples. One of them was dried in an oven at 105 °C for 24 h to determine its moisture content, while the other sub-sample was frozen at –20 °C, and later freeze-dried and ground to less than 0.5 mm before performing analyses.

2.2. Analytical methods

The following parameters were determined in the materials: electrical conductivity (EC) and pH in a water extract 1:10 (w/v), the volatile solids, which reflect the

organic matter content (OM), by loss on ignition at 430 °C for 24 h, and total nitrogen (TN) and total organic carbon (TOC) by automatic microanalysis (Navarro et al., 1991). After $\text{HNO}_3/\text{HClO}_4$ digestion, P was measured colorimetrically as molybdovanadate phosphoric acid (Kitson and Mellon, 1944), K and Na by flame photometry and Ca, Mg, Fe, Cu, Mn and Zn by atomic absorption spectrophotometry. The total fat content was determined using the traditional method of extraction in a Soxhlet with diethyl ether and later weighing. Other parameters determined in water extracts (1:20, w/v) were: water-soluble phenolic substances by a modified version of the Folin method for chemical quantification (Maestro Durán et al., 1991) and water-soluble carbohydrates by the anthrone method (Brink et al., 1959). Lignin and cellulose were determined according to the American National Standards Institute and American Society for Testing and Materials (1977a,b) and holocellulose according to Browning (1967). Hemicellulose concentration was calculated as the difference between holocellulose and cellulose. The protein content was calculated by multiplying TN by the

conversion factor of 6.25. All the analyses were made in triplicate.

2.3. Statistical analyses

Basic statistical analyses of data, correlation coefficients and regression models were made using the SPSS 11.0 program for Windows.

3. Results and discussion

3.1. Characteristics of the AL

As shown in Tables 1 and 2, the sampled AL had a moisture content higher than 56% and together with its small particle size, this made it a scarcely porous material, plastic and susceptible to compaction. These characteristics hinder and make expensive its transport and handling and may be an obstacle for its correct aeration as a composting substrate, because such a process must be carried out in favourable conditions (appropriate

Table 1
Main characteristics of the "alperujo" samples (dry weight)

Parameters	Mean	Range	CV (%)
Moisture (% fresh weight)	64.0	55.6–74.5	7.6
pH ^a	5.32	4.86–6.45	6.6
EC ^a (dS m ⁻¹)	3.42	0.88–4.76	33.9
Ash (g kg ⁻¹)	67.4	24.0–151.1	42.5
TOC (g kg ⁻¹)	519.8	495.0–539.2	2.8
C/N ratio	47.8	28.2–72.9	22.1
TN (g kg ⁻¹)	11.4	7.0–18.4	24.5
P (g kg ⁻¹)	1.2	0.7–2.2	29.7
K (g kg ⁻¹)	19.8	7.7–29.7	34.2
Ca (g kg ⁻¹)	4.5	1.7–9.2	57.3
Mg (g kg ⁻¹)	1.7	0.7–3.8	58.7
Na (g kg ⁻¹)	0.8	0.5–1.6	36.6
Fe (mg kg ⁻¹)	614	78–1462	74.9
Cu (mg kg ⁻¹)	17	12–29	28.8
Mn (mg kg ⁻¹)	16	5–39	70.2
Zn (mg kg ⁻¹)	21	10–37	36.3

CV: coefficient of variation.

^a water extract 1:10.

Table 2
Main components of the organic fraction of the "alperujo" samples (g kg⁻¹ dry weight)

Parameters	Mean	Range	CV (%)
Total organic matter	932.6	848.9–976.0	3.1
Lignin	426.3 [45.8]	323.0–556.5	16.0
Hemicellulose	350.8 [37.7]	273.0–415.8	12.7
Cellulose	193.6 [20.8]	140.2–249.0	14.8
Fats	121.0 [13.0]	77.5–194.6	28.9
Protein	71.5 [7.7]	43.8–115.0	24.5
Water-soluble carbohydrates	95.8 [10.1]	12.9–164.0	50.0
Water-soluble phenols	14.2 [1.5]	6.2–23.9	41.0

CV: coefficient of variation

Figures between brackets represent percentages with respect to the total organic matter content

humidity, nutrient balance, structure and air distribution) to obtain a useful final product. The pH values were slightly acidic (mean value 5.32), showing a small coefficient of variation (6.6%), as did moisture, while those of EC had a greater coefficient of variation (33.9%) with a mean value of 3.42 dS m^{-1} .

As regards the plant nutrient content, AL was specially rich in K (mean value of 19.8 g kg^{-1}), which is a common characteristic in olive-mill wastes and by-products. However, AL samples were poor in P, Ca and Mg compared with municipal solid wastes (MSW) and sewage sludges (SS) although similar to other vegetable wastes and manures in this respect (Cegarra et al., 1993; Pascual et al., 1997). The TN content, substantially organic and relatively low compared with the value in SS and manures, was in the range of 7.0 – 18.4 g kg^{-1} , with a mean value (11.4 g kg^{-1}) between those of OMW and OMW sludge as reported by Paredes et al. (1999). The main micronutrient in AL was Fe, with a mean value of 614 mg kg^{-1} , while Cu, Mn and Zn showed similar mean contents, but very much lower than Fe. The levels of Fe and Mn varied greatly, and these two elements showed the highest coefficients of variation. Generally, the concentrations of these nutrients were low compared with those of other materials either employed as fertilizers or as soil amendments (manures, MSW and SS), as also was the Na content (0.8 g kg^{-1} mean value).

With a low coefficient of variation, both the OM and TOC contents were much higher (932.6 and 519.8 g kg^{-1} mean values, respectively) than in OMW, OMW sludge and the other organic wastes currently used for soil amendment. The main organic constituents of the AL samples were lignin, hemicellulose and cellulose, which roughly accounted for 46%, 38% and 21%, respectively, of the total OM. Such a high lignin content and the degree of binding of this component to the other organic constituents in lignocellulosic materials, as reported by Lynch (1993), may hinder the ability of microorganisms

and their enzymes to degrade the AL, if used as a composting substrate.

Other important OM components were fats (about 13% of the total OM), hydrosoluble carbohydrates (10%) and proteins (nearly 8%). The first two may act as readily available substrate for the initial microbial growth if AL were composted, while the last would be scarcely available because of its association with the lignocellulosic fraction (Valiente et al., 1995; Molina Alcaide et al., 2001). The availability of nitrogen is a critical factor for composting; the AL samples showed relatively high TOC/TN (C/N ratio) values, which ranged between 28.2 and 72.9; hence they may be often greater than those recommended for the efficiency of composting (25–35). Lastly, the samples analysed showed a small but active fraction of water-soluble phenolic substances (about 1.5% of the total OM), which together with the lipidic fraction, has been often related with the phytotoxic and antimicrobial effects usually attributed to olive-mill wastes and by-products (González et al., 1990; Linares et al., 2001). Moreover, even if the direct application of these materials to land is an inexpensive way for disposal and recovery of their mineral and organic contents as fertilizers, the addition of AL into soil can be a source of pollution and unfavourable environment impact. This would be associated with the acidic pH, inhibition of seed germination and plant growth, antimicrobial properties and frequent unbalanced C/N ratio. These properties are not compatible with agricultural requirements, for which reason composting can be considered a good alternative for AL disposal.

3.2. Relationships between the main analytical parameters of the AL samples

A correlation matrix between the main AL analytical parameters was made in order to evaluate their degree of association (Table 3). The variation coefficients

Table 3
Correlation matrix between some parameters used for AL characterisation (dry weight)

Parameters	Ash	TN	C/N ratio	K	P	Ca	Mg	Fe	Cu	Mn	Zn
Ash	1										
TN	0.817***	1									
C/N ratio	-0.739***	0.950***	1								
K	NS	NS	NS	1							
P	0.659**	0.580**	-0.533*	-0.520*	1						
Ca	0.616*	0.676**	-0.710**	-0.579*	0.662**	1					
Mg	0.677**	0.603*	-0.631*	-0.588**	0.863***	0.850***	1				
Fe	0.444*	NS	NS	-0.651**	0.476*	0.742**	0.832***	1			
Cu	0.653**	0.447*	-0.446*	-0.547*	0.769***	0.778**	0.814***	0.621**	1		
Mn	0.495*	NS	NS	-0.628**	0.576**	0.783**	0.842***	0.925***	0.720***	1	
Zn	0.675**	0.717***	-0.747***	-0.580**	0.707***	0.836***	0.835***	0.636**	0.740***	0.664**	1

NS, not significant.

*Significant at $p < 0.05$.

**Significant at $p < 0.01$.

***Significant at $p < 0.001$.

Table 4

Mathematical models and coefficients of determination (as $100R^2$) corresponding to the fitting equations

Equations of the models fitted to data	Variance explained by the model
$C/N \text{ ratio} = 110.0015^{***} \text{ ash}^{-0.4680^{***}}$	62.4% $p < 0.001$
$TN = 0.6042^{***} + 0.0890^{***} \text{ ash}$	66.7% $p < 0.001$
$P = 0.1483^{**} - 0.0379^{NS} \text{ ash} + 0.0070^{NS} \text{ ash}^2 - 0.0003^{**} \text{ ash}^3$	59.1% $p < 0.01$

Ash, TN and P contents as % dry weight

NS, not significant, * significant at $p < 0.05$, ** significant at $p < 0.01$ and *** significant at $p < 0.001$.

of the parameters (Tables 1 and 2) generally revealed a high degree of randomness in most of the parameters analysed due to the wide variations on the factors which influence the composition of AL (production process, olive variety stage of maturity, agronomic and edaphoclimatic conditions and time of storage). In our study, none of the following parameters (dry matter, pH, EC, TOC, fat content, water-soluble carbohydrate and phenol concentrations, hemicellulose, cellulose and lignin contents) were significantly correlated; neither were they correlated with the remaining parameters analysed (data not shown). However, ash, macro and micronutrient contents and the C/N ratio showed significant correlations. Therefore, because the ash content is easy to determine, this was taken as the reference parameter to estimate other useful parameters which are much more difficult to chemically analyse. Thus, correlations between ash and the C/N ratio, TN (both $p < 0.001$) and P ($p < 0.01$) were highly statistically significant. Linear regression provided the best fit for the ash–TN relationship, but other models had to be used to fit the other relationships. Table 4 shows the regression equations obtained in the three cases and the percentage of variance explained by each model.

4. Conclusions

The AL studied was shown to be an acidic and very wet olive-mill by-product, rich in K, poor in P, Ca and Mg, and containing an intermediate level of nitrogen, most of which is organic. The main micronutrient was seen to be Fe, while Cu, Mn and Zn levels were similar but very much lower than that of Fe. Except in the case of K, both the macro and micronutrient content was lower in the AL than in most manures and other organic soil amendments.

However, the OM content was much higher, its main constituents being lignin, hemicellulose and cellulose. Other important organic components were fats, hydrosoluble carbohydrates and proteins, the last of which might be less available for microorganisms than the others if the AL is composted, due to its association to the lignocellulosic fraction. Lastly, a small fraction of water-soluble phenolic substances was en-

countered, which, together with the lipidic fraction, has been related with the phytotoxic and antimicrobial effects currently attributed to olive-mill wastes and by-products.

Highly statistically significant correlations between ash and the C/N ratio, TN and P were found and an attempt was made to establish the corresponding regression equations. This suggests that the ash content might be taken as an easy-to-determine reference parameter for cautiously estimating the other parameters, which are much more difficult to chemically analyse.

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Composting of a solid olive-mill by-product (“alperujo”) and the potential of the resulting compost for cultivating pepper under commercial conditions

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Abstract

A pollutant solid material called “alperujo” (AL), which is the main by-product from the Spanish olive oil industry, was composted with a cotton waste as bulking agent, and the compost obtained (ALC) was compared with a cattle manure (CM) and a sewage sludge compost (SSC) for use as organic amendment on a calcareous soil. The experiment was conducted with a commercial pepper crop in a greenhouse using fertigation.

Composting AL involved a relatively low level of organic matter biodegradation, an increase in pH and clear decreases in the C/N and the fat, water-soluble organic carbon and phenol contents. The resulting compost, which was rich in organic matter and free of phytotoxicity, had a high potassium and organic nitrogen content but was low in phosphorus and micronutrients. The marketable yields of pepper obtained with all three organic amendments were similar, thus confirming the composting performance of the raw AL. When CM and SSC were used for soil amendment, the soil organic matter content was significantly reduced after cultivation, while it remained almost unchanged in the ALC-amended plots.

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1. Introduction

The industrial olive oil sector generates large quantities of solid and liquid wastes and by-products in many Mediterranean countries during a short period of time (November–February). The gradual accumulation or incorrect disposal of these wastes may cause environmental problems. These materials must be treated or re-used if their environmental impact is to be reduced, enabling at the same time some of their primary components to be recovered (organic matter, nutrients, etc.).

The two-phase centrifugation system for olive oil extraction was introduced in Spain at the beginning of the 1990s. It produces a new by-product called “alperujo”

(AL), a solid material of low consistency, whose main agrochemical characteristics have been extensively reported (Albuquerque et al., 2004). Due to the rapid and generalised implementation of the two-phase system, the yearly production of AL in Spain may exceed 4 million tons. Composting as a method for preparing organic fertilisers and amendments is economically and ecologically sound and may well represent an acceptable solution for disposing of AL, at the same time increasing its value. Composting AL, prior to its application in the field, should improve the soil's agronomic quality and reduce or avoid some of the adverse effects that have been recorded when olive-mill residues are directly supplied to the soil. Some of these effects have been related to its phytotoxic and antimicrobial properties due to their phenolic and lipidic constituents (Paredes et al., 1987; Pérez et al., 1992; Linares et al., 2003), while damage to the soil's

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structural stability has also been described (Tejada et al., 1997).

Several composting experiments (Madejón et al., 1998; Rosa et al., 2001; Filippi et al., 2002) have demonstrated the effectiveness of composting to transform AL into suitable soil organic fertilisers or amendments. However, there has been very little, if any, research into the possible use of AL compost for crop production.

In the present work, we study the suitability of AL for composting as a way of transforming the by-product into a non-toxic organic amendment, as well as the potential of the AL compost for growing pepper under commercial conditions.

2. Materials and methods

2.1. Composting performance

The compost was obtained by co-composting AL and a cotton waste (CW), the latter acting as a bulking agent to increase AL porosity and improve oxygen supply during the process. A pile of about 2700 kg was prepared by mixing on a fresh weight basis (dry weight basis in parentheses) 92.6% AL + 7.4% CW (80/20). AL was provided by "Cooperativas Agrícolas Albacetenses" from Cuenca (Spain) and CW by "SAT 1371" from Torre-Pacheco (Murcia, Spain). The collected AL was very rich in organic matter (OM), mainly composed of lignin, cellulose and hemicellulose; it had a high moisture content and carbon-to-nitrogen ratio (C/N), an acid pH and low electrical conductivity (EC). It also had a considerable proportion of fats, water-soluble phenols and nutrients, mainly potassium and nitrogen (Table 1). CW had a

Table 1
Main characteristics of the "alperujo" (AL) and cotton waste (CW) used in the composting experiment (dry weight)

Parameters	AL	CW
Moisture (% f.w.)	71.3	11.5
pH ^a	4.97	6.80
Electrical conductivity ^a (dS m ⁻¹)	3.01	4.12
Organic matter (g kg ⁻¹)	952.6	933.0
Lignin (g kg ⁻¹)	449.0	232.0
Cellulose (g kg ⁻¹)	207.0	392.3
Hemicellulose (g kg ⁻¹)	379.3	207.8
Total fats (g kg ⁻¹)	116.3	21.0
Total organic carbon (g kg ⁻¹)	539.0	477.0
Total nitrogen (g kg ⁻¹)	12.2	21.3
C/N	44.2	22.4
P (g kg ⁻¹)	0.9	1.8
K (g kg ⁻¹)	15.9	17.4
Ca (g kg ⁻¹)	4.5	23.0
Mg (g kg ⁻¹)	1.6	4.2
Fe (mg kg ⁻¹)	390	1710
Cu (mg kg ⁻¹)	13	12
Mn (mg kg ⁻¹)	12	108
Zn (mg kg ⁻¹)	17	40

^a Water extract 1:10.

neutral pH, a slightly higher EC value than the AL, a high OM content (mainly composed of cellulose) and a low moisture content and C/N. It also showed a good water-retaining capacity.

A trapezoidal pile of material, approximately 1.5 m high with a 2 m × 3 m base, was composted in a pilot plant by using forced aeration combined with temperature feedback control (Finstein et al., 1985), the air being blown from the base of the pile through the holes of three PVC pipes (3 m in length and 12 cm in diameter) located at the bottom. The timer was set for 30 s ventilation every 15 min, and the maximum temperature for continuous air blowing was 50 °C. The pile was turned once during the eighth week to improve both the homogeneity of the composting substrate and the oxygen supply. The active phase of composting was considered finished when the pile temperature was stable and close to that of the ambient, which occurred after 26 weeks. Forced aeration was then stopped to allow the substrate to mature over a period of 4 months. The pile's moisture level was controlled weekly and the necessary amount of water was added to maintain a moisture content between 45% and 55%. The excess water that leached from the pile was collected and added to the pile.

The composting substrate was sampled weekly during the bio-oxidative phase and then after the maturation period, taking samples from six random sites around the pile using a cylindrical drill (1.5 m in length and 8 cm in diameter) to bore holes through the whole height of the pile. The collected samples were mixed, homogenised and subdivided into three sub-samples in the laboratory. One of the sub-samples was frozen (−20 °C) and kept for the determination of NH₄⁺-N and NO₃⁻-N, the second was dried in an oven at 105 °C for 24 h to determine its moisture content, while the third sub-sample was freeze-dried and ground to less than 0.5 mm prior to analysis.

2.2. Greenhouse culture

The compost obtained (ALC) was evaluated as an organic amendment for the production of pepper (*Capiscum annuum* cv "Orlando"), comparing it with a cattle manure (CM) and a sewage sludge compost (SSC). The field experiment was carried out under fertigation conditions in a commercial greenhouse situated in San Javier (Murcia, Spain), where pepper plants were grown on a calcareous soil with a clay loam texture (Table 2). The three organic amendments were added manually over the treatment area, tilled into the soil and mixed with a rotary soil tiller to ensure their uniform distribution. One month later, pepper seedlings were transplanted to 1.1 m × 30 m soil plots, the plant population being equivalent to approximately 2.2 plants m⁻². Thus, there were three organic treatments: C1 (with ALC), C2

Table 2
Main characteristics of the soil employed for the pepper culture in the greenhouse experiment

Parameters	Soil
pH ^a	7.47
Electrical conductivity ^a (dS m ⁻¹)	1.42
Organic matter (g kg ⁻¹)	18.4
Total nitrogen (g kg ⁻¹)	1.6
C/N	6.7
Available phosphorus (mmol P kg ⁻¹)	6.8
Available potassium (mmol K kg ⁻¹)	39.9
Cation exchange capacity (cmol kg ⁻¹)	14.7
CaCO ₃ (%)	53.2
Sand (%)	25.6
Silt (%)	38.6
Clay (%)	35.8

^a Water extract 1:5.

(with CM) and C3 (with SSC) and a non-amended control (C0). Each treatment comprised two replications separated by a border, also of 1.1 m × 30 m. The amendment ratios used for ALC (2.0 kg m⁻²) and SSC (5.0 kg m⁻²) were based on the addition of 1.5 kg m⁻² of organic matter to the soil, as supplied by CM (4.4 kg m⁻²), which is commonly used as organic amendment in local pepper production.

A basic standard fertigation program was used to supply nitrogen, phosphorus, potassium and micronutrients to all of the treatments, and crop management followed the common practices used in the area. Red and green peppers were harvested according to commercial size and shape criteria determined by the operators of the greenhouse. A comparison of both total production and fruit quality was made between treatments and the macro- and micronutrients in plant leaves were analysed at 59, 149, 190 and 219 days after planting. In addition, the effect of adding the three products as soil organic amendment was evaluated in all the treatments by determining the soil organic carbon content before and after cultivation.

Two representative leaf samples per plot were taken randomly, washed with distilled water, oven dried at 60 °C for 24 h, ground and stored for analysis. For each plot, soil samples were taken at 10 different random sites (0–40 cm depth) and combined to obtain two representative samples, which were air dried and ground to 2 mm prior to analysis.

2.3. Analytical methods

The methods used for analysing the soil and composting the organic materials have been previously described by Caravaca et al. (1999) and Paredes et al. (2002), respectively. The total organic matter (volatile solids) losses were calculated during composting according to Stentiford and Pereira Neto (1985) by taking into account the apparent increase in the ash content result-

ing from the loss of dry matter weight in order to better reflect the overall changes, as also those of the main components of the organic matter (lignin, cellulose and hemicellulose).

For plant analysis, total nitrogen was determined directly in a Carlo Erba ANA-1500 CNS analyser and the other nutrients were determined after digestion with a mixture of HNO₃/HClO₄ according to Abrisqueta and Romero (1969). Thus, phosphorus was measured colorimetrically as molybdovanadate phosphoric acid (Kitson and Mellon, 1944); potassium by flame photometry; and calcium, magnesium, iron, copper, manganese and zinc by atomic absorption spectrophotometry.

2.4. Statistical analyses

Variance and the least significant difference were calculated for the results of the composting samples to determine changes in the parameters with time, whereas the experimental data from the pepper culture were subjected to an analysis of variance, statistical differences between treatments being estimated by Duncan's multiple range test, using the SPSS 11.0 program for Windows.

3. Results and discussion

3.1. Evolution of the composting process

With respect to the temperature profile (Fig. 1), the process exhibited an initial long mesophilic period, which lasted until the eighth week with temperatures of around of 40 °C; a thermophilic step between the eighth and 12th weeks, initiated immediately after turning and coinciding with the better aeration conditions induced as a response to mixing and homogenisation of the composting substrate; and, finally, another mesophilic period, which lasted until the end of the active phase of the process (26th week). Such a long composting period could be related to the low bioavailability of N-compounds existing in AL and the high proportion of biodegradation-resistant components, such as lignin, compared with other wastes currently used for composting (manures, city refuse and sewage sludges).

During the initial phase of the process, the pH progressively increased until the 22nd week due to the degradation of acid compounds and the liberation of ammonia, after which it remained stable at around 9 (Table 3). The EC values, on the other hand, decreased from an initial value of 3.56–2.77 dS m⁻¹ during the first four weeks, then values remain still lower until the 16th week of composting, after which it rose to reach a plateau slightly higher than 3 dS m⁻¹ (Table 3). A decrease in EC during composting is rather unusual but, when

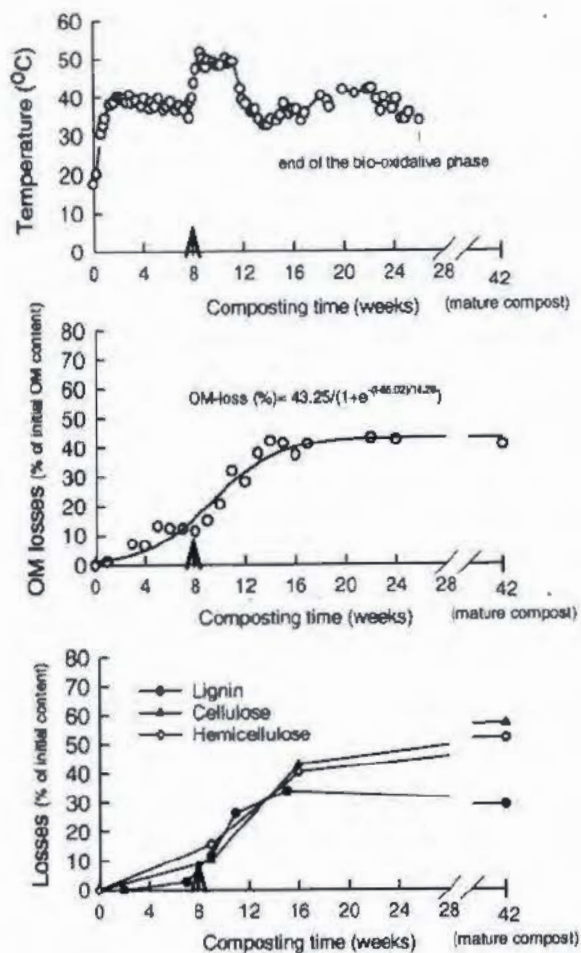


Fig. 1. Temperature profile and losses of OM, lignin, cellulose and hemicellulose during composting. The arrow indicates turning and the line of OM losses represents the curve-fitting to experimental data with a residual mean square of 12.65 and an "F" factor of 200.41 at $P < 0.001$. "t" is composting time in days.

observed (Raviv et al., 1987; Wong et al., 2001), it has been related to a decrease in the soluble nutrient ions fixed during the rapid proliferation of the aerobic microbial population, and to their precipitation as insoluble mineral salts or by ammonia volatilisation. In our experiment, a rather low degree of OM mineralisation occurred, releasing only relatively small amounts of mineral salts, which might rapidly have been removed from the soluble phase, as explained above.

During the whole composting process, the OM content decreased from 938.1 to 900.1 g kg⁻¹ (Table 3), leading to a calculated total OM-loss of 40.6% according to Fig. 1, in which experimental data were fitted to a sigmoidal model. Three different phases in OM loss related with the above discussed temperature profile could be discerned in the model: a slight but continuous increase of losses (10–15%) coinciding with the initial mesophilic period (eight weeks), a higher rate of OM degradation, which mostly occurred during the thermophilic phase, and a third step, reflecting a progressive stabilisation in OM loss due to the high lignin concentration existing in the raw material that reduced the bioavailability of the organic substrate (Lynch, 1993; Vikman et al., 2002). It should be added (Fig. 1) that lignin was much less degraded than cellulose and hemicellulose (total losses of 28.9%, 56.9% and 51.8%, respectively).

The low OM degradation rate coinciding with the initial mesophilic step could be related to insufficient substrate aeration, especially with the high initial moisture in the mixture (around 66%). It is well known that important physico-chemical changes occur during composting, affecting the volume, mass, bulk density and water content of the substrate, whose porosity (free air space) greatly influences the aeration efficiency and correct air distribution through the composting mass. When such properties are limited, turning may be an effective operating strategy for encouraging the process

Table 3
Evolution of main analytical parameters during composting (dry weight)

Composting time (weeks)	pH	EC (dS m ⁻¹)	OM (g kg ⁻¹)	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)
0	6.1	3.56	938.1	518.9	15.1	279	–
2	6.9	3.20	938.9	513.6	16.9	236	–
4	7.1	2.77	934.1	514.6	17.6	310	–
6	7.7	2.64	930.0	511.3	17.5	192	–
8	7.5	2.60	930.7	494.7	16.4	396	–
10	7.8	2.73	923.3	495.8	18.3	459	–
12	8.0	2.61	916.0	486.4	19.7	505	–
14	8.3	2.66	897.7	494.1	23.3	502	–
16	8.3	2.71	904.8	496.0	22.4	417	–
22	8.9	3.05	896.2	492.6	22.3	208	–
24	9.0	3.05	897.2	495.1	22.0	192	50
42	8.9	3.07	900.1	491.5	21.7	90	112
Lsd	0.1	0.16	4.2	6.3	0.8	39	3

–: Not detected.

EC: electrical conductivity, OM: organic matter, TOC: total organic carbon and TN: total nitrogen.

Lsd: least significant difference at $P < 0.05$.

by restoring the air-flow channels or pores, homogenising and re-inoculating the composting substrate, as was demonstrated in our experiment.

With a clear predominance of the organic forms, there was an increase in the total nitrogen concentration (TN) from 15.1 g kg⁻¹ at the start of composting until values of around 20 in the 12th week (Table 3), as a result of the OM mineralisation which reduced the weight of the pile. The increase of nitrogen led the C/N to decrease from 34.4 at the beginning to 22.7 at the end of composting, the latter value being rather high compared with other composts made of animal manures and city refuse, but reflecting the substantial proportion of lignin remaining in the mature AL compost. After the start of the thermophilic stage (eighth week), a relative increase in the NH₄⁺-N content was detected, values of 400–500 mg kg⁻¹, coinciding with the more intense rate of OM biodegradation (until the 16th week). Thereafter, the NH₄⁺-N content decreased as the composting progressed and reaching the lowest content (90 mg kg⁻¹) at the end of the process, which is well below the maximum limit of 400 mg kg⁻¹ established for a mature compost (Zucconi and de Bertoldi, 1987; Bernal et al., 1998). Nitrates, on the other hand, could hardly be detected at the end of the active phase of composting and in the mature compost, when temperature dropped (Table 3). These results agreed with those of Filippi et al. (2002), who did not detect nitrifying bacteria during AL composting.

In the early stages of composting, readily available compounds, such as sugars, starches, fatty acids, lipids and proteins, are degraded by microorganisms as the most suitable carbon and energy source. In our experiment, a decrease in the water-soluble organic carbon (WSC) and carbohydrates (WSCH) was already evident in the fourth week of composting, both parameters reaching values of around 30 and 10 g kg⁻¹, respectively (Table 4), revealing the availability of these OM fractions for microbial metabolism. Other important fractions of the initial composting substrate were total fats (91.8 g kg⁻¹) and water-soluble phenols (WSPH, 5.7 g kg⁻¹), both components being responsible, as in the case of the organic acids, for the antimicrobial and

phytotoxic effects currently assigned to olive-mill wastes and by-products (Estuán et al., 1985; Riffaldi et al., 1993; Linares et al., 2003). Until the 10th week of composting, the total fat content decreased to 15.6 g kg⁻¹ and WSPH to 4.0 g kg⁻¹, but these values were reduced to 8.8 and 2.2 g kg⁻¹, respectively, in the mature compost. Both decreases contrasted with the increase observed in the germination index (GI), an easy to quantify parameter that predicts the potential phytotoxicity of a compost. Thus, the high initial phytotoxicity tended to disappear during the process, as the decrease in the above mentioned potentially phytotoxic compounds led to the higher GI, which reached a value greater than 70% in the 10th week of composting (Table 4).

The compost obtained (ALC), clearly lacking in phytotoxicity, had considerable greater OM and lignin contents than the other two organic amendments tested (Table 5), lignin being recognised to be both poorly biodegradable and an important precursor of soil humic substances. ALC also had a considerable content of potassium and organic nitrogen, although phosphorus and micronutrients were rather low.

3.2. Greenhouse experiment

During the experiment, there was no evidence of adverse effects on plant growth or phytotoxicity symptoms in plots treated with ALC. Marketable pepper yields in plots amended with ALC, CM and SSC were similar (99.6, 98.3 and 97.7 t ha⁻¹, respectively) and rather greater than in the control (90.0 t ha⁻¹), although the

Table 5
Main characteristic of the "alperujo" compost (ALC), cattle manure (CM) and sewage sludge compost (SSC) used in the agronomic experiment (dry weight)

Parameters	ALC	CM	SSC
Moisture (% f.w.)	14	41	53
pH ^a	8.88	8.67	7.03
Electrical conductivity ^a (dS m ⁻¹)	3.07	5.31	4.63
Organic matter (g kg ⁻¹)	900.1	603.4	649.2
Lignin (g kg ⁻¹)	410.0	248.3	223.1
Total organic carbon (g kg ⁻¹)	491.5	332.2	315.2
Total nitrogen (g kg ⁻¹)	21.7	21.4	23.5
C/N	22.7	15.5	13.4
P (g kg ⁻¹)	1.5	1.0	16.8
K (g kg ⁻¹)	24.9	35.3	5.4
Ca (g kg ⁻¹)	13.4	58.5	37.5
Mg (g kg ⁻¹)	2.9	7.7	5.1
Na (g kg ⁻¹)	2.6	6.8	3.1
Fe (g kg ⁻¹)	0.7	4.3	39.4
Cu (mg kg ⁻¹)	21	32	203
Mn (mg kg ⁻¹)	46	252	204
Zn (mg kg ⁻¹)	41	175	811
Pb (mg kg ⁻¹)	4	4	78
Cr (mg kg ⁻¹)	8	9	36
Ni (mg kg ⁻¹)	8	10	31
Cd (mg kg ⁻¹)	—	—	1

—: Not detected.

^a Water extract 1:10.

Table 4
Evolution of water-soluble organic carbon (WSC), carbohydrates (WSCH) and phenols (WSPH), total fat content and germination index (GI) during composting (dry weight)

Composting time (weeks)	WSC (g kg ⁻¹)	WSCH (g kg ⁻¹)	WSPH (g kg ⁻¹)	Total fats (g kg ⁻¹)	GI (%)
0	40.7	13.6	5.7	91.8	5.0
4	32.0	11.6	4.0	62.0	52.5
10	31.4	9.0	4.0	15.6	73.0
16	29.7	10.6	2.8	15.0	74.0
42	31.4	12.6	2.2	8.8	78.0
Lsd	1.4	0.7	0.2	3.2	8.7

Lsd: least significant difference at $P < 0.05$.

Table 6
Organic matter content of soil (C0), soil + ALC (C1), soil + CM (C2) and soil + SSC (C3), before (T1) and after (T2) the pepper cultivation

Treatments	Time	Soil organic matter (g kg ⁻¹)
C0	T1	18.53c
	T2	18.49c
C1	T1	21.41a
	T2	21.28a
C2	T1	20.76a
	T2	19.06bc
C3	T1	21.53a
	T2	19.50b
Treatment	***	
Time	***	
Treatment × time	**	

Values followed by the same letters are not statistically different according to the Duncan's multiple range test at 5% probability level.

** $P < 0.01$.

*** $P < 0.001$.

differences were not statistically significant and did not affect the commercial quality of the fruit, thus confirming the beneficial effect of composting on the starting phytotoxicity of the raw AL. Nutrient leaf concentrations were also generally similar in all treatments due to the efficiency of fertigation in supplying nutrients, although the three organic amendments led to a statistically significant but rather negligible increase in the leaf nitrogen, potassium and copper contents compared with the control (data not shown). After cultivation, the soil organic matter content was significantly reduced in the plots amended with CM and SSC, but remained almost unchanged in the ALC-amended plots (Table 6), demonstrating the considerably greater resistance of the obtained compost to edaphic biodegradation.

4. Conclusions

The AL composting process involved a relatively slow biodegradation rate of organic matter, an increase in pH and decreases in the C/N and the fat, water-soluble organic carbon and phenol contents. The compost obtained was free of toxicity, rich in organic matter (mainly composed of lignin), and had a considerable potassium and organic nitrogen content but was low in phosphorus and micronutrients. It can be used as an efficient organic amendment, as was shown by its comparison with other organic materials for growing pepper. It also demonstrated a considerably greater resistance to edaphic biodegradation in the harsh thermal conditions of the greenhouse.

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UTILIZACIÓN DE ESTIÉRCOLES Y RESIDUOS VEGETALES PARA CO- COMPOSTAR ALPERUJO, EL PRINCIPAL SUBPRODUCTO DE LA INDUSTRIA EXTRACTIVA DEL ACEITE DE OLIVA EN ESPAÑA. INFLUENCIA SOBRE EL PROCESO Y LA CALIDAD DEL COMPOST.

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El alperujo, principal subproducto del sector oleícola español.

El cultivo del olivo y la actividad de la industria oleícola tienen una gran importancia económica y social en los países de la Cuenca Mediterránea, siendo España el principal país productor de aceite de oliva a nivel mundial. Dada la masiva adopción por las almazaras españolas del sistema continuo de dos fases para la extracción del aceite de oliva, éstas generan principalmente como subproducto un material sólido de escasa consistencia y muy húmedo denominado alperujo.

El destino principal del alperujo es el agotamiento del aceite residual que contiene mediante extracción química en las orujeras (a pesar de su elevada humedad y de la incorporación de sólidos finos y compuestos orgánicos que dificultan el desarrollo de las operaciones de secado y posterior extracción del aceite) y su aprovechamiento energético, una vez seco y agotado. Sin embargo y considerando los buenos resultados obtenidos en el compostaje del alpechín y otros subproductos de la industria extractiva del aceite de oliva, así como en el empleo de los composts obtenidos como enmendantes y/o abonos orgánicos (Calvet y col., 1985; Tomati y col., 1995 y 1996; Cegarra y col., 1996; Martín Olmedo, 1996; Negro y Solano, 1996; Cabrera y col., 1997; Paredes y col., 2002 y 2005), nuestro grupo de investigación se planteó el compostaje del alperujo como una opción viable para minimizar el problema ocasionado por la excesiva acumulación del mismo. En este sentido, hemos desarrollado varios proyectos en colaboración con empresas españolas e instituciones de investigación de otros países europeos sobre el compostaje del alperujo y el aprovechamiento de los productos obtenidos que demuestran el interés y la viabilidad del proceso.

Cabe añadir por último, que la alternativa de tratamiento del alperujo mediante compostaje debe redundar en beneficio para el productor de este subproducto, ya que podrá así disponer de un producto final comercializable o para su propio uso, frente al procesado en las orujeras que requiere disponer del alperujo a coste mínimo o gratuito. Lo anterior resulta especialmente cierto en explotaciones de olivo ecológico, ya que en este caso deben soportar los elevados costes derivados de la utilización de abonos orgánicos producidos a partir de materias primas "limpias", procedentes de las propias explotaciones ecológicas agrícolas y ganaderas.

Ensayos de compostaje de alperujo y caracterización de los productos obtenidos.

El objetivo de esta ponencia es exponer resumidamente los resultados obtenidos por nuestro grupo de investigación en diferentes experimentos de compostaje de alperujo, a escala de planta piloto e industrial (Tabla 1), y en ensayos agronómicos realizados con los productos obtenidos. La información que se aporta se refiere a los siguientes aspectos:

a) Caracterización agroquímica del alperujo, selección y caracterización de agentes estructurantes, indispensables para el compostaje del mismo. Los agentes ensayados fueron: un residuo de algodón, raspajo de uva, hoja de olivo y estiércoles frescos (vacuno, avícola y ovino).

b) Control y seguimiento del proceso de compostaje, estudiando la evolución de distintos parámetros analíticos con el avance del mismo y definiendo los más adecuados para tal fin.

c) Caracterización agroquímica de los composts obtenidos y evaluación agrícola de los mismos. Tal evaluación se efectuó mediante dos ensayos de cultivo: con ryegrass (*Lolium perenne* L.) en cámara de crecimiento controlado y con pimiento (*Capsicum annuum* cv "Orlando") bajo invernadero y condiciones reales de fertirrigación, comparándose en este último ensayo el compost de alperujo con dos abonos orgánicos de uso común.

Tabla 1. Composición inicial (ALP: alperujo, RA: residuo de algodón, RU: raspajo de uva, HO: hoja de olivo, EV: estiércol vacuno, CA-4: compost maduro pila 4, EA: estiércol avícola y EO: estiércol ovino), peso y manejo de las pilas de compostaje.

Mezclas	Composición (%)		Peso de las pilas (kg)	Total de volteos	Ventilación forzada
	Peso fresco	Peso seco			
Pilas 1 y 2	92,6 ALP1 + 7,4 RA	(80/20)	2.700	1	Si
Pila 3	94,6 ALP2 + 5 RU + 0,4 urea	(87/12/1)	2.600	3	Si
Pila 4	94,6 ALP2 + 5 HO + 0,4 urea	(87/12/1)	2.600	3	Si
Pilas 5 y 6	90 ALP3 + 9 EV + 1 CA-4	(87/11/2)	4.000	14	5(No) 6(Si)
Pila 7	52 ALP4 + 48 EA	(49/51)	20.000	7	No
Pila 8	65 ALP4 + 35 EO	(65/35)	20.000	7	No

Resultados.

a) Caracterización del alperujo y selección de agentes estructurantes.

El alperujo mostró valores moderados de acidez y conductividad eléctrica, así como un contenido muy elevado de materia orgánica, constituida mayoritariamente por lignina, hemicelulosa y celulosa. También su contenido de carbono orgánico fue elevado, mientras que el de nitrógeno fue generalmente bajo. Consecuentemente, la relación C/N fue en general alta en la mayoría de las muestras. Así mismo, el contenido en potasio fue alto pero, en cambio, fue bajo el de fósforo y micronutrientes (Alburquerque y col., 2004).

Otras características del alperujo, como su elevada humedad, escasa consistencia como material sólido y pequeño tamaño de partícula, le confieren una deficiente estructura física y una reducida porosidad que restringen enormemente su aptitud para ser aireado durante el proceso de compostaje. Estas propiedades, junto a su abundante contenido en grasas, riqueza en compuestos de naturaleza fenólica con acción antimicrobiana y naturaleza lignocelulósica mayoritaria de difícil biodegradación, pueden dificultar igualmente su compostaje y alargar el proceso. En consecuencia, la viabilidad del mismo dependió en gran medida de la adición de los agentes estructurantes, ya que contribuyeron a mejorar su deficiente estructura física, absorbiendo además el exceso de agua del alperujo y ajustando su elevada relación C/N. Aunque todos los agentes estructurantes dinamizaron el proceso, resultaron especialmente útiles los estiércoles y el residuo de algodón.

La aplicación de volteos a los sustratos de compostaje fue siempre eficaz y en algunas pilas decisiva para su compostaje, ya que el método de pila estática resultó ineficaz a pesar del empleo de la ventilación forzada en los ensayos de planta piloto (Alburquerque y col., 2005).

b) Control y seguimiento del proceso.

El avance del compostaje se caracterizó por un aumento del pH desde valores iniciales ácidos hasta valores próximos a 8,5 al final del proceso, pudiéndose considerar tal incremento como índice del correcto avance del mismo, especialmente durante su fase inicial. También disminuyó claramente el contenido de carbohidratos sencillos y polifenoles existentes en la fracción hidrosoluble, alcanzándose valores finales muy parecidos entre las distintas pilas, independientemente de las diferencias en la evolución de las mismas y entre los contenidos iniciales de tales compuestos.

La mayor descomposición de la materia orgánica coincidió con temperaturas claramente termófilas y siempre se ralentizó en los periodos de persistente acidez, coincidiendo con dificultades para la aireación de las pilas. Entre los tres principales componentes de la materia orgánica de los sustratos de compostaje (lignina, celulosa y hemicelulosa) la menor degradación se apreció en la lignina (14-41%), mientras que el mayor porcentaje de pérdidas se observó en la hemicelulosa (45-70%). Estas pérdidas dependieron de las condiciones de oxigenación de los sustratos y generalmente fueron más intensas a temperaturas claramente termófilas.

El proceso de compostaje incrementó el contenido de nitrógeno total, debido principalmente al efecto de concentración provocado por la pérdida de peso originada por la biodegradación de la materia orgánica, registrándose siempre un claro predominio de las formas orgánicas de este elemento sobre las inorgánicas. Las pérdidas totales de nitrógeno fueron relativamente bajas y se produjeron al coincidir un importante aumento del pH, temperaturas altas y periodos largos de ventilación forzada (ensayos de planta piloto), produciéndose las pérdidas de nitrógeno en forma de amoníaco fundamentalmente.

La relación C/N experimentó una disminución con el avance del compostaje, más pronunciado durante los periodos más activos del mismo, mostrando valores finales relativamente altos en todos los casos, pero acordes con la naturaleza lignocelulósica de los sustratos que determinó una concentración final de los biopolímeros más resistentes a la biodegradación (muy ricos en carbono).

En todos los ensayos se registró un claro ascenso de la capacidad de cambio catiónico, mientras que se apreció una rápida y drástica reducción del contenido graso, alcanzándose valores finales siempre inferiores al 2%. Sin embargo tal reducción fue menor cuando se produjeron retrasos en el aumento del pH y dificultades para la correcta aireación de las pilas.

Coincidiendo con la fuerte biodegradación y mineralización de los compuestos más lábiles durante los tres primeros meses de compostaje, la relación de humificación disminuyó para, posteriormente, aumentar y/o estabilizarse conforme progresaron los procesos de reorganización y condensación molecular propios de la humificación, a la vez que el porcentaje de ácidos húmicos aumentó claramente en todos los casos.

Inicialmente todos los sustratos mostraron un fuerte carácter fitotóxico, con un valor prácticamente nulo del índice de germinación, si bien este carácter fue desapareciendo progresivamente. La pérdida de fitotoxicidad estuvo limitada por las condiciones de aireación imperantes en los sustratos, de forma que las pilas con unas condiciones de aireación relativamente más deficientes mostraron cierto retraso en el crecimiento del citado índice.

c) Caracterización y evaluación agrícola de los composts maduros.

Los composts obtenidos (Tabla 2), muy ricos en materia orgánica predominantemente lignocelulósica, mostraron un importante contenido de potasio y aunque menos también de nitrógeno, principalmente en forma orgánica no inmediatamente asimilable.

En cambio, fue escaso su contenido de fósforo y también el de los principales micronutrientes, mientras que los controles de fitotoxicidad realizados en todos ellos mostraron siempre índices de germinación superiores al 70%.

Tabla 2. Comparación entre la composición de estiércoles de uso común y distinto origen (n=10) y los composts obtenidos del alperujo (n=8).

Parámetro (s.m.s.)	Estiércoles		Composts	
	Media	Rango	Media	Rango
pH ¹	7,8	6,5-9,1	8,7	8,3-9,0
Conductividad eléctrica ¹ (dS/m)	4,56	3,56-6,20	3,13	1,69-4,81
Materia orgánica (g/kg)	756	545-859	815	561-912
Relación C/N	12,5	8,7-19,2	19,2	13,5-22,7
Nitrógeno total (g/kg)	31,7	17,7-46,0	22,6	18,6-26,3
Fósforo (g/kg)	3,2	1,0-5,1	1,7	1,4-2,0
Potasio (g/kg)	20,9	11,0-35,9	29,3	11,0-42,7
Calcio (g/kg)	56,4	29,5-102,2	24,4	9,4-65,0
Magnesio (g/kg)	9,8	5,1-15,9	4,9	1,9-12,7
Hierro (mg/kg)	3.180	1.000-6.900	2.010	525-6.520
Cobre (mg/kg)	256	15-492	27	16-36
Manganeso (mg/kg)	280	112-402	88	38-183
Cinc (mg/kg)	231	20-576	71	38-138

¹ 1:10 (p/v)

Ensayos agrícolas.

La adición de uno de los composts de alperujo a un suelo calizo de escasa fertilidad estimuló el crecimiento de las plantas de ryegrass (Figura 1), sin que se evidenciaron en ningún caso signos aparentes de fitotoxicidad que pudieran relacionarse con la aplicación del compost y aumentando de forma estadísticamente significativa el peso fresco y seco de material vegetal en la primera y tercera cosecha. El factor limitante del cultivo fue la disponibilidad de nitrógeno, registrándose siempre los rendimientos más bajos en los tratamientos sin fertilización nitrogenada (N0), dada la escasa disponibilidad del nitrógeno del compost.

En general, la adición del compost incrementó la concentración de fósforo, potasio y cobre en el material vegetal recogido en las tres cosechas y disminuyó la de calcio y magnesio, mientras que, sólo en la tercera cosecha, también disminuyó el contenido de hierro y manganeso.

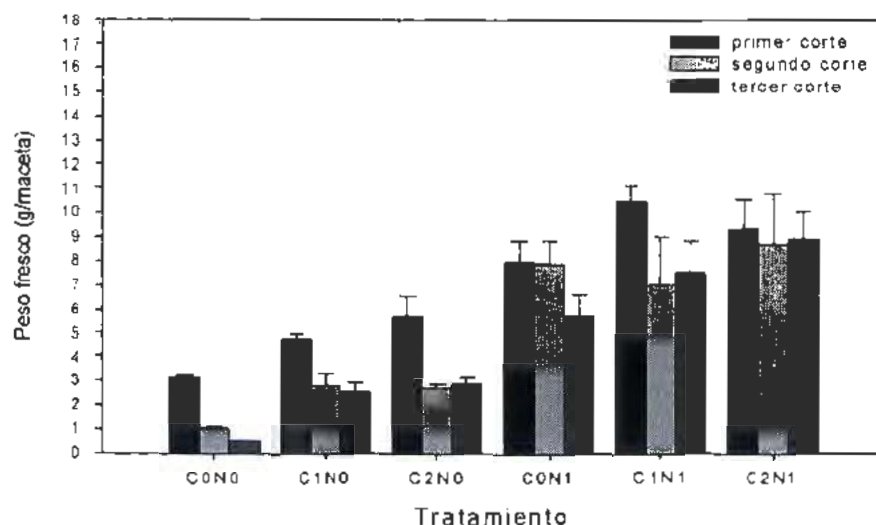


Figura 1. Rendimiento medio de las tres cosechas de ryegrass para los 6 tratamientos ensayados (valores medios + desviación estándar, n=4). Dosis de compost (C0: 0 t/ha, C1: 40 t/ha y C2: 80 t/ha) y de fertilización nitrogenada (N0: 0 kg de N/ha y N1: 60 kg de N/ha) empleadas en el ensayo.

Cuando se compararon los efectos de otro de los composts de alperujo con los de un estiércol vacuno y un compost de lodo de aguas residuales urbanas, sobre un cultivo de pimiento bajo invernadero y condiciones reales de fertirrigación, no se apreciaron apenas diferencias ni sobre el rendimiento de cultivo ni sobre el estado nutricional de las plantas, debido a que la fertilización mineral aplicada enmascaró los posibles efectos de los tratamientos orgánicos. Sin embargo, la materia orgánica aportada por los tres enmendantes que, en parte se degradó durante el ciclo del cultivo, permaneció casi inalterada en las parcelas tratadas con el compost de alperujo (Tabla 3), de acuerdo con la naturaleza lignocelulósica de éste, fuertemente resistente a la biodegradación edáfica.

Tabla 3. Análisis del contenido de materia orgánica del suelo antes (Inicial) y después (Final) del cultivo de pimiento.

Tratamiento	Materia orgánica (g/kg)	
	Inicial	Final
Control		18,53c
		18,49c
Compost de Alperujo		21,41a
		21,28a
Estiércol vacuno		20,76a
		19,06bc
Compost de Lodo de EDAR		21,53a
		19,50b
Tratamiento	***	
Tiempo	***	
Interacción	**	

Valores seguidos de la misma letra, no son significativamente diferentes a $p < 0,05$ de acuerdo con el test de Duncan. **: Significativo $p < 0,01$ y ***: Significativo $p < 0,001$.

Conclusión.

Cabe concluir, por tanto, que la transformación del alperujo en enmendante y/o abono orgánico mediante compostaje es una técnica viable en las condiciones aquí descritas, siendo suficiente para el seguimiento del proceso controlar la evolución de la temperatura, humedad, el pH, la relación C/N y el índice de germinación, a la vez que se puede prescindir de la ventilación forzada y recomendar la utilización exclusiva de volteos para abaratar costes en el desarrollo industrial del mismo. Los composts obtenidos exentos de fitotoxicidad, mostraron escaso contenido de fósforo y micronutrientes, pero notable contenido de potasio y también de nitrógeno, si bien este último fue mayoritariamente orgánico.

Fueron también muy ricos en materia orgánica de naturaleza predominantemente lignocelulósica (resistente a la biodegradación edáfica), pudiéndose considerar aproximadamente una cuarta parte de la misma de naturaleza húmica más o menos evolucionada.

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Bio-degradation of olive oil husks in composting aerated piles

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Abstract

In this study, the composting performance of two olive oil husk piles was compared using two different aeration processes: aeration by mechanical turning or by forced air-injection.

The results showed that after the maturation phase both piles had a similar chemical composition and the same level of organic matter degradation (around 55%). However, the time necessary to reach the thermophilic phase was shorter for the turned pile (2 months in comparison to 3 months for the air-injected pile) and the humification degree achieved was slightly higher (94% versus 83%).

Both piles showed the presence of an active microbial community, with an increase by 2–3 orders of magnitude in the number of cultured microbial colonies during the composting process. No significant difference in quantitative or qualitative terms was found in the microbial populations of both piles, nor was a clear succession between a mesophilic and a thermophilic population observed.

In terms of industrial application, the mechanical turning process seems to be better since it is a lower energy and time consuming process.

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Keywords: Olive oil industry; Olive husk; Composting; Humification; Microbial population; Aerated piles

1. Introduction

Olive oil extraction represents an economic and social industrial activity of high relevance in the Mediterranean countries, Spain, Italy, Greece, Tunisia, Turkey, Syria and Portugal being the main producers. Among these countries, within EC (Spain, Italy, Greece and Portugal) represent about 75% of the world production (International Olive Oil Council, www.international-oliveoil.org).

The olive oil industry is characterized by its great environmental impact due to the production of a highly polluted wastewater and/or a solid residue, depending on the olive oil extraction process.

In Portugal, until 1980s, the olive oil extraction was performed by using the traditional pressing system, which has been gradually replaced by the continuous centrifugation technology. This technology, known as

the three-phase system, separates the three main olive constituents: olive oil, vegetation water (which, together with the water added to the process to improve separation, makes up the olive oil wastewater) and a solid residue made of olive skin and stone (olive husks).

This system produces a low fat content effluent as compared with the traditional pressing operation. However, these wastewaters cause a significant negative impact in the environment and require a treatment system before being discharged into the rivers or soils. Therefore, a low water-consuming technology was suggested for olive oil extraction—the two-phase centrifugation process—aimed at reducing both the amount of process water and the output volume. This process, however, results in husks with higher moisture content and different chemical characteristics, which created a new problem for industries and a loss of market value for these sub-products (Madejon et al., 1998a; Rosa et al., 2001).

Several studies have been performed in order to provide alternative solutions for the use of these “new” husks, and two main alternatives have been proposed so

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far: the first one is husk combustion for energy production and a second one is a bio-treatment using a composting process (Madejon et al., 1998a; Vitolo et al., 1999; Filippi et al., 2002).

During the composting process, the organic fraction is partially aerobically degraded by microorganisms to carbon dioxide and water, whereas the other part undergoes a humification process which result is a stable compost possessing suitable characteristics to be used as bio-fertilizer (Vlyssides et al., 1996; Tomati et al., 1996; Paredes et al., 2000; Bertoldi and Schnappinger, 2001).

The acceptance of composting, however, depends on how well the operating strategies being employed are developed for both product quality (Tiquia et al., 2000) and environmental protection (Savage, 1996). Proper evaluation of the system is, therefore, required if an acceptable product is to be generated, and the system efficiency is to be maximized (Tiquia and Tam, 2002).

The aerobic composting technologies are windrow (turned pile), aerated static pile and in-vessel, being the former two the most commonly used. The technologies vary in the method of air supply, temperature control, mixing/turning of the material, and the time required for composting. The corresponding capital and operating costs also vary considerably.

The efficiency of composting under different aeration and/or mixing methods was tested using different sorts of organic materials such as poultry manure amended with sphagnum peat (Fernandes and Sartaj, 1997), spent pig litter and sawdust (Tiquia and Tam, 1998), poultry litter (Brodie et al., 2000), vinasse and cotton gin trash (Diaz et al., 2002a) and beet vinasse and grape marc (Diaz et al., 2002b). The results obtained in these studies demonstrated that the efficiency of composting is quite similar. The main differences seem to be related with the temperature profile and time for composting, i.e. thermophilic phase seems to occur later and to last longer in forced aerated piles. The temperature values achieved in this phase are generally higher in turned piles.

The aim of the present work is to study the composting of olive oil husks using two different aeration techniques, forced aeration by air injection and mechanical turning, and to assess their performances in terms of the main characteristics of the final compost (chemical composition, humification degree and phytotoxicity). The microbial population involved in the process was also studied.

2. Methods

2.1 Husk collection and pile construction

For the pile construction, 2.5–3.0 ton of fresh husk were collected in a two-phase olive oil milling plant. The husks were mixed with about 2% (w/w) grape stalks, and

two trapezoidal piles were prepared (2.5 m long \times 2.0 m wide \times 0.8 m high).

Two different aeration processes were tested – mechanical turning and forced air injection. In one pile (pile 1), the mixture was turned once a fortnight with a small plough (600 W, supplied by Gardena, Ulm, Germany). In the other pile (pile 2), the aeration was provided by a centrifugal ventilator (model CMB/4-200/80-1/3 hp, Solar & Palau, Porto, Portugal) connected to PVC tubes (12 cm \varnothing and 3 m long) perforated in two diametrically opposite lines, and placed at the bottom of the pile. The ventilator was controlled by means of a timer, in order to inject around $10 \text{ m}^3 \text{ h}^{-1}$ air during the first fifteen days of the composting process (corresponding to the ventilator being on 2 min h^{-1}) and $1 \text{ m}^3 \text{ h}^{-1}$ afterwards, until the thermophilic phase was achieved.

Temperature was followed by three PT100 probes (Class A, AISI 316, IP65) installed in each pile and connected to the aeration control system. In each pile, one probe was fixed at a depth of 20 cm from the top, and the other two were placed at random inside the pile, at different points. The temperature was recorded daily and the value was the average between the three readings.

2.2 Analytical methods

The samples were analysed for pH and electrical conductivity in aqueous extracts (1:10 w/v), after stirring for 15 min and allowing to stand for at least 30 min. Electrical conductivity was determined using a portable meter equipped with a conductivity sensor (model 90, Mettler Toledo Ltd., England).

All other analyses were carried out in fresh husk samples unless otherwise stated. Dry matter and moisture content were analysed by drying husk samples at 105°C for 24 h; organic matter and ashes by weight loss after ignition at 550°C for 24 h. Total nitrogen was analysed using the Kjeldhal method (Association of Official Agricultural Chemists, 1975). Fats were extracted with hexane at 60°C . The extraction process consisted in three extractions with 15-min each, followed by one extraction overnight. Fats were determined by weighing the extracts after solvent evaporation and were expressed in percentage (Greenberg et al., 1998). Total phenolic compounds were extracted with ethyl acetate at 60°C . The extraction process consisted in three extractions with 10-min each and one extraction overnight. After solvent evaporation, the extracts were dissolved in methanol, and the phenolic compounds were determined by colorimetric analysis at 765 nm, after reaction with the Folin-Ciocalteu reagent. A calibration curve was obtained with a standard solution of caffeic acid in the range 5–90 mg/l (Singleton and Rossi, 1965).

The method for total organic carbon (TOC) analysis used dichromate in acidic hot medium for organic matter oxidation. The organic carbon was determined by colorimetric analysis measuring the absorption of Cr(III) at 585 nm. A calibration curve was obtained with a standard glucose solution, which had been previously submitted to the same treatment (Tinsley, 1950). Total extractable carbon (TEC) was determined using the method referred above for TOC, after carbon extraction with alkaline sodium pyrophosphate (De Nobili and Petrussi, 1988).

Humic acids (HA) were precipitated with H_2SO_4 from the carbon extracts. The supernatant was passed through a polyvinylpyrrolidone column, where fulvic acids (FA) were adsorbed and eluted with NaOH. The fulvic fraction was added to the humic fraction, and the total humified carbon (HA + FA) was calculated using the method referred above for TOC. The humification degree was calculated using the formula $(HA + FA)/TEC \times 100$ (Sánchez-Monedero et al., 1996).

Lignin and cellulose content were determined by the American National Standard Institute (1997) on samples obtained by drying the fresh husk at low temperature ($<40^\circ C$) and then grinding it to a particle size lower than 0.5 mm.

Phytotoxicity expressed as the germination index (GI) was assayed by the *Lepidium sativum* test, according to Zucconi et al. (1985).

All analyses were performed at least thrice.

2.3. Microbial analysis

The husk samples (10 g) were suspended in 90 ml of a sterile mineral medium (Pettigrew et al., 1990) and stirred at 150 rpm for 10 min at $28^\circ C$. The suspensions were used for microbial counts being cell enumeration assessed by the determination of the number of colony forming units (CFU).

Serial dilutions of each suspension (10^{-2} – 10^{-7}) were plated in triplicate on different agar media: Tryptic Soy Agar (TSA) (Difco, Detroit, USA), Rose-Bengal Chloramphenicol Agar Base (CRB) (Difco) and 1/50 strength TSA Polymixin B (Merck Laboratories, Darmstadt, Germany) for enumeration of the heterotrophic bacteria, fungi and actinomycetes, respectively. The incubation period was 72 h, except for actinomycetes, which was 15 days. The incubation temperature was 28 and $50^\circ C$.

2.4. Degradation experiments

The detection of the cellulolytic microorganisms was performed by screening the endoglucanase activity (CMCase) (Teather and Wood, 1982). The test substrate was carboxymethylcellulose (Sigma), which was incorporated as the sole carbon source into the mineral agar

medium at 0.1%. The incubation time was 3 days at 28 or $50^\circ C$.

Phenol-degrading microorganisms were detected in mineral agar medium (Pettigrew et al., 1990) with caffeic or syringic acids at a final concentration of 200 mg l^{-1} . Caffeic and syringic acids were used as test substrate and were added to the mineral medium after sterilization by filtration. The plates were incubated at 28 or $50^\circ C$ for 3–5 days.

2.5. Strain identification

The characterization and identification of the isolated bacterial strains were based on colony morphology, gram staining, spore formation, catalase and cytochrome oxidase enzyme activities. Several physiological characteristics were also determined by the Analytical Profile Index (API system), with commercial test kits, namely Api 20 E, Api 20 NE and Api 50 CHB (bioMérieux Vitek Inc., Marcy-l'Étoile France).

3. Results and discussion

Under optimal conditions, composting proceeds through three phases: the mesophilic phase, the thermophilic phase, which can last from a few days to several months, and the cooling and maturation phase which lasts for several months (Tuomela et al., 2000). Concomitantly, the waste went through physico-chemical and microbial changes that at the end of the composting process will result in a rich-humus complex mixture, known as compost.

In the present study, olive husks were supplemented with grape stalks, which are a low cost residue and the most abundant one in the region where the husks are produced. The addition was made to decrease the moisture content and to create a higher amount of aeration channels in the piles.

Table 1 presents the average values of the chemical parameters analysed in olive husks samples and in grape stalks. Due to the high total organic carbon to total nitrogen ratio of both wastes, the initial mixture had a C/N ratio of 46, higher than that recommended for composting. Values between 25 and 35 are referred in the literature as being the most favourable for an efficient composting process (Tomati et al., 1996; Paredes et al., 1996; Barrington et al., 2002; Charest and Beauchamp, 2002).

3.1. Temperature patterns

The temperature within the piles was measured daily throughout the process and the temperature profiles of both piles are shown in Fig. 1. These results allow the identification, in both piles, of three different phases

Table 1
Chemical characterization of olive husk and grape stalk

Parameters	Olive husk		Grape stalk	
	Average	Range	Average	Range
pH (H ₂ O)	4.9	4.6–5.2	4.9	4.6–5.2
Conductivity (1:10, 25 °C) (mS cm ⁻¹)	1.78	1.50–2.28	2.11	2.00–2.24
Humidity (% f.w.)	61.8	58.3–66.9	8.6	7.3–10.4
Ashes (% f.w.)	2.6	2.0–3.4	5.2	4.4–6.6
MO (% f.w.)	97.4	96.6–98.0	94.8	93.4–95.3
TOC (% d.w.)	55.9	43.7–72.1	51.5	50.4–52.2
Total nitrogen (% d.w.)	1.05	0.95–1.17	1.19	1.08–1.27
C/N ratio	53	45–66	43	41–46
Lignin (%) (d.w.)	41.2	39.4–43.7	55.4	55.3–55.5
Fats (% d.w.)	3.76	0.98–8.12	n.d.	n.d.
Phenolic compounds (% d.w.)	0.54	0.33–0.81	n.d.	n.d.

The results are the average of three independent analyses on five olive husk samples and three grape stalk samples. For each sample, standard error <5% (fats) and <1% (remaining parameters).

n.d.: not determined.

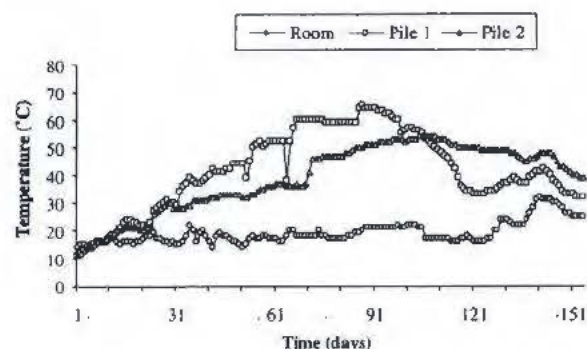


Fig. 1. Temperature profiles in the two piles along the composting process. (Pile 1—aeration by mechanical turning; pile 2—forced aeration.)

occurring sequentially in time, namely a mesophilic, a thermophilic and a maturation phase.

In pile 1, where mechanical turning provided the aeration, it was observed a progressive and smooth temperature increase. After the 4th turning (day 53), the temperature rose more rapidly and reached a maximum value of 65 °C in the thermophilic phase. This phase lasted for about one month with temperatures in the range of 60–65 °C.

Following this period the decrease in temperature was rapid and the process was considered complete after 4 months, when values close to room temperature were reached. Then, the mixture was allowed to rest for more 2 months in order to complete the maturation phase.

In pile 2, during the initial phase, the temperature increased more slowly than in pile 1. Since after 2 months it appeared to have stabilized at around 40 °C, the mixture was turning mechanically on day 71. It is worth note that the temperature difference between the two piles was about 20 °C, at that time.

A temperature increase was then verified, the thermophilic phase being rapidly reached with a maximum temperature of 54 °C. Several authors have reported the re-activation of the composting process in aerated static piles, immediately after a mechanical turning reflected by a marked temperature increase, which is explained by the incorporation of external material into the pile, providing degradable substrate for the microbial biomass (Brodie et al., 2000; Paredes et al., 2002; Garcia-Gomez et al., 2003).

The following phase of temperature decrease was again slower than in pile 1 and room temperature was reached only after five more months (Fig. 1).

Temperature has been widely recognized as one of the most important parameters in the composting process (Mckinley and Vestal, 1985; Strom, 1985). Moreover, temperature has been correlated with microbial activity (Tiquia et al., 1996) and with most of the important compost properties, including C/N ratio and pH between others (Tiquia et al., 1998).

In this study two main features were observed: a very slow beginning of the composting process, reflected in a 13 day-period where the temperature in both piles was not significantly different from the room temperature (IC 95%—room: 14.97–16.41, pile 1: 13.75–16.55 and pile 2: 13.88–17.20, for $n = 13$) and a long mesophilic phase in both piles with a very slow temperature increase. This was particularly truth in pile 2, where only after 85 days, a temperature of 50 °C was attained.

According to several authors high initial C/N (optimal values between 25 and 35) will cause a slower beginning of the process and the required composting time to be longer than usual (Madejon et al., 1998a; Tuomela et al., 2000).

In fact, Madejon et al. (1998a) studying the composting of olive husks from the two-phase process, supplemented with wheat straw, with an initial C/N ratio of about 47, added urea to reach a C/N ratio of about

35–40. However, the same authors referred that if the lignin component due to chopped stones was excluded, the initial ratio fell to 29. In this case, the very slow beginning of the composting process could not be explained by the high C/N ratio and other factors may be involved.

In this study, the particular evolution of the temperature pattern might be related with the negative synergistic effect of some initial parameters as the low pH and the high fat content (Fig. 2 and Table 2), contributing this last one to inhibit the oxygenation and to difficult the absorption of water to the pile, as referred by Garcia-Gomez et al. (2003).

Comparing both piles, aeration provided by mechanical turning appears to be more effective than air forced injection in terms of time required to attain the thermophilic phase, maximum temperatures reached and length of time to reach the maturation phase.

Similar results have been described in the literature. Poultry manure amended with sphagnum peat was composted by natural, forced and passive aeration (Fernandes and Sartaj, 1997). The results showed that in

the case of forced aeration the temperature stayed above 55 °C less time than in natural and passive aeration and longer time was necessary to reach this temperature in forced aerated pile.

Diaz et al. (2002a) compared the composting efficiency of vinasse and cotton gin using two different aeration systems: static aerated pile and turned pile, and found differences in temperature changes between both composting systems; a faster increase of temperature in the turned pile (54 °C at 7 days) than in the static pile (45 °C at 21 days) was observed. The same authors, in a similar work (Diaz et al., 2002b) but using a composting mixture of beet vinasse and grape marc reported that the thermophilic phase was longer in the aerated static pile (25 days) than in the turned pile (10 days).

3.2. Chemical monitoring

Some of the most representative chemical parameters were analysed during the composting process and are shown in Table 2.

The biodegradation of organic matter occurred in both piles, especially what concerns the content in phenolic compounds and fats. For phenols, which are an important component of olive husks, a reduction of about 83.7% and 77.6% respectively in pile 1 and pile 2, was achieved in the end of the mesophilic phase. A marked decrease was also observed in the lipid content with a reduction of about 75% in both piles at the end of the thermophilic phase. Madejon et al. (1998a) obtained similar results working with a mixture of olive husks amended with wheat straw that were composted in a static pile, reporting a reduction of about 100% for phenols and 60% for lipids during the thermophilic phase. Filippi et al. (2002) in a composting experiment performed with olive husks amended with poplar sawdust and bark chips reported a rapid reduction of the lipidic and phenolic fractions

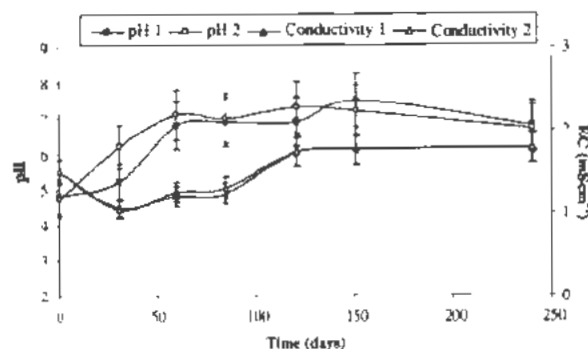


Fig. 2. Evolution of pH and electrical conductivity in the piles along the composting process (Pile 1: aeration by mechanical turning; pile 2: forced aeration).

Table 2
Chemical parameters during the composting process in piles 1 and 2 (dry weight basis)

Day	Temperature (°C)	Fats (%)	Phenolics (%)	Ashes (%)	C/N	Lignin (%)	Cellulose (%)	Holocellulose (%)
Pile 1								
0	10	8.12	0.49	2.56	46	43.7	40.8	63.2
31	28	9.49	0.20	2.23	56	47.1	44.2	68.8
72	60	4.34	0.08	3.58	36	48.5	44.6	69.2
121	34	2.03	0.06	4.47	25	50.2	42.4	64.5
240	28	0.40	0.05	5.79	17	60.5	53.5	68.9
Pile 2								
0	10	8.12	0.49	2.56	46	43.7	40.8	63.2
31	29	8.89	0.13	2.49	45	49.1	44.9	70.0
90	51	4.83	0.11	4.53	40	51.5	50.5	76.3
125	45	1.81	0.06	4.44	37	47.7	50.0	71.7
240	28	0.31	0.06	5.32	18	59.5	53.7	68.0

The values are the mean of at least three independent analyses with a standard error <5% (fats, cellulose and holocellulose) and <1% (remaining parameters).

The increase that was observed in ash concentration clearly shows that effective organic matter degradation occurred during the composting process. Organic matter (OM) losses can be calculated based on the initial (X_1) and final (X_2) ash contents, according to the formula (Paredes et al., 2000):

$$\text{OM losses (\%)} = 100 - 100[X_1(100 - X_2)]/[X_2(100 - X_1)]$$

There were indeed OM losses of 57% and 53% in piles 1 and 2 respectively from the initial process stage to the final maturation phase. This variation is consistent with the pronounced decrease in the C/N ratio, mainly in the maturation phase, where values of 17 and 18 were obtained for piles 1 and 2, respectively.

With similar initial values for C/N ratio (40–45) Madejon et al. (1998a) and Filippi et al. (2002) obtained values of about 30 at the end of the composting process of olive husks. Madejon et al. (1998a) related this high value with the presence of the grinded stones, hardly to be microbiologically degraded.

The lower ratio obtained in this work is explained by the fact that the amount of carbon is reduced by way of partial conversion to CO_2 , while nitrogen continues to be recycled (Golueke, 1992).

The decomposition of OM brought about an increase in pH and EC in both piles (Fig. 2). Values of pH increase from 4.8 (pile 1) and 4.7 (pile 2) to values close to neutrality at the end of the thermophilic phase in both piles. As referred by Paredes et al. (2000), the increase in pH occurred as a consequence of the biodegradation of acid-type compounds such as those with carboxylic and phenolic groups and the mineralization of organic compounds, such as proteins, amino acids and peptides to inorganic compounds. Increase in EC has been reported by several authors (Madejon et al., 1998b; Paredes et al., 2000) being correlated with water evaporation and the consequent salt concentration.

No degradation of lignin, cellulose and hemicellulose was detected throughout the process (Table 2). The non-biodegradation of lignin could be related to the fact, besides its relatively low biodegradability, that this component of olive husks is due to chopped stones and presents a reduced surface to microbial attack, as referred before. Low levels of lignin degradation (about 20%) were obtained in the composting of this waste (Madejon et al., 1998a). An increase in the lignin content in parallel with the composting process of olive oil wastewater mixed with other wastes was reported by Monteoliva-Sánchez et al. (1996).

Concerning cellulose and hemicellulose, the lack of microbial attack of these polymers may be related with nutrient limitation, particularly nitrogen, stressed by the long mesophilic phase that occurred in both piles.

In order to assess the compost stability some humification parameters were analysed and it was verified that the degradation of organic matter corresponded to

an increase in the humification degree. In fact, the humification degree increased from 69% to about 94% in pile 1 and to 83% in pile 2. This observation is in agreement with the increase in the germination index from 1.0% to 42.4% and to 53.7% in piles 1 and 2 respectively, at the end of the thermophilic phase, indicating a relevant decrease in substrate phytotoxicity. The results obtained at the end of the maturation phase showed a complete loss of toxicity.

3.3. Microbial monitoring and degradation studies

Raw olive husks were predominantly colonized by mesophilic bacteria, including actinomycetes and mesophilic fungi (Table 3). At the end of the composting process, in both piles, an increase in the population of all the mesophilic groups was detected, ranging from 10^5 – 10^7 /g husk in the beginning of the process to 10^8 – 10^9 CFU/g husk at the end.

Although in low number, thermophilic microorganisms were firstly detected in pile 2 and this fact is coincident with the increase in pH to values close to neutrality observed in this pile, what did not occur in pile 1 (Fig. 2).

An interesting finding is the fact that thermophilic CMC degraders follow a pattern similar to that displayed by thermophilic fungi as can be seen in Tables 3 and 4. Moreover, their number is closely coincident in both piles, suggesting that, in the present case, thermophilic CMC degraders could belong to that group of microorganisms.

The high number of colonies detected with CMCase activity did not correlate with a decrease in cellulose and hemicellulose contents (Table 2). This fact can be related with the model substrate used. Carboxymethylcellulose is a water-soluble substrate and could lead to false positive degraders.

Mesophilic phenol degraders were present in a growing number during the composting process, following a pattern close resembling that displayed by the mesophilic bacteria. This group of microorganisms seem to be the main responsible for the removal of phenolic compounds, since during the mesophilic phase a reduction of about 60% and 73% was attained for piles 1 and 2, respectively. Three bacterial strains were isolated as phenol-degraders and were identified as *Pseudomonas aeruginosa* and *Ralstonia pickettii* (syringic acid) and *Pseudomonas cepacia* (caffeic acid).

The composition of the microbial populations in both piles did not show significant differences in qualitative and quantitative terms, namely in respect to the number of bacteria, actinomycetes and fungi. The predominant microbial strains that were present along all of the composting process were identified as *Ps. testaceorom*, *Ps. putida*, *Bacillus* sp., *Aspergillus* sp. and *Cladosporium* sp.

Table 3
Log₁₀ numbers/g husks (fresh weight basis) of total mesophilic and thermophilic microorganisms in the different phases of the composting process

Days	Temperature (°C)	M. bacteria	M. actinomycetes	M. fungi	T. bacteria	T. actinomycetes	T. fungi
<i>Pile 1</i>							
0	10	8.04	7.76	8.00	n.d.	n.d.	n.d.
31	28	8.18	8.00	7.70	n.d.	n.d.	n.d.
72	60	9.08	9.00	8.42	8.00	7.48	7.00
121	34	9.63	8.34	8.83	6.00	7.34	6.00
174	33	9.45	8.52	8.62	7.85	6.48	n.d.
240	28	10.00	8.78	9.15	8.95	7.60	7.00
<i>Pile 2</i>							
0	10	8.04	7.76	8.00	n.d.	n.d.	n.d.
31	29	9.20	9.00	7.72	5.90	5.00	5.00
90	51	9.04	8.73	9.40	8.42	5.00	7.11
125	45	9.54	9.48	8.08	8.78	7.00	7.60
195	32	9.53	8.60	8.85	8.18	6.60	7.00
240	28	10.45	8.85	9.56	8.96	7.95	7.00

The values are the mean of three independent analyses with a standard error $\leq 10\%$.

M—Mesophilic; T.—Thermophilic; n.d.—not detected under the experimental conditions.

Table 4
Log₁₀ numbers/g husks (fresh weight basis) of phenolic and carboxymethylcellulose degraders (mesophilic and thermophilic microorganisms) in the different phases of the composting process

Days	Temperature °C	M. caffeic acid degraders	M. syringic acid degraders	M. CMC degraders	T. caffeic acid degraders	T. syringic acid degraders	T. CMC degraders
<i>Pile 1</i>							
0	10	7.30	7.38	n.d.	n.d.	n.d.	n.d.
31	28	6.95	7.71	5.30	n.d.	n.d.	n.d.
72	60	7.30	8.38	7.48	n.d.	8.30	7.70
121	34	9.08	9.26	8.92	7.48	7.85	7.00
174	33	9.43	9.38	7.08	6.00	7.45	n.d.
240	28	8.95	9.56	8.78	7.85	8.34	7.30
<i>Pile 2</i>							
0	10	7.30	7.38	n.d.	n.d.	n.d.	n.d.
31	29	8.70	8.54	7.00	n.d.	n.d.	5.00
90	51	8.28	7.70	7.78	7.36	7.59	7.00
125	45	8.15	9.70	6.70	7.28	9.00	7.30
195	32	8.40	9.20	8.12	7.30	7.96	7.83
240	28	9.54	9.92	9.00	7.95	8.54	7.30

The values are the mean of three independent analyses with a standard error $\leq 10\%$.

M—Mesophilic; T.—Thermophilic; n.d.—not detected under the experimental conditions.

In the thermophilic phase *Penicillium* sp., *Aspergillus* sp. and *Serratia marcescens*, thermophilic strains generally presents in this composting phase (Tuomela et al., 2000; Heerden et al., 2002), were also isolated. The bacterial isolate was identified with excellent identification to the genus level (API 20 E). All strains belonging to *Serratia* group occur in the natural environmental and can grow at temperatures between 10 and 36 °C at pH 5–9. The strains are facultative anaerobic and produce extracellular enzymes that hydrolyze DNA, lipids and proteins. Ortho-nitro-phenyl-galactoside (ONPG) is hydrolyzed by most strains (Holt et al., 1984).

In the maturation phase, when the temperature decreased to 28 °C, the microbial diversity increased,

having been identified in this phase the bacterial strains *Ps. paucimobilis* and *Ps. maltophilia*.

Table 5 presents the results of bacteriological, biochemical and growth tests for the gram-negative bacterial strains (API 20 NE). These strains are included in the genus *Pseudomonas* that can be characterized by its high nutritional versatility showing the ability for growth in simple mineral media at expense of a single carbon compound as sole carbon sources. In addition to low molecular weight compounds, a variety of macromolecules can be degraded by means of extracellular enzymes. Many fatty acids and aromatic compounds can be used for growth by *Pseudomonas* species (Holt et al., 1984). Gram-positive bacteria, *Bacillus* sp., were present along the whole composting process. They are

Table 5
Identification of gram-negative strains predominated in the composting process

Characteristic	<i>Ps. testosteroni</i>	<i>Ps. putida</i>	<i>Ps. paucimobilia</i>	<i>Ps. maltophilia</i>
Gram reaction	–	–	–	–
Morphology	Rods	Rods	Rods	Rods
Endospore formation	–	–	–	–
Oxidase test	+	+	±	±
Reduction of nitrates to nitrites or to nitrogen	–	–	–	–
Indole production	–	–	–	–
Glucose acidification	–	–	–	–
Arginine dihydrolase	–	+	–	–
Urease test	–	–	–	–
Esculin hydrolysis	–	–	+	+
Gelatin hydrolysis	–	–	–	+
β-Galactosidase	–	–	+	+
Glucose assimilation	–	+	+	+
Arabinose assimilation	–	+	+	+
Mannose assimilation	–	+	+	+
Mannito. assimilation	–	–	+	–
N-acetyl-glucosamine assimilation	–	–	+	+
Maltose assimilation	–	–	–	+
Gluconate assimilation	–	+	–	–
Caprate assimilation	+	+	–	–
Adipate assimilation	–	–	–	–
Malate assimilation	+	+	+	+
Citrate assimilation	+	+	–	+
Phenyl-acetate assimilation	–	+	–	–

Ps. *Pseudomonas*, (+), present, (–), absent, (±), weakly positive

capable of producing endospores, which are very resistant to heat, radiation and chemical disinfection (Houg, 1993), becoming metabolically active when a suitable substrate is made available and play an important role in the biological cycling of carbon and nitrogen. These bacterial groups are typical of compost environments and different species of *Pseudomonas* and *Bacillus* have been isolated during this biological process (Tuomela et al., 2000; Hassen et al., 2001).

In the present study, and conversely to some literature (Golueke, 1992; Beffa et al., 1996; Tuomela et al., 2000; Hassen et al., 2001), it was not visible a significant succession between the mesophilic and thermophilic populations. The reason for this pattern is probably related with the temperature evolution. The length of the mesophilic and thermophilic phases and peak temperature seem to have a marked effect in the number and diversity of the microbial population.

As described by Tiquia et al. (2002) during the composting of yard trimmings the number of total aerobic heterotrophs, actinomycetes and fungi decreased dramatically when pile temperature started peaking at 70 °C. Also, Heerden et al. (2002) founded significant differences in population densities at 5 and 30 cm within citrus waste composting pile, corresponding to important differences in temperature profiles, being reported values between 60 and 70 °C only at 30 cm within the pile.

4. Conclusions

Despite the high humidity and consequent compact structure of olive oil husk, this study showed that the composting of this agriculture residue in piles is feasible and it takes about 6 months to complete. However, before the process can develop efficiently, it is necessary to add a structuring agent and promote an adequate aeration of the mixture.

When the aeration system by mechanical turning is compared with the forced aeration provided by a ventilator, the former appears to be more at an industrial scale. In fact, although there were no significant differences in terms of chemical parameters for both processes, mechanical turning allowed for higher temperatures, corresponding to a more rapidly developing process and to higher humification efficiency. Furthermore, this process is energetically more economic since it requires reduced workmanship and a low initial investment.

The microbial results showed that the microbiology of the compost is quite complex, being very difficult to correlate the microbial populations isolated during the different phases of the process with their role in the degradation of some of the chemical substances present in the husk. To understand this correlation, composting phases/microorganisms/biodegradation, other methodologies (molecular technology, PLFA profiles, biochemistry and immunology) should be used, which will

undoubtedly allow for the development of a new understanding of this complex and fascinating biological process.

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Measuring detoxification and maturity in compost made from “alperujo”, the solid by-product of extracting olive oil by the two-phase centrifugation system

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Abstract

Olive-mill wastes and by-products from the edible olive oil industry contain a high non-stabilised organic load, including organic acids, phenolic compounds and fats with antimicrobial and phytotoxic properties, which make them unsuitable for direct agricultural application. The most abundant olive-mill by-product in Spain is “alperujo” (AL), a solid material with a lack of consistency and low porosity due to its high water content and small particle size, which can be suitably composted by adding bulking agents. Six piles were prepared by mixing AL with cotton waste, grape stalk, olive leaf and fresh cow bedding, then successively composted, five of them managed by forced ventilation assisted by mechanical turning and the sixth only turned. After monitoring the process, the relationship between the germination index (GI) and the presence of some potentially phytotoxic compounds, as well as several maturity and stability indices, was assessed. As composting advanced, the GI increased and both the fat and water-soluble phenol contents decreased, indicating a gradual detoxification as the composts matured. The values of the maturity and stability indices were frequently different from the values reported for other composts.

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Keywords: Composting; “Alperujo”; Phytotoxicity; Germination index; Maturity and stability indices

1. Introduction

The agricultural food industry generates a great variety and quantity of wastes and by-products, which can potentially be used as soil fertilisers and amendments due to their high contents of organic matter and plant nutrients.

Most countries of the Mediterranean Basin annually produce vast quantities of olive-mill wastes and by-products from the edible olive oil industry, whose high content of non-stabilised organic load, which includes organic acids, phenolic compounds and fats with antimicrobial and phytotoxic properties, may affect the physical, chemical and biological properties of a soil if applied directly

(González et al., 1990; Riffaldi et al., 1993; Martín et al., 2002). Following the large-scale introduction of the new two-phase centrifugation system, which greatly reduces the generation of pollutant wastewater compared to the classical three-phase system, “alperujo” (AL) has become the most abundant by-product of the Spanish olive oil extraction industry and its current disposal represents a great economic and technical problem for producers. AL is a solid material with a lack of consistency and low porosity due to its high water content and small particle size, which makes it difficult and expensive to handle and transport, and even to extract the residual oil it contains.

Much research has been carried out into the composting of olive-mill wastewater from the three-phase system (Tomati et al., 1995; Paredes et al., 2000), the solid cake resulting from both press and three-phase systems (Calvet

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et al., 1985; Ait Baddi et al., 2004), the flocculated solid fraction of olive-mill wastewater (Negro and Solano, 1996; García Gómez et al., 2003) and AL (Madejón et al., 1998; Filippi et al., 2002; Baeta Hall et al., 2005), as a result of which valuable final products potentially suitable as soil organic fertilisers and amendments have been obtained. Due to the above-mentioned deficiency of physical structure in AL, several organic wastes were added as bulking agents in the above experiments to improve composting.

Before a compost can be used safely in agricultural or horticultural production, its stability and maturity should be assessed. Stability is usually defined in terms of the bioavailability of the organic matter, referring exclusively to the resistance of the compost organic matter to further degradation (Brewer and Sullivan, 2003). Materials with a high proportion of bioavailable organic matter generate a high level of microbial activity and are considered unstable. Maturity is defined, loosely, as the suitability of the material for plant growth and often has been associated with the degree of compost humification (Cooperband et al., 2003).

Phytotoxicity is one of the most important criteria for evaluating the suitability of organic materials for agricultural purposes. Also, testing the phytotoxicity of composting substrates is a valuable way of evaluating the stage of the process reached (decomposition, stabilisation and curing phases) as well as its efficiency and conditions. Phytotoxicity has been related mainly to the presence of different compounds in the added organic matter, such as phenolic substances, organic acids, ammonia/ammonium and heavy metals. The germination index (GI), which combines the measure of relative seed germination and relative root elongation of cress seed (*Lepidium sativum* L.), has been used widely to evaluate both the phytotoxicity and maturity of compost (Zucconi et al., 1981a,b; Bernal et al., 1998; Wong et al., 2001).

This study examines the relationship of the GI with the content of some potentially phytotoxic compounds during AL composting, and with other parameters including several indices commonly used to define compost maturity and stability.

2. Materials and methods

2.1. Composting performance

Six trapezoidal piles (approximately 1.5 m high with a 2 × 3 m base) of about 3 tons (piles 1–4) and 4 tons (piles 5–6) were prepared by mixing three selected AL samples (AL1, AL2 and AL3) with four bulking agents (CW, cotton waste; GS, grape stalk; OL, olive leaf and FCB, fresh cow bedding) on a fresh weight basis (dry weight basis in brackets) as indicated below:

- Piles 1 and 2: 92.6% AL1 + 7.4% CW (80/20).
- Pile 3: 94.6% AL2 + 5.0% GS + 0.4% urea (87/12/1).
- Pile 4: 94.6% AL2 + 5.0% OL + 0.4% urea (87/12/1).

- Piles 5 and 6: 90% AL3 + 9% FCB + 1% mature compost from pile 4 (87/11/2).

In piles 1, 2, 5 and 6, the initial values of the C/N ratio ranged from 33.1 to 34.4, whereas in piles 3 and 4 they were much greater than those recommended for the composting process: thus, they were lowered to a similar value by adding urea (36.6 and 35.3, respectively).

Three composting experiments were carried out in a pilot plant during a period of nearly 3 years (piles 1, 2, 3–4 and 5–6, successively) using the Rutgers strategy (Finstein et al., 1985) combined with mechanical turning, except in pile 5, which was only turned. Forced ventilation of the piles was provided during the bio-oxidative phase (active phase) of the process through a blower (1/6 HP), operated on a timer-schedule with on/off cycles of 0.5 min/14.5 min in piles 1 and 2, 5 min/15 min in piles 5 and 6 and, in piles 3 and 4, 1 min/5 min during the first three months and 0.5 min/29.5 min during the rest of the bio-oxidative phase. Despite the addition of bulking agents, the use of the static pile system alone was shown at an early stage to be unsuitable for AL composting due to the deficient physical structure of the resulting substrates, which restricted the oxygen distribution within the composting mass. In fact, the static system favoured substrate compaction and the subsequent low porosity and deficient air distribution caused by the blockage of pores meant that forced ventilation was clearly insufficient to maintain the correct aerobic conditions. This explained the need for mechanical turning in addition to the forced ventilation, since, in this way, substrate oxygenation would be improved by reducing compaction and increasing porosity, at the same time homogenising and re-inoculating the substrates. Thus, the piles composted first (1–2) were turned in the eighth week; those processed next (3–4), in the fourth, tenth and twenty first weeks; whereas piles 5 and 6 were turned fourteen times during the entire process. The bio-oxidative phase was considered finished when the pile temperature was stable and close to that of the atmosphere. This occurred after 26 weeks (piles 1, 2, 5 and 6) or 34 weeks (piles 3 and 4); then, air blowing and turning were stopped to allow the mixtures to mature.

In order to avoid high temperatures that might limit the process, the ceiling temperature for continuous air blowing was 50 °C (pile 1), 60 °C (pile 2) and 55 °C (piles 3, 4 and 6). The moisture content was controlled periodically and maintained at 40–55% by adding the necessary amount of water and the excess water leached from the piles was recirculated.

Composting materials were sampled weekly during the bio-oxidative phase and then at the end of the maturation period, from randomised sites around the pile. The representative samples were homogenised and subdivided into three sub-samples in the laboratory. One of them was frozen (–20 °C) and kept for the determination of inorganic nitrogen (NH_4^+ and NO_3^-), the second was dried in an oven at 105 °C for 24 h to determine its moisture content

and the third sub-sample was freeze-dried and ground to less than 0.5 mm prior to analysis.

2.2. Analytical methods

The water-soluble organic carbon (WSC) was determined, for a 1:20 (w/v) deionised water extract, by using a Skalar-Formacs^{HT} TOC analyser; NH_4^+ was extracted from the previously-stored frozen sub-samples with 2 M KCl and determined by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992) while NO_3^- was measured by using an ion-selective electrode in the water extract; organic nitrogen (ON) was calculated as the difference between total nitrogen (TN), determined using a Carlo-Erba NA 1500 CNS elemental analyser for solid samples, and inorganic nitrogen; cation exchange capacity (CEC) was determined with BaCl_2 -triethanolamine (Lax et al., 1986) and the volatile solids, which reflect the organic matter content (OM), by loss on ignition at 430 °C for 24 h. Other parameters, including pH, total organic carbon (TOC), lignin, cellulose, fat content, water-soluble phenols (WSPH) were determined according to the methods previously described by Albuquerque et al. (2004). OM losses were calculated according to the equation of Stentiford and Pereira Neto (1985), taking into account the apparent

increase in the ash content resulting from the loss of dry matter: $\text{OM-loss (\%)} = 100 \times [(\text{OM}_i/\text{Ash}_i - \text{OM}_t/\text{Ash}_t) / (\text{OM}_i/\text{Ash}_i)]$, where OM_i and OM_t are the organic matter contents in the sample initially and at time t , respectively, and Ash_i and Ash_t are the ash contents of the sample initially and at time t , respectively.

Phytotoxicity was evaluated by modifying the original method of Zucconi et al. (1981b), which combines the measurements of seed germination and root elongation of cress (*Lepidium sativum* L.). GI measurements were made by using water extracts obtained as follows: samples (4 g of dry material) were moistened to 60% moisture content; after being allowing to stand for 30 min, more deionised water (54 ml) was added and the samples were stirred mechanically for 30 min and then filtered through 0.45-µm Whatman filter papers. Eight cress seeds were evenly distributed on filter paper in Petri dishes (10-cm diameter) and moistened with 1 ml of the obtained extract. Ten replicates per sample were incubated in an oven for 48 h at 27 °C in dark conditions; later, the number of germinated seeds and their root length were measured and results expressed as a percentage of germination and root elongation of a control (seeds moistened with 1 ml deionised water and incubated in the same conditions) as follows: $\% \text{GI} = (\% \text{germination} \times \% \text{root elongation}) / 100$.

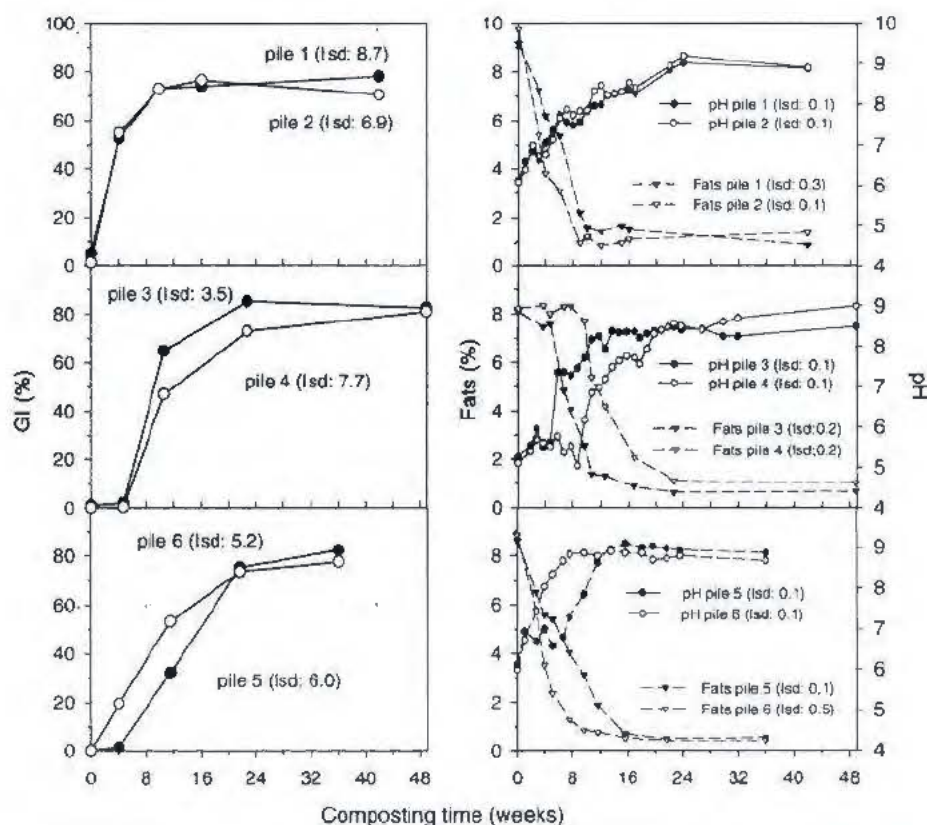


Fig. 1. Evolution of the germination index (GI), fat content and pH during AL composting (LSD: least significant difference at $P < 0.05$).

2.3. Statistical analyses

Basic statistical analyses of data and correlation coefficients were made using the SPSS 11.0 program for Windows. Analysis of variance and the least significant difference were calculated for the results of the composting samples, to determine changes in the parameters with time.

3. Results and discussion

3.1. Phytotoxicity

In our piles, very low initial GI values were recorded in the early composting stage (<2%, Fig. 1), suggesting a severe phytotoxicity in the substrates. In piles 1 and 2, which showed a similar behaviour throughout the process, the GI had increased by over 50% by the end of the first month, reaching the highest values during the thermophilic period (Fig. 2). In contrast, no increases were detected in piles 3 and 4 during the first month of composting, coinciding with the low temperatures provoked by the difficulty in aerating the substrates in this experiment, whose physical

structure was hardly improved by the bulking agents added to AL. However, an immediate increase in the GI followed the first turning; this increase was delayed in pile 4 as was the evolution of other parameters, such as temperature (Fig. 2), OM-loss (Table 1), pH and fat content (Fig. 1), due to the extremely deficient aeration conditions induced by the olive leaves used as bulking agent in pile 4. In addition, this material could have contributed to increasing phytotoxicity through the formation of low-molecular weight organic acids, as was reported by Manios et al. (1989). After the third month, the GI was higher than 50% in both piles but always remained lower in pile 4 until the end of composting. Moreover, the GI values were lower in pile 5 (only mechanical turning) than in pile 6 (both forced ventilation and mechanical turning) almost throughout the entire bio-oxidative phase, which might have been related to the comparatively restricted oxygen supply to pile 5, again coinciding with similar behaviours of pH, OM-loss and fat content. The acid pH, the delay in both OM and fat biodegradation and the temperature decrease all resulted from the lack of the fully-aerobic conditions required for composting. Phytotoxin production is a characteristic of the initial stage of OM decomposition, the intensity and duration of this process being mainly dependent on oxygen availability (Zuccconi et al., 1985). As composting advances, different experiments (Fiquia et al., 1997; Wong et al., 2001) have shown that GI values increase, particularly when an adequate oxygen supply is used. In fact, phytotoxic decomposition products are liberated and accumulated under deficient-oxygenation conditions (Patrick, 1971; Harper and Lynch, 1982; Zuccconi et al., 1985; Michel and Reddy, 1998). However, these compounds are increasingly degraded by the intense aerobic microbial activity developed under appropriate temperature, oxygen, water and nutrient conditions.

Several experiments performed with olive-mill wastes and by-products have linked phytotoxicity to organic acids of low-molecular weight, lipids and phenols (Estaún et al., 1985; Madejón et al., 1998; Filippi et al., 2002; Linares et al., 2003; Ait Baddi et al., 2004). Throughout our composting experiments, the increases of pH and total decreases of fats (7–20 fold) and WSPH (2–4 fold) contrasted with the above-mentioned increases in GI. Thus, the water extracts employed to determine GI at the beginning of our experiments could have included lipidic compounds, either emulsified or partially solubilised, which would have contributed to low GI values by diminishing the moisturising effect on the cress seeds. In fact, Dinel et al. (1996) revealed that lipids may influence negatively the hydrological properties of growing substrates, thus reducing biological activities such as germination and seedling growth. As regards WSPH, it has been reported (Greco et al., 1999) that the oxidoreductive enzymes contained in AL may remove phenols by oxidising low-molecular weight phenolic compounds, which polymerise to produce less soluble substances. Also, it has been claimed (Martínez Nieto et al., 1993) that several microbial groups

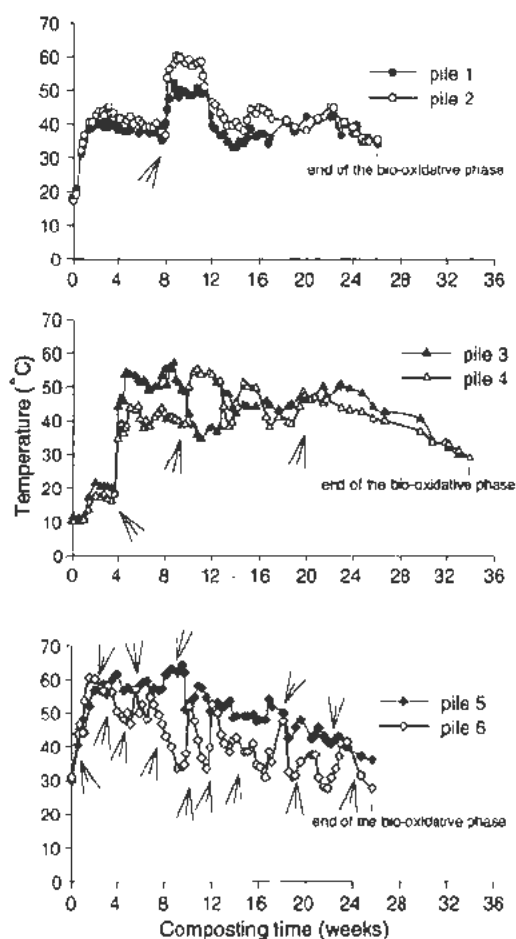


Fig. 2. Temperature profile during the bio-oxidative phase of composting (arrows indicate turnings).

Table 1
Values of several composting parameters

Pile	Time (weeks)	OM-loss (%)	WSPH (g kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	WSC (g kg ⁻¹)	WSC/ON	CEC ^a (meq 100 g ⁻¹)	CEC/TOC (meq g ⁻¹)
1	0	0.0	5.7	279	40.7	2.74	52.2	0.94
	4	6.5	4.0	310	32.0	1.85	42.9	0.78
	10	20.6	4.0	459	31.4	1.76	60.1	1.12
	16	37.3	2.8	417	29.7	1.35	69.1	1.26
	42	40.5	2.2	90	31.4	1.46	87.9	1.61
	LSD	3.0	0.2	55	1.4	0.13	6.7	0.14
2	0	0.0	6.1	306	44.4	2.91	54.6	0.98
	4	1.8	5.0	297	34.5	2.16	44.9	0.84
	10	18.7	4.2	648	26.8	1.46	63.9	1.18
	16	34.7	3.4	443	26.0	1.19	76.4	1.39
	42	43.0	3.4	86	29.6	1.36	95.3	1.73
	LSD	7.8	0.7	41	2.6	0.17	3.6	0.06
3	0	0.0	14.2	146	94.8	7.07	29.2	0.55
	5	4.4	9.0	279	62.4	4.42	39.3	0.75
	11	24.1	7.7	472	31.6	2.29	55.7	1.06
	23	43.1	4.1	281	27.8	1.52	80.6	1.50
	49	50.0	3.3	178	32.7	1.45	103.1	1.96
	LSD	9.7	0.4	30	1.5	0.24	3.7	0.07
4	0	0.0	15.8	131	110.8	7.91	30.3	0.57
	5	3.1	11.3	305	69.3	4.84	37.6	0.68
	11	6.1	8.5	509	43.7	2.69	43.4	0.80
	23	30.6	5.9	447	37.0	1.66	60.4	1.14
	49	34.4	4.1	185	35.0	1.54	77.2	1.45
	LSD	2.8	0.6	37	2.9	0.20	4.0	0.07
5	0	0.0	9.0	1282	74.2	5.70	21.9	0.41
	4	17.7	7.0	761	69.3	4.95	30.1	0.56
	12	30.5	5.4	785	40.0	2.18	53.5	0.98
	22	45.2	2.9	166	26.1	1.22	76.2	1.44
	36	54.1	2.3	114	26.4	1.01	101.5	1.87
	LSD	5.5	0.5	30	4.3	0.28	4.9	0.10
6	0	0.0	9.0	1089	83.6	6.27	27.3	0.51
	4	29.5	6.6	313	65.2	4.06	41.8	0.77
	12	44.9	4.8	362	37.4	1.87	59.8	1.12
	22	51.2	3.4	207	30.4	1.47	73.6	1.37
	36	60.5	2.9	119	27.0	1.03	110.0	2.01
	LSD	1.6	0.3	45	2.3	0.23	13.1	0.22

WSPH, WSC: water-soluble phenols and organic carbon, respectively; ON: organic nitrogen; CEC: cation exchange capacity and TOC: total organic carbon.

^a Ash-free material. LSD: least significant difference at $P < 0.05$.

can contribute to phenol degradation, both processes leading to detoxification of the composting substrates. During maturation, the WSPH content remained nearly constant (Table 1), indicating a final balance between the release of these compounds to the aqueous phase (mainly through lignin degradation) and the above processes.

As shown in Table 2, correlations between values of GI and those of fats, WSPH, NH₄⁺-N and pH (Fig. 1 and Table 1) in different stages of composting have been found. Significant inverse correlations between the GI and both the fat and WSPH contents were obtained in all the piles, and with NH₄⁺-N in pile 5, where the highest NH₄⁺-N concentration was detected throughout the process. These results confirm the inhibitory effect of these components on seed germination, as mentioned above.

The same statistical approach applied to GI and pH also led to significant direct correlations (piles 1, 3, 4 and 5), again in agreement with the preceding discussion. This result may be related to the existence of phytotoxic compounds having an acid character (for example, organic acids) or to the possible inhibition of the microbial population responsible for the degradation of the phytotoxic components at low pH values.

3.2. Maturity and stability parameters

Our composts reached C/N values clearly higher than 12 (Table 3), the limit value established by Iglesias Jiménez and Pérez García (1992b) and Bernal et al. (1998) for composts made, respectively, with city refuse and a wide range

Table 2
Significant correlations between the germination index and other composting parameters (correlated points = 5)

Parameter	Pile 1	Pile 2	Pile 3	Pile 4	Pile 5	Pile 6
pH	0.929*	NS	0.992**	0.986**	0.886*	NS
Fat content (%)	−0.958*	−0.998***	−0.994**	−0.978**	−0.900*	−0.919*
WSPH (%)	−0.898*	−0.919*	−0.881*	−0.937*	−0.968**	−0.989**
NH ₄ ⁺ -N (mg kg ^{−1})	NS	NS	NS	NS	−0.924*	NS
C/N	−0.919*	NS	NS	−0.973**	−0.980**	−0.975**
WSC (%)	−0.961**	−0.980**	−0.907*	NS	−0.962**	−0.990**
WSC/ON	−0.966**	−0.960**	−0.923*	−0.906*	−0.948*	−0.976**
CEC (meq 100 g ^{−1})	NS	NS	0.899*	0.919*	0.977**	0.922*
CEC/TOC (meq g ^{−1})	NS	NS	0.895*	0.920*	0.979**	0.921*
OM-loss (%)	NS	NS	0.970**	0.918*	0.951*	0.961**

NS: not significant.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

Table 3
Maturity and stability parameters in AL composts ($n = 6$, dry weight)

Parameters	Mean	Range	CV (%)
C/N ratio	20.0	16.6–22.7	13.3
(Final C/N)/(initial C/N)	0.58	0.50–0.67	12.9
NH ₄ ⁺ -N (mg kg ^{−1})	129	86–185	33.4
NO ₃ [−] -N (mg kg ^{−1})	74	31–112	50.8
Cellulose/lignin ratio	0.44	0.40–0.52	9.6
OM-loss (%)	47.1	34.4–60.5	20.3
CEC (meq 100 g ^{−1} OM)	95.8	77.2–110.0	12.3
CEC/TOC (meq g ^{−1})	1.77	1.45–2.01	12.2
WSC (g kg ^{−1})	30.3	26.4–35.0	11.1
WSC/ON	1.31	1.01–1.54	17.6
GI (%)	79	71–83	5.8

CV: coefficient of variation; CEC: cation exchange capacity; TOC: total organic carbon; WSC: water-soluble organic carbon; ON: organic nitrogen and GI: germination index.

of organic wastes, thus reflecting the substantial proportion of lignin (around 40%) resisting biodegradation during AL composting. Due to the high resistance of lignin, the cellulose/lignin ratio decreased during the process to 0.5 and below, in agreement with Komilis and Ham (2003), who defined this ratio as a relatively accurate maturity indicator. Also, a mean value of the (Final C/N)/(initial C/N) ratio similar to that proposed for city refuse compost (0.60) by Iglesias Jiménez and Pérez García (1989) was recorded in our case (Table 3).

AL compost showed a quite low mineral nitrogen content, while the common belief is that a good compost should contain substantial quantities of mineral nitrogen, preferably more NO₃[−]-N than NH₄⁺-N. In our case, the latter ranged from 86–185 mg kg^{−1}, clearly lower than the maximum value (400 mg kg^{−1}) suggested by Zucconi and de Bertoldi (1987) and Bernal et al. (1998). Also, a value of the NH₄⁺-N/NO₃[−]-N ratio lower than 0.16 has been suggested as an appropriate maturity index Bernal et al. (1998), although this was of limited use for us due to the low NO₃[−]-N values detected in our experiments.

Two other parameters commonly used as indicators of the progress towards maturity and stability were analysed:

WSC and WSC/ON ratio (Table 1). Both clearly decreased from widely different initial values to values relatively similar and nearly constant, showing the equilibrium between the rates of depolymerisation of biopolymers and the mineralisation of the resultant fractions. However, the values still remained higher than those proposed by Bernal et al. (1998) and Hue and Liu (1995) for WSC (<17 g kg^{−1} and <10 g kg^{−1}) and WSC/ON (<0.55 and <0.70), respectively.

Maturity was also confirmed by the considerable increase in CEC and the CEC/TOC ratio observed during composting (Table 1), changes in both parameters being particularly apparent in the piles showing the greatest OM-losses (3, 5 and 6). Values were much lower in pile 4 than in pile 3, which, once again, might be related to the better aeration conditions existing in the latter. Our CEC values (Table 3) were undeniably higher than the 60 and 67 meq 100 g^{−1} presented respectively by Harada and Inoko (1980) and Iglesias Jiménez and Pérez García (1992b) for city refuse composts, whereas those of the CEC/TOC were relatively close to the minimum values reported for municipal composts (1.9 meq g^{−1}) and manures (1.7 meq g^{−1}) by Iglesias Jiménez and Pérez García (1992a) and Roig et al. (1988), respectively.

Since increasing maturity leads to a decrease in phytotoxicity, the GI was correlated with the maturity and stability parameters, as was shown previously for fats, WSPH, NH₄⁺-N and pH. It can be seen that the GI was inversely correlated with the C/N ratio (piles 1, 4, 5 and 6), with the WSC (piles 1, 2, 3, 5 and 6) and with the WSC/ON ratio, in all the piles (Table 2). Bernal et al. (1998) found that WSC/ON is the most suitable parameter of those studied for describing the maturity and stability of different composts.

In addition, the GI was correlated directly with the CEC and CEC/TOC in piles 3, 4, 5 and 6, reflecting the progressive humification of the organic substrate, since this process produces functional groups leading to increased CEC values, whereas the significant correlations obtained between the GI and the OM-loss in the same piles could

be related to the overall OM biodegradation and the progressive decomposition of phytotoxic compounds.

4. Conclusions

As composting advanced, the GI increased and the concentration of both fat and water-soluble phenols decreased, indicating a progressive detoxification. However, the less oxygen available to substrates, the greater the delay in the increase in GI and the longer the delays in temperature and pH increases and in OM and fat biodegradation.

Detoxification contributed to maturity, as shown when correlating the GI with other parameters commonly used as indicators of the progress of composting towards stability and maturity. The values of these parameters indicate that all the composting substrates reached an acceptable degree of maturity and stability, even if these values were often considerably different from the data reported for other composts, thus indicating the peculiar nature of the AL compost, which showed considerably higher C/N, WSC, WSC/ON and CEC values.

The long composting period observed should be related to the low bioavailability of nitrogen compounds in AL and to the high proportion of resistant components, such as lignin, in comparison to other wastes currently used for composting. For the application of these results to real-scale composting, the economical significance of such a long period, clearly greater than that employed for normal matrices, should be taken into account.

Although all the bulking agents contributed to the composting advance, CW and FCB showed the best results. These composting experiments underline the need for mechanical turning in order to homogenise and re-inoculate the substrates, as well as to re-establish the material's structure, to permit a better-distributed ventilation. The slight delay in the evolution of some composting parameters, observed when only turning was employed, was overcome when forced ventilation was coupled to mechanical turning. However, production costs would be much lower without using forced air, since the compositions of the two end-products were comparable.

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APLICACIÓN AL SUELO DE COMPOST DE ALPERUJO

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DEGRADACIÓN Y TRANSFORMACIÓN DE SUBPRODUCTOS DE ALMAZARA EN PROCESOS DE CO – COMPOSTAJE Y APLICACIÓN COMO ENMIENDA AL SUELO

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Resumen

La doble necesidad de integrar rentabilidad y medio ambiente en cultivos de olivar ecológico ha promovido la búsqueda de soluciones para resolver el problema de escasez de fertilizantes y manejo de subproductos en este sector. Bajo estas condiciones se ha considerado el compostaje del alperujo, principal subproducto de la extracción de aceite de oliva, como una alternativa buena para solventar el problema de manejo de subproductos y obtener una enmienda que mejore sus condiciones de cultivo. Se han realizado unas experiencias previas para compostar el alperujo, mezclándolo con hoja de olivo, estiércol, compost maduro y una harina cárnica. Se ha estudiado durante este proceso la evolución de los procesos más importantes, como temperatura, descomposición y humificación. Se observó que la mezcla que mejor comportamiento tuvo fue la formada con una harina cárnica, ya que alcanzó la madurez más rápidamente, necesitó menos días de descomposición y la calidad físico química que alcanzó fue muy buena. También se observa que la modelización del compostaje con cinéticas de primer orden fue buena. Paralelamente al ensayo de compostaje se realizó una prueba de aplicación de compost de alperujo, mostrando valores esperanzadores de cara al futuro, como enmienda orgánica de calidad, al incrementar la fertilidad del suelo.

Palabras clave: Alperujo, compost, temperatura, carbono orgánico, cinética de descomposición, días de descomposición, humificación, fertilidad.

Introducción

En regiones como Andalucía, que representa más del 60 % de la producción española de aceite de oliva y prácticamente ronda el 30 % de la producción mundial [1], año tras año, se produce un problema de manejo con la gran cantidad de subproductos generados durante esta actividad. Problema acrecentado por el corto espacio de tiempo en que estas industrias desarrollan su actividad, generalmente entre los meses de Noviembre y Febrero. Estos dos factores: cantidad producida y tiempo en el que se desarrolla la actividad, propician que se profundice en el estudio de estrategias que tiendan a minimizar el impacto que, sobre el medio ambiente y sobre la economía de este tipo de industrias, pueda derivar la producción del alperujo, subproducto más importante obtenido durante el proceso de extracción del aceite de oliva por el método de dos fases.

Han sido diferentes las estrategias estudiadas para dar un uso adecuado al alperujo, entre los que cabe destacar la aplicación directa del alperujo al suelo sin recibir ningún tratamiento [2,3]; extracción de antioxidantes u otros compuestos con alto valor añadido (quitina, quitosan o hidroxitirosol) [4,5,6]; vermicompostaje [7]; generación eléctrica [8] o co-compostaje [9,10,11,12].

Dado que en las principales zonas olivareras hay un riesgo alto de padecer procesos erosivos [13] y que la tecnología más próxima a prácticas medioambientalmente correctas y sostenibles es el compostaje o co-compostaje con otros agentes, nuestros esfuerzos se han centrado en esta estrategia.

En zonas como en el valle del Guadiato, en Córdoba, el cultivo ecológico del olivo es el más extendido y cuenta con una gran tradición e innovación, como demuestra el desarrollo de la trampa OLIFE para el control de *Dacus* sp. [14], así como el contar con uno de los aceites de oliva virgen ecológico español más preciados en el mercado internacional. De acuerdo con los principios básicos de la agroecología, el consumo de fertilizantes o enmiendas de síntesis está restringido y se debe potenciar, en la medida de lo posible, el consumo de productos lo más cercanos posible a la explotación. Por lo tanto la utilización del alperujo producido en esta zona y otros agentes acondicionadores de la masa a compostar, igualmente producidos en la zona, como son hoja de olivo, estiércol vacuno y harinas cárnicas, en procesos de compostaje es una práctica de gran interés, que puede solventar las necesidades fertilizantes de sus explotaciones y que implica un coste mínimo al integrar el bajo coste de la producción del compost, el ahorro en el coste para los cooperativistas pertenecientes a la principal almazara de la zona (OLIFE), así como un ahorro en la aplicación de fertilizantes al estar añadiendo al suelo unas cantidades importantes con la enmienda.

Con la presente comunicación se pretende hacer un análisis de los parámetros más importantes del proceso de compostaje, estimar la evolución que puede tener la fracción orgánica durante el proceso, así como realizar un acercamiento previo a la aplicación de esta enmienda en olivares ecológicos.

Material y métodos.

El valle de los Pedroches se encuentra al norte de la provincia de Córdoba en una extensión situada al pie de la Sierra Morena cubierta por una vasta dehesa y donde se ubica una gran superficie de olivar ecológico, principalmente en las laderas. En esta zona el cultivo del olivo se ve limitado por el escaso desarrollo del suelo, así como por la orografía del terreno, con pendientes por encima del 10 % y por un clima muy riguroso, con carácter continental, con temperaturas mínimas bajo cero y una pluviometría media anual de entre 400 y 500 mm. Al desarrollarse la mayor parte de la agricultura de esta zona bajo cultivo ecológico, los fertilizantes y enmiendas que pueden aplicar los agricultores son reducidos. De ahí que se haya seleccionado el compostaje o maduración del alperujo para mejorar la fertilidad de sus explotaciones.

Como experiencia previa se han realizado una serie de mezclas con el alperujo, procedente de la cooperativa OLIFE (Pozoblanco, Córdoba), cuyas características se muestran en la tabla 1, con otros agentes estructurantes y elementos ricos en Nitrógeno, para facilitar la actividad transformadora y degradadora de los microorganismos. Estas mezclas estuvieron compuestas por Alperujo, compost de alperujo y estiércol, estiércol y una harina cárnica en las proporciones detalladas en la Tabla 2.

Tabla 1. Características físico – químicas del alperujo empleado.

Humedad	%	65.0
Materia orgánica	% sms	97.3
pH		4.93
CE	dS/m	6.54
N _{Kjeldahl}	% sms	1.26
C/N	"	45.40
P ₂ O ₅	"	0.19
K ₂ O	"	2.07
Ca ⁺⁺	"	0.33

Mg ²⁺	"	0.07
Na ⁺	"	1.41
Fe	mg kg ⁻¹ sms	268
Mn	"	14.8
Cu	"	16.9
Zn	"	13.8

Para la realización de los ensayos de compostaje se construyeron unas pilas de forma piramidal, con las mezclas obtenidas por triplicado, en un sistema de pilas estáticas con volteo manual.

La temperatura interior de las pilas de compostaje se monitorizó con dos sensores de termopares conectados a una centralita HOBO y situados en dos profundidades diferentes (10 y 20 cm sobre el fondo).

Tabla 2. Mezclas realizadas durante el primer año de ensayo

Mezcla 1

Materiales	% Humedad	C _{org} (sms)	N _{Kjeldahl} (sms)	kg
A	65.0	57.2	1.3	80.0
E	45.0	28.1	2.3	20.0
H	40.0	50.5	1.4	10.0
H _t		59.1		
C/N		32.2		

Mezcla 2

Materiales	% Humedad	C (sms)	N (sms)	kg
A	65.0	57.2	1.3	70.0
C	40.0	21.1	1.2	30.0
H	40.0	50.5	1.4	15.0
H _t		55.2		
C/N		34.4		

Mezcla 3

Materiales	% Humedad	C (sms)	N (sms)	kg
A	65.0	57.2	1.3	60.0
C	40.0	21.1	1.2	15.0
E	45.0	28.1	2.3	15.0
H	40.0	50.5	1.4	20.0
H _t		54.3		
C/N		30.7		

Mezcla 4

Materiales	% Humedad	C (sms)	N (sms)	kg
A	65.0	57.2	1.3	80.0
Hn	10.3	56.5	12.0	3.0
H	40.0	50.5	1.4	17.0
H _t		59.1		
C/N		27.8		

Se muestrearon las pilas de compostaje con una periodicidad quincenal en cinco puntos diferentes y se homogeneizó la mezcla para su análisis en laboratorio.

El contenido de carbono orgánico se evaluó con el método de Walkley - Black, Nitrógeno total por el método Kjeldahl tras digestión con sulfúrico concentrado y fósforo disponible por el método de Olsen tras formar un compuesto coloreado y medida en espectrofotómetro de ultravioleta visible [15].

Sodio, Potasio, Calcio, Magnesio y metales (Cu, Mn, Fe, Zn) fueron determinados según los métodos oficiales del MAPA [16], por calcinación por vía húmeda y medida en espectrofotómetro de llama. Na y K se midieron por emisión y el resto de elementos por absorción atómica.

Humedad, pH y CE se midieron según los métodos oficiales de análisis propuestos por el MAPA [16].

Para la caracterización y extracción de ácidos húmicos y fúlvicos se emplearon las metodologías descritas por Duchaufour (1977) y Schnitzer (1990) citados por Mustin (1987) [17] modificadas, con extracción con pirofosfato potásico, posterior alcalinización con sosa, retirada del precipitado y posterior acidificación con ácido sulfúrico concentrado.

El análisis de la fitotoxicidad de los tratamientos realizados, se estimó por el método de Zucconi et al. [18] con semillas de mastuerzo evaluando el índice de germinación.

Para estudiar la evolución de la materia orgánica presente en las pilas de compostaje se seleccionaron dos métodos diferentes. Por un lado se ajustó esta evolución a una cinética Michaelis - Menten (1) (MM) [19,20,21,22] y se estimó la calidad de su ajuste con el índice de Nash - Sutcliffe [23]. Por otro se aproximó esta evolución a un comportamiento lineal, al cambiar la escala de tiempo real por una serie de días de descomposición efectivos [24,25,26], obtenidos a partir de considerar un día efectivo de descomposición como aquel que satisface simultáneamente las condiciones de superar una humedad del 25 % y una temperatura superior a 32 ° C, considerados a partir de otros trabajos [17,25,26,27,28].

$$\frac{dC}{dt} = -\xi_c^* \cdot C \quad (1)$$

C = contenido en carbono orgánico; t = tiempo; ξ_c^* = constante de descomposición

Paralelamente se realizó también una primera experiencia de aplicación de los compost obtenidos a partir de alperujo en un olivar de la zona bajo cultivo ecológico, para servir de base de un ensayo que actualmente se está llevando a cabo en una finca de olivar tradicional en Montoro, Córdoba y otra en olivar ecológico en Pozoblanco.

El ensayo consistió en dos aplicaciones de compost de alperujo, en dos años consecutivos, a dosis A: 10 kg compost por árbol (color azul), Tratamiento B: 5 kg alperujo por árbol (color rojo) Tratamiento C: 0 kg alperujo por árbol. (Testigo- color verde) (Figura 2)

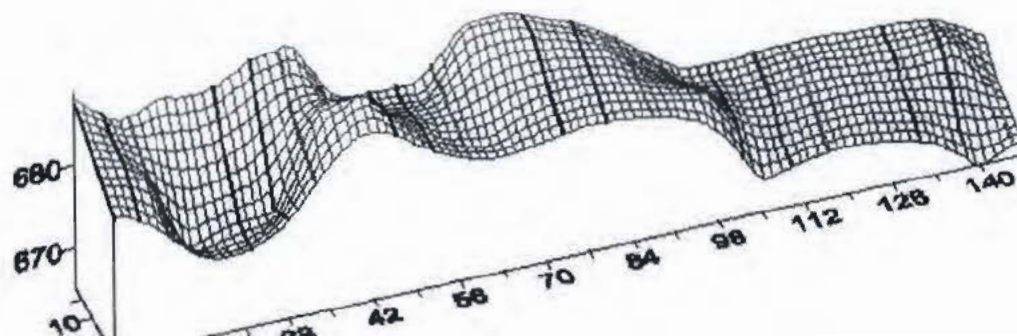


Figura 2. Esquema de las parcelas de ensayo.

Resultados.

Se observa que inicialmente las pilas atraviesan una fase mesofílica de corta duración tras la que discurre una primera fase termofílica que concluye con el volteo realizado el día 15 del proceso (Figura 3). Esto se debe a que la descomposición de compuestos orgánicos fácilmente degradables, unido al descenso en la población microbiana termofílica como consecuencia del enfriamiento producido por el volteo, así como un contenido menor de humedad, decelera el metabolismo microbiano. Este efecto es menos acusado en la mezcla 2, mientras que la mezcla 4 es la que más acusa este efecto [10,21,28]. Tras el volteo realizado durante la sexta semana, así como tras la adición de agua en los días posteriores, se produce una reactivación en las pilas de compostaje reactivación que en primer lugar sufre la mezcla, que posteriormente se desarrolla en el resto de mezclas, y que se mantiene durante un espacio de corto más breve en la mezcla 4. Tras esta nueva fase termofílica, paulatinamente la temperatura en todas las pilas desciende hasta aproximarse a la temperatura ambiente, fase comúnmente denominada como de enfriamiento y maduración.

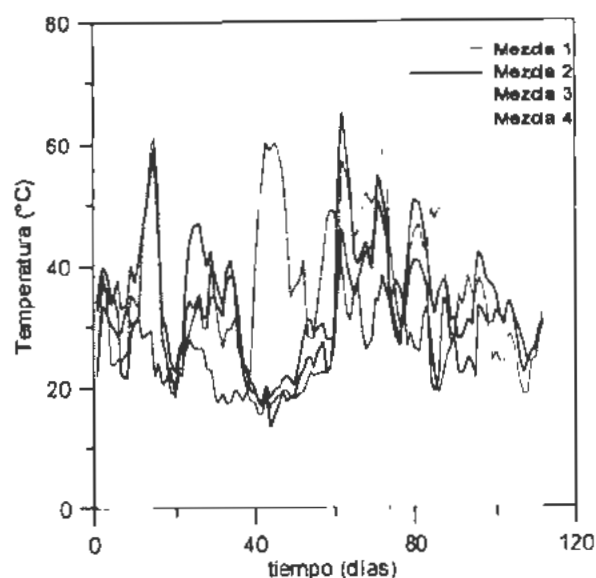


Figura 3. Evolución de la temperatura en la parte superior de las pilas de compostaje.

La materia orgánica evoluciona, durante el proceso de descomposición, llegando a producirse unos descensos en el contenido en carbono orgánico de entre el 30 y el 50 % (Tabla 3).

Tabla 3. Parámetros incluidos en los ajustes de descomposición.

Trtmt	C ₀ (% sms)		ξ_r^* (semanas ⁻¹)	E _{Nash-Summe}	Días de Descomposición	ΔC (% sms)
	observado	estimado				
1	51.8	47.8	0.0393	0.9	55	44.6
2	50.8	48	0.0447	0.89	58	47.5
3	49.4	46.3	0.0332	0.87	55	43.2
4	54.9	52.2	0.0297	0.86	42	31.1

Carbono Orgánico (C₀); constante de degradación (ξ_r^*); descenso en carbono orgánico (ΔC)

Esta evolución puede ajustarse a cinéticas de descomposición de primer orden, tipo Michaelis – Menten, como hace van Ginkel [20] u otros autores, como Paredes y col. [21] o García – Gómez y col. [22], en sus trabajos sobre compostaje (Figura 4 a). El ajuste obtenido tiene bastante calidad, como se puede observar en los índices de Nash–Sutcliffe de la tabla 3. Se observa cómo la constante de degradación para el tratamiento 4 es la menor de todos los valores, mientras que este valor es máximo en el tratamiento 2, en el que, atendiendo a los datos de carbono orgánico registrados durante el proceso, se aprecia una mayor descomposición (Tabla 3).

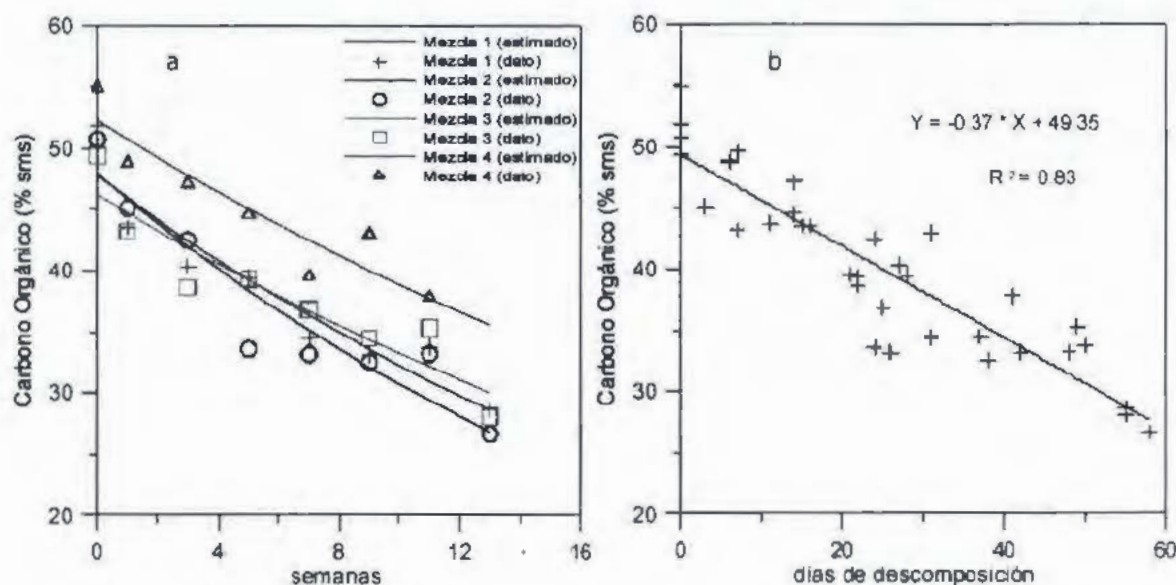


Figura 4. Evolución del carbono orgánico durante el proceso de compostaje. Ajuste a cinética Michaelis – Menten (a). Ajuste lineal al número de días de descomposición (b)

Autores, como Stroo [24] o Steiner [25], estudian la evolución en la descomposición de los residuos orgánicos a través de un cambio en la escala de tiempos, permutando los días por días de descomposición, calculados a través de dos factores dependientes de la humedad y de la temperatura óptima para la descomposición y de los datos registrados de pluviometría y temperatura. Giráldez y col. [26] aplican estos días de descomposición a un residuo de trigo, mostrando una relación lineal entre la evolución de la descomposición del residuo y los días de descomposición. Esta relación tiene un buen ajuste también en el caso de sistemas de descomposición forzados, como es el compostaje, tal y como se puede comprobar en la figura 4 b. Del análisis de los días de descomposición se observa que igualmente a lo reflejado por la constante de degradación en el caso de ajustes exponenciales, como MM, el tratamiento 4 es el que menor número días de descomposición atraviesa, refrendado por el menor descenso en el contenido de materia orgánica que se produce, mientras que el tratamiento 2 sufre la mayor degradación.

Tabla 4. Características del extracto húmico al inicio y al final del proceso

	Extracto húmico (%)		AH (%)		AH/AF		COT	
	I	F	I	F	I	F	I	F
Ttmtto 1	15,23*	8,71	7,86*	5,04	1,07	1,44	51,77*	30,45
Ttmtto 2	13,30*	7,34	6,41*	3,43	0,96	0,94	50,75*	29,85
Ttmtto 3	11,55	8,00	5,67	5,18	1,02	1,85	49,42*	29,07
Ttmtto 4	12,29*	9,00	6,02	5,42	0,96	1,58	54,94*	32,32

* diferencias significativas entre valores iniciales (I) y finales (F) $p < 0.05$.

Ácidos Húmicos (AH), Ácidos Fúlvicos (AF), Carbono Orgánico total (COT)

La actividad microbiana durante el proceso de compostaje produce una disminución y transformación de la materia orgánica, tal y como se observa en la tabla 4. El extracto húmico disminuye significativamente en todos tratamientos, salvo en el 3, donde estas diferencias no son significativas. Este extracto varía sus características como consecuencia del proceso y se producen una serie de polimerizaciones y policondensaciones [29], pasando en todos los casos, salvo en el tratamiento 2, a formar una mayor proporción de compuestos más estables y complejos, como los ácidos húmicos, tal y como se observa de la relación AH/AF. Esta diferencia en el caso del tratamiento se puede deber a una mayor degradación y un menor extracto húmico, tal y como se observa en las tablas 3 y 4. esta degradación incide en una menor polimerización y consiguientemente en una menor proporción de ácidos húmicos y fúlvicos.

Los compost obtenidos durante el proceso de maduración presentan un buen aspecto, granulado y de color oscuro, y unas buenas cualidades agronómicas: alto contenido en materia orgánica y potasio, pH alcalino, valores medios de salinidad y nitrógeno orgánico y valores bajos de fósforo, mostrando valores similares a los aportados por otros autores [9,30,31] (Tabla 5). Su relación C/N se sitúa próximo a 15, dando muestras de la madurez alcanzada y la toxicidad inicial que presentaba el alperujo queda reducida en gran medida.

Tabla 5. Características físico-químicas de los compost obtenidos.

Tratamiento	1	2	3	4
Humedad	25.2	27.6	24.8	26.2
M O (% sms)	48,7 a	45,3 a	47,7 a	64,3b
PH	8.41	8.24	8.31	7.85
CE (dS m ⁻¹)	5.32 a	3.72 b	4.33 b	4.70 a
NO ₃ ⁻ (% sms)	0,06	0,08	0,09	0,09
N _{kjeldahl}	1,89 a	1,86 a	2,12 a	2,89 b
C/N	15,17	14,34	13,25	13,09
P ₂ O ₅ (% sms)	0,40 a	0,24 b	0,33 ab	0,32 ab
K ⁺	2,46 a	1,83 b	1,86 a	2,20 ab
Na ⁺	4,88 a	1,69 b	4,64 a	2,63 b
Ca ⁺⁺ (% sms)	3,41	2,72	3,25	1,94
Mg ⁺⁺	0,40 a	0,17 b	0,32 a	0,16 b
Fe mg kg ⁻¹	1519 a	1960 b	1363 ac	991 c
Cu	36,3 a	31,9 a	32,2 a	126,6 b
Mn	82,0	71,8	82,7	56,2
Zn	300	321	242	141

Letras diferentes indican diferencias significativas entre tratamientos ($p < 0,05$)

Los valores de concentración de metales pesados, a pesar de no llegar a ser limitantes en el caso de enmiendas orgánicas para laboreo tradicional, sí que pueden llegar a superar algunos límites establecidos en la etiqueta ecológica [32], como en el caso del Cu, para el tratamiento 4 y del Zn para el tratamiento 2. Estos excesos pueden ser debido a haber empleado hojas de olivo que pueden haber sido tratados anteriormente.

En el ensayo en campo para obtener unos datos previos a la planificación de un ensayo de aplicación de compost de alperujo en olivar, se observó como la aplicación repetida de este tipo

de compost, repercute positivamente en las características del suelo, factor muy importante en suelos con escasa fertilidad como es el caso (Tabla 6). El contenido en materia orgánica se vio incrementado en todos los casos como consecuencia de la aplicación de la enmienda, estableciéndose unas diferencias significativas con respecto al valor inicial. El incremento en el testigo se debe a un aporte externo desde las parcelas de mayores dosis como consecuencia de no haber incorporado con el primer horizonte del perfil el compost y a una mala distribución del mismo. Estos valores coinciden con los mostrados por otros autores [2,3]. Se observan también diferencias significativas entre el K en suelo para el tratamiento a dosis máxima y el valor inicial. La capacidad de intercambio catiónico y el pH también aumentan en los suelos tratados con compost, no obstante, las diferencias que existen no son significativas y del estudio de las nuevas parcelas destinadas a la evaluación de esta enmienda se mostrarán resultados más consistentes, tanto para el análisis en suelo como en las características fisiológicas y nutritivas del árbol.

Tabla 6. Características del suelo antes y después de aplicar compost de alperujo.

	P	K % sms	MO	pH _{H2O}	CIC mol _c kg ⁻¹
10 kg / arbol	20.95a	162.60a	3.59a	6.48a	0.142a
5 kg / arbol	15.88a	134.97ab	3.27a	6.67a	0.141a
0 kg / arbol	15.63a	136.33ab	3.79a	6.69a	0.234a
Inicial	7.20a	76.28b	1.66b	6.26a	0.117a

Letras diferentes indican diferencias significativas entre tratamientos ($p < 0,05$)

Conclusiones.

La aproximación y estimación de la evolución del carbono orgánico durante el compostaje quedan bien reflejadas con cinéticas de primer orden y reducción a días de descomposición.

Como consecuencia del proceso se produce una estabilización y transformación de la materia orgánica y se obtiene una enmienda orgánica de gran calidad, que aporta grandes cantidades de materia orgánica y potasio y medias de nitrógeno.

La aplicación continuada de alperujo comportado al olivar produce mejoras en las propiedades químicas del suelo, aumentando, principalmente, el contenido en materia orgánica y potasio.

La aplicación de esta enmienda en olivares en pendiente debe ser lo más homogénea posible y se recomienda su incorporación superficial al suelo.

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Fitotoxicidad y valor fertilizante de enmendantes diferentes orgánicos

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INTRODUCCION. - En general, la incorporación de enmendantes orgánicos a un suelo mejora las propiedades físicas, químicas y biológicas de este y en último término mejora su productividad (PEREZ GARCIA *et al.*, 1986). El conocimiento del grado de estabilidad de la materia orgánica de los enmendantes orgánicos es uno de los aspectos más importantes para el entendimiento de su actuación sobre las propiedades químicas, físicas y bioquímicas del suelo así como sobre el desarrollo vegetal, después de su aplicación. Los materiales orgánicos de reciente producción (denominados frescos) tales como lodos de depuradora, residuos sólidos urbanos, estiércoles, etc. generalmente pueden producir efectos negativos sobre la germinación de semillas y el crecimiento vegetal al presentar gran contenido de compuestos fácilmente biodegradables y compuestos semidegradados de naturaleza hidrosoluble con carácter fitotóxico, tales como: ácidos orgánicos de bajo peso molecular, fenoles, etc. (WILSON Y DALMAT, 1986). Otros problemas relacionados con fitotoxicidad pueden ser: conductividad elevada (PEREZ GARCIA *et al.*, 1986); exceso de amonio (GODDEN *et al.*, 1985); elevado contenido de metales pesados (ANID, 1986), así como otros metabolitos de naturaleza orgánica. Esto hace que sea conveniente el estudio de los enmendantes orgánicos a utilizar previo a su aplicación a suelos. Con el fin de facilitar y simplificar su conocimiento, en la bibliografía se proponen diversos índices químicos que indican la madurez o estabilidad de la materia orgánica tales como: C/N, C hidrosoluble/N (GARCIA *et al.*, 1992a); contenido de amonio (SPHON, 1978); C extraíble/C orgánico; C fúlvico/C húmico (SEQUI, 1986). A pesar de ello, ningún índice es totalmente concluyente por lo que para evaluar el grado de madurez de los enmendantes orgánicos es recomendable el uso de va-

rios índices, con el fin de conseguir una información lo más completa posible del material.

En el presente trabajo se ha llevado a cabo el estudio de diversos parámetros químicos y bioquímicos de enmendantes orgánicos de distinta procedencia y con diferente grado de estabilidad de su materia orgánica, con el fin de establecer el efecto positivo o negativo de estos enmendantes sobre la germinación, elongación de raíces y el crecimiento vegetal.

MATERIALES Y METODOS. - Se han utilizado seis enmendantes orgánicos de distinto origen: dos materiales, leonardita y turba, como enmendantes más edafizados y los otros cuatro de formación reciente, un lodo de depuradora, un residuo sólido urbano, un estiércol caprino y un compost tomado a partir de una mezcla de un lodo de depuradora y la fracción orgánica de un residuo sólido urbano (en una relación 1:1 de su contenido en carbono orgánico total, COT).

El COT, el C extraíble (Cex) con pirofosfato a pH 9,8 (p:v, 1:10), la fracción de éste, soluble a pH 2, y el C hidrosoluble se determinaron por el método de oxidación por el dicromato (YEOMANS y BREMNER, 1988). El carbono del extracto obtenido con pirofosfato, que precipita a pH 2 (C de ácidos húmicos, CAH) se obtuvo por diferencia entre el C del extracto total y el de la fracción que permanecen soluble a pH 2. El N se determinó por el método Kjeldahl, el $N-NH_4^+$ extraído con KCl 2N, se midió con electrodo de ión selectivo y el $N-NO_3^-$ extraído con agua a 50°C (p:v, 1:10) se midió espectrofotométricamente (BOLARIN *et al.*, 1982). El P y K total se determinaron a partir de la digestión nítrico-perclórica de los enmendantes: el P total y el extraído con bicarbonato sódico 0.5 M a pH = 8.5 se determinaron por el método de MURPHY y RILEY (1962) y el K total y el extraído con acetato amónico 0.1 M, por fotometría de llama. La conductividad eléctrica y el pH se midieron directamente en extracto acuoso 1:10 (p:v) y los ácidos orgánicos de bajo peso molecular por HPLC después de eliminar los tenoles por Seppak (SOUTHERN, 1987). Las actividades ureasa, proteasa que hidroliza $N\alpha$ -benzoyl-L-argininamida (proteasa BAA) y fosfatasa se determinaron sobre 0.2 g de muestra molida y seca según el método de NANNIPIERI *et al.* (1980) y la β -glucosidasa fue medida según el método de MASCIANDARO *et al.* (1994).

La posible fitotoxicidad y valor fertilizante de estos materiales se determinó mediante un ensayo de germinación y otro de crecimiento vegetal: ambos se realizaron por cuadruplicado. El experimento de germinación se realizó en placas Petri utilizando semillas de ryegrass (*Lolium perenne* L. Cv. Argo) y tomate (*Lycopersicum esculentum* tipo Daniela). En cada placa se colocaron 15 semillas y 2 ml del extracto acuoso (peso:volumen 1:10) de cada uno de los materiales orgánicos. Las placas se mantuvieron en oscuridad a 28°C durante 5 días determinando entonces el

porcentaje de semillas germinadas y la longitud de raíces. El índice de germinación se calculó a partir de la fórmula: $IG = \% G \frac{Lm}{Lc}$ donde G es el porcentaje de semillas germinadas con respecto al control, Lm es la longitud media de las raíces de las semillas germinadas en el tratamiento y Lc longitud media de las raíces de las semillas germinadas en el control. El ensayo de crecimiento vegetal se realizó por cuadruplicado sobre contenedores en los que se colocaron 500 g de suelo al cual fueron incorporados los distintos materiales en dosis adecuada para aumentar hasta un 0.5% y un 2% el contenido de materia orgánica del mismo. El suelo utilizado fue un suelo calizo con un bajo contenido en materia orgánica y nutrientes, formado sobre un sustrato litológico de margas y que puede ser clasificado según el Soil Taxonomy (1975) como un Xeric Torriorthen (Tabla 1). Contenedores con suelo solo se utilizaron como control. En cada contenedor se colocaron 0.5 g de semillas de ryegrass (*Lolium perenne* L. Cv. Argo). Este cultivo se realizó en una cámara de crecimiento, con un 90% de humedad relativa y una temperatura 20-30°C. El fotoperiodo fue de 12 horas con una intensidad de 40.000 lux. El control de la humedad en los contenedores se realizó de una forma periódica. La experiencia tuvo una duración de 39 días, realizando dos cortes, uno a los 21 días y otro al final del experimento. A partir de la primera semana, se tomaron medidas de la altura de las plantas, cada dos días con el fin de determinar la velocidad media de crecimiento en distintos periodos del desarrollo. Por otra parte se determinaron los rendimientos de los distintos tratamientos (peso fresco y peso seco a 60°C), en los dos cortes efectuados.

TABLA 1. Análisis granulométrico y características físicas, químicas y físico-químicas del suelo.

Análisis granulométrico	
Arena gruesa (%)	20.50
Arena fina (%)	15.70
Limo (%)	39.50
Arcilla (%)	27.90
Análisis físico-químico	
pH (H ₂ O)	8.21
CE (dSm ⁻¹)	1.21
CaCO ₃ total (%)	52.00
CaCO ₃ activo (%)	17.00
Materia orgánica (%)	0.44
Carbono orgánico (%)	0.25
N total (%)	0.02
K ₂ O (%)	3.85
P ₂ O ₅ (%)	0.09

RESULTADOS Y DISCUSION. — Las características de los materiales orgánicos estudiados (Tabla 2) ponen de manifiesto la diferente naturaleza de los mismos, ya que los valores de cualquier parámetro difieren notablemente entre los materiales. Así, el pH oscila de fuertemente ácido, en la leonardita, a ligeramente básico como en el compost y el estiércol. Es de destacar el elevado valor de CE del estiércol lo que puede constituir, a determinadas dosis, un inconveniente para el desarrollo vegetal (CHANYASAK, 1982). En cuanto al contenido en

macronutrientes (N, P, K) tanto los contenidos totales de estos elementos como de sus fracciones utilizables por las plantas, son, en general, superiores en los materiales frescos que en los más humificados. Cabe resaltar los altos contenidos en N y P del lodo lo que le confiere un efecto fertilizante considerable (DE BERTOLDI *et al.*, 1982). Por otra parte, el contenido de amonio del lodo supera el nivel de 0.4 g Kg^{-1} establecido por FINDENEGG (1987) como valor límite de toxicidad para las plantas.

TABLA 2. — Características de los enmendantes orgánicos (peso seco, 105°C).

Parámetros	Leonardita	Turba	Compost	Estiércol	Lodo	RSU
pH	3.20	5.17	7.52	7.57	6.52	6.06
CE, dS m^{-1}	2.75	1.23	3.00	6.60	3.47	4.90
N, g kg^{-1}	5.60	9.20	12.60	17.30	49.20	15.80
N- NH_4^+ , g kg^{-1}	0.15	0.44	0.11	0.12	1.20	0.28
N- NO_3^- , g kg^{-1}	0.03	0.62	0.63	1.27	1.30	0.87
P, g kg^{-1}	0.22	1.20	9.11	1.88	7.45	4.33
Pext, g kg^{-1}	0.02	0.54	0.60	0.48	2.08	0.45
K, g kg^{-1}	3.00	3.00	5.50	32.50	2.80	9.50
Kext, g kg^{-1}	0.16	2.30	4.00	26.50	1.80	7.00
Materia orgánica g Kg^{-1}	692.50	950.40	365.00	698.00	743.30	548.60
COT, g kg^{-1}	347.40	487.40	122.50	260.70	364.40	217.20
Cex, g kg^{-1}	190.50	40.80	19.10	31.40	75.70	38.10
C _{sol} , g kg^{-1}	175.40	29.80	6.00	7.60	16.60	5.80
C sol. pH 2 g kg^{-1}	15.10	11.00	13.10	23.80	59.10	32.30
C _{sol} x 100/Cext	92	73	31	24	22	15
CH g kg^{-1}	0.01	1.40	1.00	10.80	30.30	21.10
C/N	62.04	52.97	9.72	15.07	7.41	13.74
CH/N	0.01	0.15	0.08	0.62	0.61	1.33

COT = C orgánico total; CAH = C de ácidos húmicos;
Cex = C extraíble con $\text{Na}_2\text{P}_2\text{O}_7$; CH = C hidrosoluble.

Los valores de COT y sus diferentes fracciones muestran también una amplia variedad entre los materiales estudiados. Por otra parte, en base a los valores de la relación CAH/Cex considerados como índices de humificación, estos materiales podrían clasificarse en tres grupos: los que han sufrido un proceso intenso de humificación, como leonardita y turba; aquellos materiales que no han estado sometidos a dicho proceso como estiércol, lodo y RSU, denominados frescos y el compost que ocupa una posición intermedia entre estos dos grupos. Los valores de C hidrosoluble (CH) y

de la relación CH/N, consideradas como índice de madurez de residuos orgánicos (GARCÍA *et al.*, 1992a), son mayores en la leonardita, turba y compost que en el estiércol, lodo y RSU, lo cual pone de manifiesto la mayor estabilidad de la materia orgánica de aquellos materiales.

Se han identificado algunos ácidos de bajo peso molecular, los cuales son citados entre otros por DEVLEESCHAUWER *et al.* (1981), como inhibidores de la germinación y crecimiento vegetal. En los materiales más humificados incluyendo al compost se han detectado trazas o pequeñas cantidades, mientras que son los materiales frescos los que presentan los valores más altos (Tabla 3), al no haber sufrido estos últimos, un ataque microbiano tan prolongado como es el caso de los materiales más humificados.

TABLA 3. — Contenido de ácidos orgánicos de bajo peso molecular en los enmendantes orgánicos (mg kg⁻¹ peso seco).

	Leonardita	Turba	Compost	Estiércol	Lodo	RSU
Fórmico	Trazas	Trazas	14	418	79	97
Acético	Trazas	11	5	762	306	29
Propiónico	Trazas	5	38	1018	4068	1632
Isobutírico	Trazas	Trazas	7	263	423	116
Butírico	Trazas	Trazas	116	898	6973	3725

El contenido en metales pesados en los diferentes materiales varía mucho en función del origen de los materiales orgánicos estudiados. En general, la leonardita y la turba presentan concentraciones de metales pesados inferiores a la de los residuos urbanos o su compost (Tabla 4). En cualquier caso, el nivel de metales pesados en estos materiales está por debajo de los niveles máximos establecidos por la Unión Europea para el uso de lodos en agricultura (Council Directive 86/278/EEC) y por la legislación Española para el uso de fertilizantes y afines (BOE Real Decreto 72/1988 del 5 de Febrero).

La Tabla 5 refleja el estado bioquímico de los diversos materiales en estudio. En ella se observa una disminución de actividad enzimática en función de la estabilidad de la materia orgánica: materiales frescos mayor actividad que materiales estabilizados; este hecho confirma la hipótesis de que las hidrolasas estudiadas dependen de la biomasa microbiana: es por ello que el compost mues-

tra una menor actividad que los materiales de partida (Lodo y RSU), puesto que el propio proceso de compostaje conlleva la disminución de materia orgánica lábil y por consiguiente la pérdida de biomasa microbiana (GARCÍA *et al.*, 1992b). La leonardita, material sumamente estable, muestra los valores más bajos de actividad enzimática. Diversos autores han puesto de manifiesto la posible inhibición de diversas actividades enzimáticas por metales pesados (DICK and TABATABAI, 1993). En nuestro caso, a pesar del contenido en metales pesados de los materiales frescos, estos presentan una elevada actividad bioquímica posiblemente debido a que el contenido de metales pesados no llega a umbrales de inhibición o a que esta inhibición se vea ampliamente superada por la cantidad de sustratos susceptibles a ser hidrolizados, presentes en los materiales frescos. Otro hecho destacable, es el elevado valor de actividad fosfatasa presente en el lodo frente al resto de materiales frescos, lo que es atribuible al elevado contenido de compuestos fosforados presentes en los lodos, procedentes de detergentes que actúan como sustrato induciendo la síntesis del enzima.

TABLA 4. - Contenido de metales pesados de los enmendantes orgánicos (mg Kg⁻¹).

	Cr	Fe	Mn	Ni	Pb	Zn	Cu	Cd
Leonardita	24	14544	34	19	26	40	22	ND
Turba	15	1080	35	ND	10	34	4	ND
Compost	261	11565	404	221	198	535	184	ND
Estiercol	16	2319	108	37	18	94	14	ND
Lodo	38	4061	70	22	85	415	151	ND
RSU	250	5498	209	178	77	281	77	ND

Límite máximo permitido:

Unión Europea

(Lodos) - - - 300-400 750-1200 2500-4000 1000-1750 20-40

Leg. Española

(Fert. y afines) - - - 400 1200 4000 1750 40

ND = no detectado

Ensayo de germinación. - La germinación de semillas es un test recomendado por diversas administraciones de protección medioambiental para la determinación de efectos tóxicos, al ser el período de germinación uno de los más sensibles al estrés ambien-

tal durante el desarrollo vegetal. Pero cada planta puede ser sensible a un tipo de toxicidad diferente, es por esto que distintas administraciones medioambientales, (Food and Drug Administration, (FDA), 1987; Organization for Economy Cooperation and Development, 1984), recomiendan al menos el uso de dos semillas. En el presente estudio siguiendo la normativa de FDA se han utilizado dos semillas una monocotiledonea (ryegrass) y una dicotiledonea (tomate). Los materiales frescos inhiben significativamente la germinación en relación al control (Figura 1), lo cual es atribuible a la existencia en estos materiales de ácidos orgánicos de bajo peso molecular (Tabla 4) y otros compuestos de naturaleza fitotóxica. Este efecto inhibitor es particularmente acusado por las semillas de tomate en el caso del estiércol y el RSU debido a que esta semilla es más sensible que la de ryegrass a la alta conductividad eléctrica de estos materiales (PEREZ *et al.*, 1986). De hecho se ha encontrado una correlación negativa entre el índice de germinación de esta semilla y los valores de CE ($r = -0.9566$, $p = 0.01$). Asimismo, se ha observado una correlación negativa entre el índice de germinación y la relación CH/N, considerada como índice de madurez ($r = -0.7550$, $p = 0.05$).

TABLA 5. - Actividad específica ureasa, proteasa BAA, fosfatasa y β -glucosidasa de los enmendantes orgánicos (actividad en función de su C orgánico total, COT).

	Ureasa ($\mu\text{g NH}_4^+ / \text{g COT h}$)	Proteasa BAA ($\mu\text{g NH}_4^+ / \text{g COT h}$)	Fosfatasa ($\mu\text{mol PNF} / \text{g COT h}$)	Glucosidasa ($\mu\text{mol PNF} / \text{g COT h}$)
Leonardita	1	2	8	4
Turba	24	1	276	410
Compost	47	22	1945	677
Estiércol	676	66	3662	3326
Lodo	826	63	21859	4575
RSU	650	61	3742	2200

PNF = p-nitrofenol.

La turba es la que presenta el índice de germinación más alto en ambas semillas, siendo significativamente superior al control en el caso del tomate, lo que pone de manifiesto la acción positiva de algún compuesto orgánico presente en este material. La existencia de este tipo de compuestos no puede ser descartado en el resto de materiales ya que pueden estar encubiertos por otros fac-

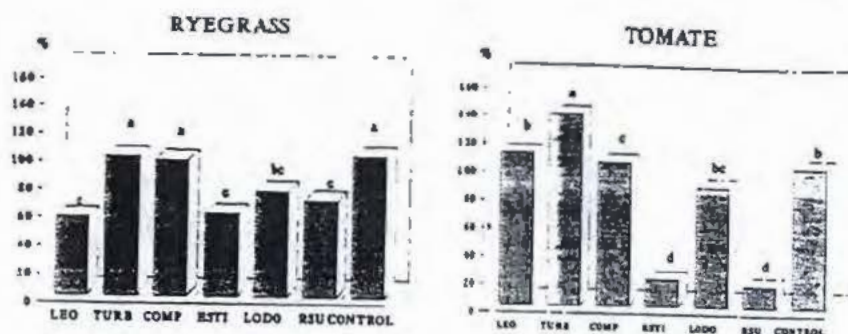


FIG. 1. - Indices de germinación (IG) de las semillas de ryegrass y tomate sobre los extractos acuosos (1:10, p:v) de los materiales orgánicos (Expresados como % del IG del control). Los datos correspondientes a barras con igual letra no son significativamente diferentes al nivel de probabilidad del 95% según el test de Tukey.

tores negativos (conductividad eléctrica, ácidos orgánicos, fenoles, pH excesivamente ácido, etc.).

Ensayo de crecimiento. - La influencia que los materiales ensayados han tenido en el crecimiento vegetal se muestra en las Figuras 2 y 3. En el primer corte (Figura 2), se observó que en la primera semana se producía una disminución del crecimiento vegetal con respecto al control en los tratamientos con materiales frescos que no han sufrido un proceso de humificación, siendo esta disminución más marcada en la dosis mayor, lo que pone de manifiesto la existencia en estos materiales de inhibidores del crecimiento. El crecimiento vegetal, en los materiales más humificados (leonardita y turba), era igual o ligeramente superior al control independientemente de la dosis, lo que indica la no presencia de

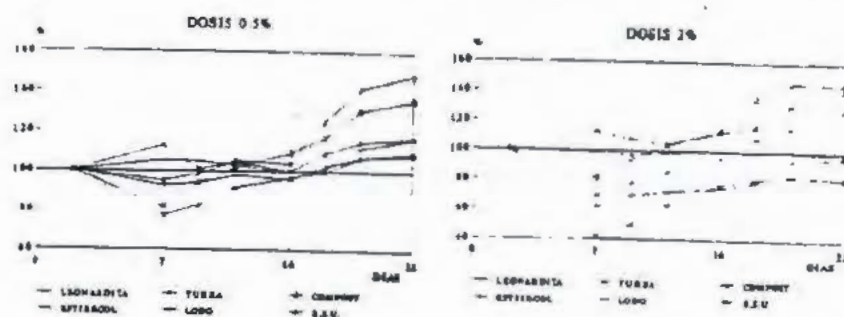


FIG. 2. - Crecimiento del ryegrass, en relación al control, en los distintos tratamientos a lo largo del cultivo. 1º corte.

compuestos que tengan un efecto depresivo sobre el desarrollo. El compost puede considerarse como un estadio intermedio entre los materiales frescos y los más humificados, produciendo una ligera inhibición con respecto al control pero ésta no se ve afectada por la dosis.

A partir de la primera semana, se observó una paulatina recuperación del crecimiento vegetal en los tratamientos con materiales frescos con respecto al control, indicativo de la desaparición de compuestos fitotóxicos. Sin embargo, el tratamiento con lodo no experimentaba la recuperación de una forma tan rápida, debido al elevado contenido del lodo en amonio y ácidos orgánicos de bajo peso molecular (Tabla 2), en particular de ácido propiónico, el más tóxico para el crecimiento vegetal (KATAYAMA *et al.*, 1985). Esto es indicativo de que la fitotoxicidad no ha desaparecido por completo durante los primeros días del cultivo, cuando ésta desaparece los tratamientos con lodo adquieren un crecimiento considerable, llegando incluso a superar al control y resto de tratamientos, a pesar del retraso de las primeras fases. Esto puede ser debido fundamentalmente a su elevado contenido en macronutrientes particularmente N y P (Tabla 2).

En la dosis de 0,5%, antes del primer corte (a los 21 días), todos los tratamientos presentan un crecimiento superior al control, indicativo de la desaparición de la práctica totalidad de los posibles inhibidores que en un principio podrían tener los materiales en origen, actuando las propiedades positivas de los materiales. Esta actuación positiva puede ser de una forma directa a través del aporte nutricional o fitohormonal, o de forma indirecta por la mejora de las propiedades físicas del suelo. En la dosis 2%, los tratamientos con lodo y residuo sólido urbano a los 21 días, a pesar de haber mejorado el crecimiento con respecto al control a lo largo del cultivo, todavía se encuentran por debajo del mismo, ya que posiblemente los compuestos fitotóxicos presentes en estos materiales aún no se han degradado por completo, particularmente en el caso del RSU.

A partir del segundo corte (Figura 3), todos los tratamientos, a excepción de la leonardita, presentan crecimientos superiores al control, independientemente de la dosis, lo que indica la eliminación total o al menos en parte de compuestos fitotóxicos, comenzando a predominar las propiedades positivas frente a las negati-

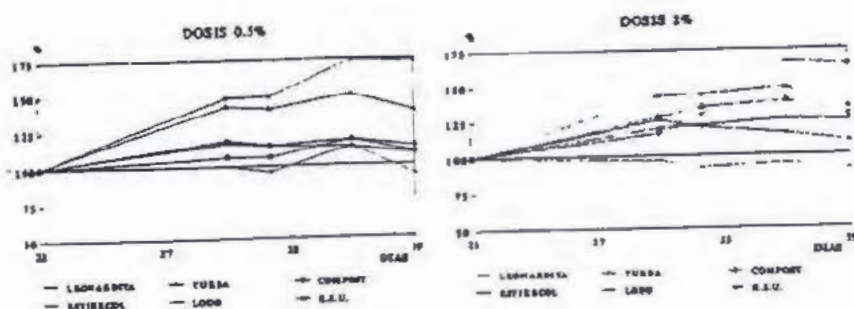


FIG. 3. - Crecimiento del ryegrass, en relación al control, en los distintos tratamientos a lo largo del cultivo. 2º corte.

vas de los materiales de partida. Los tratamientos que dan lugar a los valores de crecimiento más altos con respecto al control son los correspondientes a los materiales frescos, al ser estos últimos los que presentan más nutrientes.

En relación a los rendimientos (Figura 4), se observó que en el primer corte, los tratamientos con dosis elevadas de los materiales frescos producían rendimientos similares o inferiores al control, mientras que los materiales más humificados, incluyendo al compost, superaban al control. Estos mismos materiales frescos, al ser incorporados en menores cantidades dejaban de producir inhibición. En el segundo corte, se observaba una clara recuperación en los rendimientos obtenidos con los materiales (valores significativamente iguales o superiores al control). En el caso de los mate-

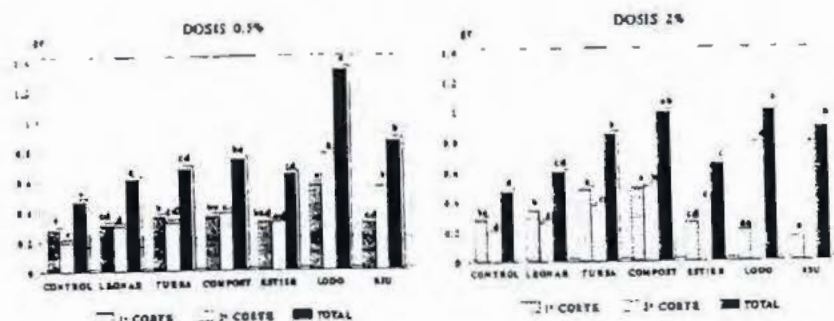


FIG. 4. - Rendimientos de ryegrass con los distintos tratamientos. Para cada corte y dosis, los datos correspondientes a barras con igual letra no son significativamente diferentes al nivel de probabilidad del 95% según el test de Tukey.

riales frescos y compost se advertía un aumento con respecto al primer corte y en el caso de los materiales humificados y el control, una disminución. Esto puede ser debido a que en el tiempo transcurrido desde la adición de los materiales frescos al suelo, éstos han sufrido una maduración suficiente para que comiencen a actuar los macro y micronutrientes y para que se degraden, al menos en parte, las sustancias fitotóxicas; mientras que en el caso de los materiales humificados, el propio cultivo va agotando los nutrientes con el paso del tiempo (GARCIA *et al.*, 1992c).

En cuanto a la dosis de 0,5%, cabe indicar que en ambos cortes, la mayoría de los tratamientos presentan rendimientos significativamente superiores al control, mostrando la existencia de un efecto residual, en especial en el caso del lodo, con liberación de N y P.

En cuanto al rendimiento total, el lodo y el residuo sólido urbano, independientemente de la dosis, presentan los mayores rendimientos, a pesar de la inhibición inicial que tenía lugar en la dosis alta. Sin embargo, el tratamiento con estiércol se ve claramente influenciado por la elevada conductividad eléctrica de este material por lo que el rendimiento que produce es menor que el de los otros compuestos orgánicos.

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RESUMEN. - Se han caracterizado química y bioquímicamente dos materiales orgánicos muy evolucionados: una turba y una leonardita; y cuatro materiales de formación reciente: un lodo, la fracción orgánica de un residuo sólido urbano (RSU), un estiércol y un compost obtenido a partir de una mezcla de lodo y RSU. La posible fitotoxicidad de estos materiales así como su valor agronómico se ha estudiado mediante un ensayo de germinación de semillas de ryegrass y tomate sobre extracto acuoso de los materiales, y otro de crecimiento de ryegrass en suelo tratado con dos diferentes dosis de material. Los materiales frescos inhibían la germinación, particularmente la del tomate indicando la presencia de sustancias fitotóxicas en ellos. La CE y la relación C hidrosoluble/N de los materiales se correlacionaban negativamente con el índice de germinación. A la dosis baja no se observaba depresión del rendimiento pero a dosis alta, los materiales frescos (lodo, estiércol y RSU) deprimían el rendimiento del primer corte de ryegrass mientras que los de los materiales estabilizados (leonardita, turba y compost) superaban al control. En el segundo corte todos los tratamientos y dosis superaban al control. Los efectos fitotóxicos de los materiales frescos desaparecían con el tiempo después de su adición al suelo, mostrando estos materiales un importante efecto positivo residual sobre el cultivo.



Short Communication

Effects of a compost made from the solid by-product (“alperujo”) of the two-phase centrifugation system for olive oil extraction and cotton gin waste on growth and nutrient content of ryegrass (*Lolium perenne* L.)

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Abstract

A pot experiment was conducted on a low-fertility calcareous soil in order to evaluate the effect on ryegrass growth and nutrient uptake of an organic fertiliser obtained by composting “alperujo” and cotton gin waste. Compost, alone and combined with nitrogen fertilisation, was added to the soil at three rates and three harvests were obtained.

The compost application enhanced plant growth in the first and third harvest. However, the additional nitrogen fertilisation clearly improved soil productivity due to the scarce availability of this nutrient in the compost. Also, a general increase in the plant contents of phosphorus and potassium in the first two harvests was recorded, whereas treatments with the maximum compost rate showed the highest plant content of copper in the last two harvests. Decreases in calcium in the last two harvests, in magnesium in all of them and in iron and manganese in the last harvest were also observed.

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Keywords: Olive-mill by-product “alperujo”; Compost application; Ryegrass; Plant growth; Plant nutrient content

1. Introduction

The role of organic matter and its application to soil have changed in modern agriculture where chemical fertilisers have become the major source of crop nutrients. The use of intensive agricultural methods generally leads to fertility loss, soil erosion, water contamination, soil compaction and organic matter content decline. A common characteristic of the Mediterranean soils is their low organic matter content, which is highly correlated with their potential productivity and fertility due to its direct influence on physical, chemical and biological soil properties. Thus, the use of organic wastes through soil systems

for different land purposes seems to be the best way to improve soil organic content and composting, as a method to transform organic wastes and by-products either into organic fertilisers or amendments, has received increasing attention in modern sustainable agriculture (Felipó, 1996; Sequi, 1996).

The olive oil production industry has a great socio-economic importance in Mediterranean countries. In Spain, the biggest olive oil producer in the world, this industrial sector generates large quantities of organic wastes and by-products during the short olive harvest season, whose gradual accumulation or incorrect disposal may have damaging effects on the environment. Nowadays, the most abundant by-product from the olive oil extraction process in Spain is “alperujo” (AL), a wet solid material obtained from continuous two-phase centrifugation, which is the

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most recent technology employed for olive oil extraction (Albuquerque et al., 2004). For its appropriate composting, addition of several bulking agents such as grape stalk (Baeta-Hall et al., 2005), poplar sawdust and bark chips (Filippi et al., 2002) and wheat straw (Madejón et al., 1998) has been performed in order to improve its scarce porosity; in all cases, end-products with a high content of organic matter and significant amounts of plant nutrients were obtained. Also, cotton gin waste has been tested for composting another olive-mill waste by Paredes et al. (2002).

Effects on plant growth of other composted olive-mill wastes, different from AL, have been evaluated already (Cegarra et al., 1996; Martín-Olmedo et al., 1995), but little information exists about the effect of AL-compost application on plant nutrient uptake and crop production, the subject of the present work, where perennial ryegrass was used as the test plant.

2. Methods

2.1. Compost preparation

Cotton gin waste (CW), which is a cellulosic material widely produced in southern Spain, was added to the AL as bulking agent in the following proportion on a fresh weight basis (dry weight basis in brackets): 92.6% AL + 7.4% CW (80/20) and composted by using the Rutgers system (Finstein et al., 1985), as was reported in detail by Cegarra et al. (2000).

2.2. Soil and compost characteristics

The soil used in the experiment was collected from 0–30 cm depth of a low fertility calcareous clay loam soil (Table 1) located in Murcia, south-eastern Spain, classified as a Haplic Calcisol (FAO, 1988) and sieved through a 2 mm mesh.

The compost showed a basic pH value and was very rich in organic matter, even though nearly half was composed of lignin that caused a relatively high C/N ratio (Table 2). It had a considerable content of potassium and nitrogen, mainly in organic form and poor in available forms

Table 1
Main characteristics of the Spanish soil used in the experiment

Parameter	Value
pH ^a	8.04
Electrical conductivity ^a (dS m ⁻¹)	0.16
Organic matter (g kg ⁻¹)	8.3
Total nitrogen (g kg ⁻¹)	0.6
C/N ratio	8.0
CaCO ₃ (%)	27.0
Sand (%)	23.2
Silt (%)	40.3
Clay (%)	36.5

^a Water extract 1:5.

Table 2

Main characteristics of the compost made from AL and CW employed in the experiment (d.w.)

Parameter	Value	Parameter	Value
pH ^a	8.90	P (g kg ⁻¹)	1.4
Electrical conductivity ^a (dS m ⁻¹)	3.09	K (g kg ⁻¹)	27.5
Organic matter (g kg ⁻¹)	889.5	Ca (g kg ⁻¹)	12.8
Lignin (g kg ⁻¹)	424.6	Mg (g kg ⁻¹)	2.8
TOC (g kg ⁻¹)	489.0	Fe (mg kg ⁻¹)	910
TN (g kg ⁻¹)	21.9	Cu (mg kg ⁻¹)	21
C/N ratio	22.3	Mn (mg kg ⁻¹)	44
NH ₄ ⁺ - N (mg kg ⁻¹)	86	Zn (mg kg ⁻¹)	38
NO ₃ ⁻ - N (mg kg ⁻¹)	108	GI (%)	71

TOC: total organic carbon; TN: total nitrogen and GI: germination index.

^a Water extract 1:10.

(NH₄⁺ and NO₃⁻), whereas the content of other nutrients was low when compared with manures and other composts. Moreover, the compost showed a relatively high value of the germination index (GI), which combines the measure of relative seed germination and relative root elongation of cress seed (Zucconi et al., 1981), suggesting the absence of phytotoxicity.

2.3. Growth chamber trial

The experiment on ryegrass was conducted in a controlled environment chamber at a day/night temperature of 25 °C/20 °C, a 16 h photoperiod and a relative humidity kept at 70%. It was carried out in round pots with a surface area of 75 cm², each of which had a capacity of 500 g of soil. The ryegrass seeds were surface sown on the pots (0.3 g per pot, equivalent to 40 g m⁻²) and a sand layer was spread over the seeds to facilitate germination. Four pots were used for each treatment and all pots were irrigated daily with distilled water to field capacity. Ryegrass was grown by using six different treatments, which were arranged as a 3 (compost application rates) × 2 (with and without nitrogen fertilisation) factorial. The treatments were a combination of the following three compost rates: C0 (0 t/ha), C1 (40 t/ha, 30 g per pot) and C2 (80 t/ha, 60 g per pot) and the following levels of nitrogen fertiliser: N0 (0 kg N/ha) and N1 (60 kg N/ha, 78 mg urea + 26 mg NH₄NO₃ per pot) and were called: C0N0, C0N1, C1N0, C1N1, C2N0 and C2N1.

The compost (17% moisture) was incorporated before sowing ryegrass seeds, while the complementary nitrogen fertiliser was applied in two fractions: 80% of the total as urea (46% N) before sowing and the remaining 20% as NH₄NO₃ (33.5% N) immediately after the first harvest. Moreover, all treatments received 60 kg P₂O₅/ha and 100 kg K₂O/ha by adding 86 mg KH₂PO₄ (52% P₂O₅ and 34% K₂O) and 92 mg K₂SO₄ (50% K₂O) per pot, before sowing.

The ryegrass was harvested three times at 31, 61 and 87 days from sowing. At each harvest, the aerial parts of the plants were cut approximately 1 cm above the soil surface and the fresh plant material was weighed, washed with

distilled water, oven dried at 60 °C for 24 h and reweighed. The dried plant material was later ground and stored for nutrient analysis.

2.4. Analytical methods

Soil texture was revealed by sieving and sedimentation, using the hydrometer method (Gee and Bauder, 1986). Total nitrogen (TN) and organic carbon (TOC) concentrations of the soil, compost and plant material were measured with a Carlo Erba automatic microanalyzer (Navarro et al., 1991). Organic matter of soil was determined by multiplying TOC by 1.72, and that of compost by ashing at 430 °C. For determination of CaCO_3 , CO_2 released by addition of HCl was measured with a calcimeter. Values of pH and electrical conductivity (EC) were determined for 1:5 (soil) and 1:10 (compost) aqueous extracts. $\text{NH}_4^+ - \text{N}$ was extracted with 2 M KCl from the frozen sub-samples of compost and determined by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992) whereas $\text{NO}_3^- - \text{N}$ was measured by using an ion-selective electrode on the compost water extract. Phytotoxicity was determined from the germination index (GI) according to Zucconi et al. (1981). Lignin was analysed according to the American National Standards Institute and American Society for Testing and Materials (1977).

After nitric acid-perchloric acid digestion (Abrisqueta and Romero, 1969), phosphorus was measured colorimetrically as molybdovanadate phosphoric acid (Kitson and Mellon, 1944), potassium by flame photometry and calcium, magnesium, iron, copper, manganese and zinc by atomic absorption spectrometry.

2.5. Statistical analyses

Analysis of variance was used to evaluate the effects of the compost and nitrogen fertiliser additions and their interactions on both plant growth and nutrient content. When the interaction between both factors was significant, a subsequent analysis was carried out to test the simple effect of one of the main factors at a particular level of the other. Duncan's multiple range test was employed to determine statistical differences in plant yield and nutrient content for both main and simple effects.

3. Results

3.1. Effect of compost on plant growth

Ryegrass growth was evaluated by determining the plant fresh weight. The interaction between the two factors considered, compost and nitrogen fertilisation, was not significant in any of the three harvests (Table 3). Plant yield in the first harvest was found to be higher in the compost-added treatments than in the control (C0) and non-significant differences were observed between yields at the C1 and

Table 3

Main effects of AL-compost (C) and N fertilisation (N) on fresh weight (g pot^{-1}) of ryegrass foliage at the three harvests

Factor	First harvest	Second harvest	Third harvest	Accumulated yield
<i>Compost (C)</i>				
C0	5.52b	4.40	3.08c	13.00b
C1	7.60a	4.86	5.02b	17.48a
C2	7.47a	5.71	5.90a	19.08a
	***	NS	***	***
<i>Nitrogen fertilisation (N)</i>				
N0	4.50	2.17	1.96	8.63
N1	9.23	7.82	7.37	24.42
	***	***	***	***
C × N	NS	NS	NS	NS

For each factor values in the same column followed by the same letter are not significantly different according to the Duncan's multiple range test at 5% probability level. NS: not significant and *** $P < 0.001$.

C2 application rates, whereas there were not significant differences between compost rates (C0, C1 and C2) in the second harvest. In the third one, a similar behaviour to the first harvest was observed again, but plant yield at the C2 application rate was significantly greater than at the C1.

Table 4

Main effects of AL-compost (C) and N fertilisation (N) on the phosphorus (g kg^{-1}), iron and copper contents (mg kg^{-1}) of ryegrass foliage at the three harvests

Factor	First harvest	Second harvest	Third harvest
<i>Phosphorus</i>			
C0	3.2b	2.7b	2.2b
C1	3.6a	3.0b	2.9a
C2	3.6a	3.4a	3.0a
	***	**	*
N0	3.7	3.5	3.4
N1	3.3	2.6	2.0
	***	***	***
C × N	NS	NS	NS
<i>Iron</i>			
C0	178	180	210a
C1	206	173	127b
C2	189	180	136b
	NS	NS	***
N0	206	173	151
N1	176	184	168
	NS	NS	*
C × N	NS	NS	NS
<i>Copper</i>			
C0	13	18b	15b
C1	14	16b	15b
C2	12	34a	23a
	NS	**	*
N0	14	20	18
N1	12	25	17
	NS	NS	NS
C × N	NS	NS	NS

For each nutrient and factor, values in the same column followed by the same letter are not significantly different according to the Duncan's multiple range test at 5% probability level. NS: not significant, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

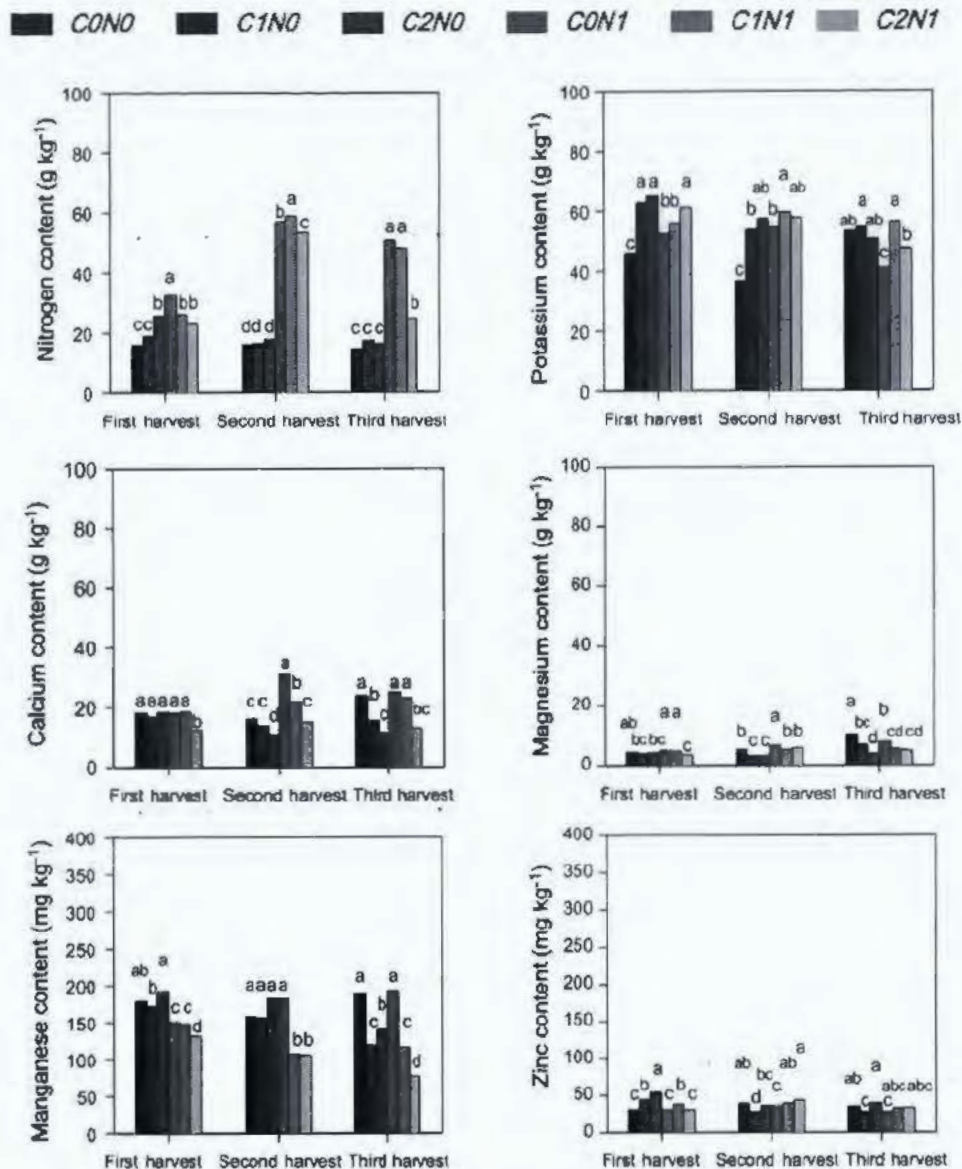


Fig. 1. Contents of nitrogen, potassium, calcium, magnesium, manganese and zinc of ryegrass foliage in the six treatments at the three harvests (for each harvest, vertical bars with the same letter are not significantly different according to Duncan's multiple range test at 5% probability level).

Also, the accumulated yields were greater in the compost treatments than in those not amended with compost and, as might be expected, a general increase in the ryegrass fresh weight was recorded in all the harvests when nitrogen fertilisation was used. Furthermore, yields in the first harvest were generally higher than in the other two harvests, which was more evident in the treatments without nitrogen fertilisation.

3.2. Effect of compost on plant nutrient content

The interaction compost \times nitrogen fertilisation was not significant for phosphorus, iron and copper in any of the harvests (Table 4), whereas it was significant for the

remaining plant nutrients tested. A general increase in the phosphorus content of the plants was observed after compost application, but the treatments including nitrogen fertilisation lowered the contents. Also, the C2 application rate clearly increased plant copper concentration except in the first harvest and both C1 and C2 application rates of compost resulted in decreased iron content only in the third harvest.

As was expected, nitrogen fertiliser treatments caused the highest plant nitrogen concentration, mainly in the second harvest (Fig. 1). Among the treatments not supplemented with nitrogen, the C2 application rate resulted in the highest concentration of this nutrient in the first harvest. However, C2 in combination with nitrogen

fertilisation (C2N1) led to the lowest concentration of nitrogen in the second and third harvests, when compared to the two other treatments including nitrogen (C0N1 and C1N1). As a general trend, the compost application caused an increase in potassium, mostly in the first and second harvests, and a decrease of calcium and magnesium. Manganese also showed a clear decrease, except in the first and second harvests obtained without nitrogen fertilisation. No trends in zinc concentrations were observed with any of the three harvests (Fig. 1).

4. Discussion

Due to the low fertility of the soil employed, the initial availability of nutrients and their progressive depletion determined that the highest plant yields were obtained in the first harvest. The soil compost application without adding nitrogen (C1N0 and C2N0 treatments) increased plant yield, indicating a beneficial effect on crop production, but this effect was rather low compared to that of the nitrogen fertilisation (C0N1, C1N1 and C2N1). Moreover, plants not treated with nitrogen (C0N0, C1N0 and C2N0) clearly showed nitrogen deficiency ($<20 \text{ g kg}^{-1}$) in the last two harvests, according to Bolton et al. (1976) who reported a critical value of 25 g kg^{-1} for concentration of this nutrient in ryegrass. When supplemented with nitrogen fertilisation, the C2N1 treatment led to lower plant nitrogen content than C0N1 and C1N1, which could be related to higher microbial competition for available soil nitrogen at the C2 application rate. The above results suggest that soil nitrogen availability was a critical factor for plant growth and nutrient uptake in this experiment. Thus, the scarce supply of available nitrogen provided by the AL-compost (a lignocellulosic material mainly containing organic nitrogen, Table 2), as opposed to the fertilisers employed, should be related to the presumably slow mineralisation of the compost organic nitrogen. In longer-term experiments, Cabrera et al. (2005) and Madejón et al. (2001) found that a considerable amount of the organic nitrogen in calcareous soils amended with other composted olive-mill wastes was highly resistant to mineralisation.

The application of AL-compost to soil increased the content of phosphorus in the ryegrass plants. In this respect, it must be noted that high soil carbonate content and pH are unfavourable characteristics making phosphorus less available for plant nutrition (Hinsinger, 2001), thus applying the compost in the present experiment may have improved phosphorus plant availability. Several reactions may have been involved to increase availability, including formation of phospho-humic complexes, protective coatings over colloidal sesquioxides with reduction in phosphate adsorption as well as chelate complexes with Ca from the insoluble Ca-phosphates that predominate in calcareous soils, following the release of phosphate to water soluble forms (Stevenson, 1994). Similar results were found by Cegarra et al. (1996) in an experiment involving a chard (*Beta vulgaris* L.) culture

and the addition of another composted olive-mill waste to a calcareous soil. Moreover, the lowest phosphorus concentration in ryegrass was registered in the treatments with nitrogen fertilisation, which was much more apparent in the third harvest. The lower phosphorus concentration could be explained by either the substantial increase of plant biomass because of the nitrogen fertilisation (dilution effect) or the depletion of phosphorus availability due to its progressive consumption. The compost application additionally increased the plant potassium contents, which were higher than the optimal content reported for ryegrass (Bergmann, 1992) in all the treatments, in agreement with Cegarra et al. (1996) who reported the great effectiveness of composts made with other olive-mill wastes for supplying potassium to plants.

Treatments with the maximum application rate of compost showed the lowest plant contents of calcium and magnesium, but they never reached deficient levels according to Bergmann (1992). The decrease can be attributed to either an antagonist effect with potassium or a chelating action of the compost organic matter (Nogales et al., 1984). As regards to the micronutrients, the compost addition caused an increase in plant copper in agreement with Kabata-Pendias (2001), who reported that low-molecular weight compounds liberated during decay of plant residues may greatly increase the availability of this micronutrient to plants, but a decrease in both iron and manganese plant content probably due to the formation of poorly soluble and unavailable chelates (Linehan, 1978; Madrid, 1999). However, all values were found to be within or above the optimal range defined by Bergmann (1992).

5. Conclusions

Under the conditions of the present experiment, the low fertility of the soil employed and the slow supply of nitrogen from AL-compost were the most important factors influencing the growth and development of ryegrass. The compost tested, a lignocellulosic material containing nitrogen scarcely bio-available in the short term, demonstrated its capacity to stimulate plant yield even without adding nitrogen, but this effect was rather low compared to that of the additional nitrogen fertilisation.

Also, compost addition generally increased the plant concentrations of phosphorus and potassium and decreased the contents of calcium and magnesium. For the micronutrients, an increase of copper and a decrease of iron and manganese were detected. Compost addition had no effect on zinc, which showed a non-defined trend throughout the experiment.

Even if further experiments should obviously be conducted, it can be concluded that the application of this compost, supplemented with nitrogen fertilisation, would be suitable and would contribute to the enhancement of productivity in the agricultural soils of southern Spain, which strongly demand supplementary organic fertilisation.

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CARACTERIZACIÓN Y COMPOSTAJE DE ALPECHÍN

Characterization of olive mill wastewater (alpechin) and its sludge for agricultural purposes

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Abstract

Ten samples of olive mill wastewater (OMW) taken from different mills in southern Spain and other ten of OMW sludges from evaporation ponds were analysed. The aim was to study the composition of these wastes and to find relationships which would make it possible to use easily determinable parameters to ascertain their composition.

Compared with other organic wastes, these materials had a high potassium concentration, a similar organic matter content and notable levels of nitrogen, phosphorus, calcium, magnesium and iron. The highest potassium concentrations were observed in the OMWs, while the sludges showed higher levels of the other nutrients, especially iron. The dry matter of the OMWs was significantly correlated with most of the parameters studied but, in the sludges, the only correlation was between the ash content and the total organic carbon and total nitrogen concentrations. The regression equations obtained permitted a rapid characterization of the OMWs from their dry matter content. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Olive-mill wastewater; Sludge; Organic carbon; Plant nutrients; Fats; Polyphenols; Carbohydrates

1. Introduction

Olive oil extraction produces vast amounts of liquid and solid wastes. The elimination of olive mill wastewater (OMW) is one of the main environmental problems related to the olive oil industry in Mediterranean countries, where Spain and Italy are the greatest producers. During recent years, the OMW production of Spain has been approximately $2.1 \times 10^6 \text{ m}^3 \text{ year}^{-1}$, which is generated during a few months of the year (November–February). This liquid waste comes from the vegetable water of the fruit and the water used in the different steps of oil production and contains olive pulp, mucilage, pectin, oil, etc. suspended in a relatively stable emulsion.

To solve the problems associated with OMW, different elimination methods have been proposed based on evaporation ponds, thermal concentration and physico-chemical and biological treatments, as well as its direct application to agricultural soils as an organic fertilizer (Fiestas Ros de Ursinos and Borja Padilla, 1992; Martínez Nieto and Garrido Hoyos,

1994). However, the most frequently used methods nowadays are the direct application to agricultural soils and storage in evaporation ponds, which produces a sludge.

OMW contains a high organic load, substantial amounts of plant nutrients and is a low cost source of water (Cegarra et al., 1997), all of which favour its use as a soil fertilizer. However, many authors have observed phytotoxic effects in plants when this waste is used directly as an organic fertilizer and have therefore warned against its direct application (Zucconi and Bukovac, 1969; Jelmini et al., 1976). Such negative effects are associated with its high mineral salt content, low pH and the presence of phytotoxic compounds, especially phenols. Negative effects have also been recorded on soil properties, including the immobilization of available nitrogen (Pérez and Gallardo-Lara, 1987; Saviozzi et al., 1991), the displacement of the exchange complex calcium by potassium in an anisot. increased salinity (López et al., 1996) and decreased plant-available magnesium, perhaps because of the antagonistic effect of potassium (Pérez et al., 1986).

Therefore, before an OMW can be used properly and safely it must be characterized. The aim of the

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study described in this paper was to characterize OMW and OMW-sludge samples from different mills in southern Spain in an attempt to find relationships which would make it possible to estimate the composition of these wastes from easily determined parameters.

2. Methods

Ten OMW samples were collected directly from the centrifuge of different mills in southern Spain and the ten OMW sludges from different evaporation ponds. All the samples were homogenized in a mixer and stored in a cool environment (4°C) prior to analysis. Electrical conductivity (EC) and pH were determined directly in the OMW samples and in a 1:5 (w/v) water-soluble extract of the OMW sludge samples, density (*d*) by weighing an exactly measured volume of OMW, dry matter content (DM) by drying at 105°C for 12 h, organic matter (OM) by loss on ignition at 430°C for 24 h (Navarro et al., 1993) and total nitrogen (TN) and total organic carbon (TOC) by automatic microanalysis (Navarro et al., 1991). After HNO₃/HClO₄ digestion, the P content was measured colorimetrically (Kitson and Mellon, 1944), Na and K by flame photometry and Ca, Mg, Fe, Cu, Mn and Zn by atomic absorption spectrophotometry. Polyphenols were extracted with ethyl acetate (Balice and Cera, 1984) and determined by the Folin method (Maestro Durán et al., 1991),

carbohydrates by the anthrone method (Brink et al., 1960) after deproteinizing the sample with Pb(CH₃-COO)₂ (Ebell, 1969) and fats by extraction with petroleum ether (Ministerio de Sanidad y Consumo, 1985). These methods for phenols, carbohydrates and fats were not suitable for determination on sludges.

3. Results and discussion

3.1. Characteristics of the olive oil industry wastes

The analyses made of OMW and OMW sludge samples are summarized in Table 1. As was to be expected, the percentage of dry matter of OMW sludges was statistically higher than that of OMWs, while no significant differences were observed in the pH, EC, OM and TOC between the sludges and the OMWs. The similar OM and TOC values might indicate the low degree of mineralization suffered by the organic matter of OMW during its storage in the evaporation ponds, while simultaneous processes of degradation and reorganisation may have taken place during this time. This was suggested by Saiz-Jiménez et al. (1986), who observed the simultaneous degradation of high molecular weight fractions to produce fractions of a smaller molecular weight and the synthesis of compounds which were more resistant to degradation. Pérez et al. (1992) also pointed to the lower phenol

Table 1
Analysis of OMW and OMW sludge samples (dry weight)

	OMWs			OMW sludges		
	Mean	Range	CV	Mean	Range	CV
Dry matter (%) ^a	7.19 b	4.12–16.38	3.87	48.00 a	14.23–94.69	43.38
pH	5.17 a	4.80–5.50	4.83	5.41 a	4.85–5.87	7.86
EC (dS/m)	5.50 a	4.00–13.98	41.73	6.74 a	1.53–9.03	53.76
OM (%)	64.60 a	58.45–70.63	6.33	71.06 a	43.84–94.26	26.43
TOC (%)	47.52 a	43.61–53.45	6.46	47.90 a	31.08–63.21	25.73
TN (%)	0.88 b	0.58–1.13	25.18	1.74 a	0.60–2.73	38.92
P (%)	0.19 a	0.06–0.32	51.73	0.14 a	0.06–0.30	45.37
K (%)	5.24 a	3.30–6.94	23.18	1.41 b	0.78–3.10	57.85
Na (%)	0.15 a	0.04–0.48	114.97	0.06 a	0.02–0.13	63.71
Ca (%)	0.42 b	0.32–0.53	18.66	2.87 a	0.51–10.22	107.24
Mg (%)	0.18 b	0.06–0.22	26.10	0.36 a	0.09–0.67	52.44
Fe (mg/Kg)	951 b	652–1482	31.83	4501 a	394–12096	97.74
Cu (mg/Kg)	21 b	14–44	40.63	61 a	14–203	91.51
Mn (mg/Kg)	15 b	1–53	110.07	97 a	19–288	91.15
Zn (mg/Kg)	57 a	31–82	31.55	37 b	18–55	37.30
d (g/cm ³)	1.02	1.01–1.06	1.30	nd	nd	nd
Fats (%)	4.27	0.55–11.37	84.41	nd	nd	nd
Polyphenols (%)	2.21	1.32–3.99	35.80	nd	nd	nd
Carbohydrates (%)	12.22	3.37–32.91	76.79	nd	nd	nd

^a Referred to fresh weight.

CV: Coefficient of variation. nd: Not determined (see Section 2).

Mean values followed by the same letter are not significantly different (*p* < 0.05) between the groups of wastes.

Table 2

Correlation matrix between the main analytical parameters of OMW samples referred as fresh weight ($n = 10$)

	Dry matter (g/l)	Ash (g/l)	EC (dS/m)	d (g/cm ³)	TOC (g/l)	TN (g/l)	P (g/l)	K (g/l)	Ca (g/l)	Fe (mg/l)	Cu (mg/l)	Mn (mg/l)	Zn (mg/l)	Fats (g/l)	Poly-phenols (g/l)
Ash	0.982***	1													
EC	0.968***	0.972***	1												
d	0.967***	0.928***	0.948***	1											
TOC	0.995***	0.978***	0.956***	0.950***	1										
TN	0.877***	0.873***	0.857**	0.783**	0.909***	1									
P	0.876***	0.933***	0.911***	0.772**	0.883***	0.867***	1								
K	0.937***	0.969***	0.975***	0.904***	0.925***	0.800**	0.914***	1							
Ca	0.890***	0.824**	0.824**	0.867***	0.893***	0.845**	0.690*	0.726*	1						
Fe	0.869***	0.840**	0.783**	0.819**	0.878***	0.771**	0.697*	0.763**	0.795**	1					
Cu	NS	NS	0.676*	NS	NS	NS	0.689*	NS	NS	NS	1				
Mn	0.762**	0.835**	0.792**	0.697*	0.734*	NS	0.772**	0.878***	NS	NS	NS	1			
Zn	0.911***	0.908***	0.915***	0.916***	0.897***	0.719*	0.824**	0.917***	0.650*	0.767**	0.632*	0.768**	1		
Fats	0.795**	0.816**	0.733*	0.694*	0.827**	0.769**	0.731*	0.798**	NS	0.854**	NS	0.713*	0.748**	1	
Polyphenols	0.925***	0.962***	0.918***	0.858**	0.928***	0.810**	0.897***	0.965***	0.724*	0.857**	NS	0.856**	0.875***	0.905***	1
Carbohydrates (g/l)	0.728*	0.734*	0.678*	0.761**	0.728*	NS	0.653*	0.684*	NS	0.694*	NS	NS	0.808**	NS	0.708*

*, **, ***: Significant at $p < 0.05$, 0.01, 0.001, respectively. NS: Not significant.

Data not shown were not statistically significant.

content of an OMW stored in an evaporation pond compared with that of a fresh OMW, and suggested that this may have been due to the polymerization of the low molecular weight phenols.

As regards the macronutrient content, the sludges had higher levels of TN, as has been reported previously by Saiz-Jiménez et al. (1986) in a study to identify proteins in OMW and OMW sludge. This might have been due to the biological fixation of nitrogen during storage of OMW in evaporation ponds since OMW is considered by many authors to be suitable substrate for the growth of free nitrogen fixers (Paredes et al., 1987; García-Barrionuevo et al., 1992).

The levels of K were lower in the sludges than in the OMWs, probably because of the highly soluble nature of this element, which would lead to its filtering

through the permeable substrate in the bottom of the ponds. The higher Ca levels noted in the sludges may have been due to mixing with the calcium present in the soil where the ponds were constructed, since the soils of southern Spain are calcareous.

The levels of micronutrients varied greatly although they were generally higher in the sludges. The high iron content of the sludges may, as in the case of Ca, have been due to mixing with the mineral components in the bottom of the ponds.

The fat, polyphenol and carbohydrate contents and density of OMW were also determined (Table 1), the fat and carbohydrate levels, particularly, varying greatly.

The two groups of wastes analysed generally showed high concentrations of K, similar quantities of OM and

Table 3

Correlation matrix between the main analytical parameters of OMW sludge samples referred as dry weight ($n = 10$)

	Dry matter (%)	Ash (%)	pH	EC (dS/m)	TOC (%)	TN (%)	Mg (%)	Fe (mg/Kg)
pH	0.683*	0.648*	1					
EC	NS	0.696*	NS	1				
TOC	NS	-0.942***	-0.773**	NS	1			
TN	NS	-0.906***	-0.708*	NS	0.918***	1		
P	NS	0.652*	NS	NS	NS	NS		
Ca	NS	0.697*	0.636*	NS	-0.723*	NS		
Fe	NS	NS	NS	NS	NS	NS	0.813**	1
Cu	NS	NS	NS	-0.678*	NS	MS	0.658*	NS
Mn	NS	NS	NS	NS	NS	NS	NS	0.663*
Zn (mg/Kg)	NS	0.884***	NS	NS	-0.850**	-0.764**	NS	NS

*, **, ***: Significant at $p < 0.05$, 0.01, 0.001, respectively. NS: Not significant.

Data not shown were not statistically significant.

Table 4

Parameters of the linear regression and correlation coefficient values between dry matter (DM) and electrical conductivity (EC), organic matter (OM), total organic carbon (TOC), the main macro and micronutrients and polyphenols in OMW. $Y = a + bX$; $X = DM$ (g/l). ($n = 10$)

Y	a	b	r
EC (dS/m)	1.516*	0.071***	0.9682***
Ash (g/l)	-4.798 NS	0.427***	0.9822***
TOC (g/l)	1.220 NS	0.455***	0.9952***
TN (g/l)	0.160 NS	0.006***	0.8764***
P (g/l)	-0.081 NS	3.237***	0.8756***
K (g/l)	-0.916 NS	0.066***	0.9365***
Ca (g/l)	0.080 NS	3.074***	0.8898***
Fe (mg/l)	16.994 NS	0.667**	0.8686***
Zn (mg/l)	-0.701 NS	0.068***	0.9114***
Polyphenols (g/l)	-1.438*	0.044***	0.9247***

*, **, ***: Significant at $p < 0.05$, 0.01 , 0.001 , respectively. NS: Not significant.

notable levels of N, P, Ca, Mg and Fe compared with those found by Cegarra et al. (1993) in manures and composts made from municipal solid wastes, which are commonly used as organic fertilizers. According to that, these wastes from the olive oil industry could be used as organic fertilizers in agricultural soils both for their elimination and for improvement of soil fertility. However, the notable contents of mineral salts, fats and polyphenols in OMW could limit their direct application to soils.

3.2. Relationships between the main analytical parameters of OMW and OMW sludge samples

The great variation found in the characteristics of these wastes (see coefficients of variation, Table 1), means that they should be characterized fully before being used for agricultural purposes. This is particularly true in the case of the sludges since their composition depends not only on the factors which influence the composition of OMW but on such factors as the time of storage, depth of the pond (which decides whether the organic matter degrades aerobically or anaerobically), climatic conditions, loss through

Table 5

Parameters of the linear regression and correlation coefficient values between ash and total organic carbon (TOC) and total nitrogen (TN) in OMW sludges. $Y = a + bX$; $X = Ash$ (%) (dry weight). ($n = 10$)

Y	a	b	r
TOC (%)	65.784***	-0.618***	-0.9418***
TN (%)	2.682***	-0.033***	-0.9060***

***: Significant at $p < 0.001$.

seepage, etc. However, since a full analysis would be very time consuming, it would be useful to establish equations which would make it possible to ascertain most parameters from one of them easily determined.

Correlations between the main parameters of the OMWs, referred to fresh weight, are shown in Table 2, where those between dry matter and ash, EC, d, TOC, principal nutrients and polyphenols are highly statistically significant ($p < 0.001$). However, in the case of the sludges significant correlations ($p < 0.001$) were found only between ash and TOC, TN and Zn and between TOC and TN (Table 3).

Table 4 shows the values for the coefficient of correlation and of the parameters of the regression equations obtained between dry matter and EC, ash, TOC, principal nutrients and polyphenols for the OMWs, while Table 5 shows the regression equations between ash and TOC and TN for OMW sludges. In both groups of equations the high r values, of between 0.9952 and 0.8686, indicate a high level of correlation ($p < 0.001$). In the case of the OMWs, it was found that, except for EC and polyphenols, the intercept (a) was not statistically significant so that the ash, TOC, TN, P, K, Ca, Fe and Zn contents could be determined by multiplying the concentration of dry matter by the slope (b). In the case of the sludges, on the other hand, the intercept (a) was significant for all parameters so that an equation must be used to calculate the concentration of TOC and TN from the ash content.

3.3. Conclusions

Both groups of wastes showed generally notable contents of organic matter and substantial quantities of plant nutrients compared with those found in manures and city refuse composts.

The great variation in most of the parameters determined in both the OMWs and the sludges means that they should be characterized fully before they are used for agricultural purposes. In the case of the OMW, their composition can be easily ascertained by determining the dry matter content, using the highly significant correlations observed between this parameter and the ash, EC, principal nutrients and polyphenols. In the case of OMW sludges, only the TOC and TN concentrations can be calculated by reference to the ash content, a routine method which is much more straightforward and less costly than those usually used for their analysis.

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Use of Olive Mill Wastewater Compost for Crop Production

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Vast amounts of olive mill wastewaters (OMW) are produced in Mediterranean countries, where their treatment and disposal are becoming a serious environmental problem. Increasing attention has been paid to discovering a use for OMW and a wide range of technological treatments are available nowadays for reducing their pollutant effects and for their transformation into valuable products, the most suitable procedures being found to involve recycling rather than the detoxication of these wastes. Direct application of OMW to soil has been considered as an inexpensive method of disposal and recovery of their mineral and organic components but, because of their organic acid and phenol contents, OMW are also a source of pollution. By using composting technologies, it is possible to transform either fresh OMW or sludge from pond-stored OMW mixed with appropriate plant waste materials (carriers) into organic fertilizers (composts) with no phytotoxicity to improve soil fertility and plant production, the process involving the microbial degradation of the polluting load of the wastes. Results of field and pot experiments using OMW-composts to cultivate horticultural and other crops have shown that yields obtained with organic fertilization are similar, and sometimes higher, to those obtained with a balanced mineral fertilizer. A comparison between the macro and micronutrient contents of plants cultivated with organic or mineral fertilizers did not generally reveal important differences. However, the cases of iron and manganese are worth mentioning as their bio-availability may be linked to the soil humic complexes originated by the OMW organic fertilizers. © 1997 Elsevier Science Limited. All rights reserved

INTRODUCTION

Vast amounts of olive mill wastewaters (OMW) are produced in Mediterranean countries, where their treatment and disposal are becoming a serious environmental problem. Increasing attention has been paid to discovering a use for OMW and a wide range of technological treatments are available nowadays for reducing their pollutant effects and for their transformation into valuable products. Different methods based on thermal concentration, physico-chemical and biological treatments of the OMW as well as their direct application to agricultural soils as a fertilizer have been widely tested, the most suitable procedures being found to involve recycling rather than detoxication of these wastes.

If soil characteristics are appropriate, the OMW are probably best used as a fertilizer since this is an inexpensive method of disposal and

important advantages may be derived for soil fertility, among which the following can be cited (Fiestas Ros de Ursinos, 1986; Tomati and Galli, 1991):

- the effective use of plant nutrients contained in the wastes, mainly K, but also N, P and Mg;
- a low cost source of water, taking into account the increasing scarcity of hydraulic resources for irrigation; and
- supply of organic matter (OM), which enhances microbial activity and improves the physical and chemical properties of soil.

As disadvantages, the following may be cited:

- the high content of mineral salts and the presence of organic compounds, such as fatty acids and polyphenols in the OMW, both factors being detrimental to soil fertility; and
- the difficulty of storing and disposing of the

large amounts of this liquid waste which is produced in a short, and often rainy, period of time.

According to the above considerations, the following recommendations for using OMW as fertilizer have been made (Fiestas Ros de Ursinos, 1986):

- the by-product should be applied at a certain distance from trees;
- doses should not exceed $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ (OMW from traditional olive oil mills) and $100\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ (those obtained by the continuous system);
- applications should be made in a stepwise fashion;
- at least 1 month should elapse between the application and the sowing for seeding yearly crops; and
- the by-product must never be added when the crops are in the sprouting period.

Some studies have been carried out on the direct application of OMW to soils as fertilizer either as a fresh liquid or sludge and their effect on soil characteristics and crop production (Morisot and Tournier, 1986; Pérez and Gallardo-Lara, 1987; García-Rodríguez, 1990; Saviozzi *et al.*, 1991; Riffaldi *et al.*, 1993). However, very little research has been performed as on OMW composting and the use of such compost for crop production (Amirante and Di Renzo, 1990; Cabrera *et al.*, 1990). More work is necessary to improve our knowledge of OMW composting, which probably is the best method for recycling these pollutant liquid wastes.

Composting is as a controlled biooxidative process that involves a heterogeneous organic substrate in the solid state, which evolves through a thermophilic stage and the temporary release of phytotoxins, leading to the production of carbon dioxide, water, mineral salts and stabilized OM containing humic-like substances. By using this method, it is possible to transform either fresh OMW or sludge from pond-stored OMW mixed with appropriate plant waste materials (carriers) into organic fertilizers (composts) with no phytotoxicity to improve soil fertility and plant production.

In the following paper, the characteristics of the raw materials needed for OMW composting, the effect on the composting process of adding fresh OMW to the carriers, the origin and main

characteristics of the OMW composts, and the effect of these composts on the yield and nutritional status of different crops are examined.

CHARACTERISTICS OF RAW MATERIALS FOR OMW COMPOSTING

Composting of OMW may be performed starting from fresh samples or sludges which have been stored in ponds for months or even years. As regards their potential use as fertilizer, OMW samples obtained by the continuous system contain substantial amounts of K ($2\text{--}4\text{ g l}^{-1}$) which is useful for compost preparation (Table 1), although their N and P contents are very low (<0.8 and 0.24 g l^{-1} , respectively), as is the level of the principal micronutrients (data not shown). As also shown in Table 1, the fat content ranges from 0.26 to 4.79 g l^{-1} , that of sugars from 1.4 to 15.9 g l^{-1} , and polyphenols from 0.86 to 1.61 g l^{-1} . The exception is the sample No. 1, which was taken from a factory that still uses the traditional method of extracting oil (discontinuous system) and which exhibited much higher values for the above-mentioned parameters.

A statistical analysis of some chemical characteristics of fresh and pond-stored OMW samples (Table 2), shows that the values of the coefficient of variation and the range of most of the parameters studied are usually higher in the sludges than in the fresh OMW, as is to be expected considering that the former are influenced by the changeable climatic conditions during their storage. The mean value of the K content is clearly lower in the sludges probably due to its great mobility, which facilitates its percolation through the more or less permeable layers of soil at the bottom of ponds. However, the statistical values of the Ca content are higher in the stored samples, which may be explained by its lower mobility compared with the K, its special suitability for linking OM and also because it is a common component of soil minerals, which may easily mix with the olive wastes during their storage in ponds. In addition, the mean values of the Fe content is clearly higher in sludges than in fresh samples, which is beneficial in the production of OMW compost because this micronutrient is of paramount interest for plant growth, particularly in calcareous soils. Lastly, it must be noted that the mean value of N content is higher in the stored than in the fresh samples,

Table 1. Some Characteristics of the Fresh OMW Samples

Sample No.	Water content (%)	Dry matter	Organic matter	Organic C	K g l ⁻¹	Fats	Sugars	Polyphenols
1	82.70	172.94	101.25	80.0	11.33	13.15	24.5	6.90
2	94.43	55.71	32.68	24.0	3.59	0.82	2.8	1.31
3	95.40	45.94	30.07	22.5	3.19	2.83	2.7	0.94
4	93.76	62.41	40.50	28.7	3.74	1.03	5.4	1.03
5	93.42	65.78	42.78	33.2	2.76	3.42	15.9	1.15
6	95.84	41.54	28.93	22.0	2.00	4.72	1.4	1.30
7	92.24	77.60	47.66	37.4	4.16	0.55	9.1	1.61
8	92.28	77.10	50.19	37.7	2.54	4.79	5.9	1.48
9	90.57	94.30	66.61	43.4	3.86	1.63	8.3	1.25
10	95.33	46.65	31.12	20.7	2.21	0.26	15.4	0.86

Table 2. Coefficient of Variation (CV), Mean Value and Range of Some Characteristics of the Samples of Fresh OMW (f) and OMW Sludges (s) (% d.m.)

	CV		Mean V		Range	
	f	s	f	s	f	s
Organic matter	6.3	26.4	64.61	71.06	58.55-70.64	43.84-94.26
Organic C	6.2	25.7	47.52	47.90	43.08-52.96	31.08-63.21
Nitrogen	23.1	38.9	0.84	1.74	0.54-1.09	0.60-2.73
Phosphorus	52.6	45.3	0.19	0.14	0.06-0.31	0.06-0.30
Potassium	23.2	57.8	5.24	1.41	3.29-6.94	0.78-3.10
Sodium	115.5	64.9	0.14	0.06	0.03-0.48	0.02-0.13
Calcium	18.7	107.2	0.42	2.87	0.32-0.53	0.51-10.22
Magnesium	40.0	52.3	0.19	0.36	0.06-0.36	0.09-0.67
Iron	31.9	97.0	0.09	0.45	0.06-0.15	0.05-1.21

which suggests both a selective biodegradation of the organic compounds not containing N and a degree of biological N-fixation during the storage of olive wastes. Nevertheless, the fact that the average OM content is higher in the sludges (71.06% against 64.61% in fresh samples) points to the elaboration of organic compounds rather than their biodegradation during OMW storage, biological fixation probably being responsible for most of the increased N content in stored samples.

The characteristics of the carriers are of paramount interest for OMW composting,

basically because of their differing capacity to hold the OMW. Table 3 and Table 4 show some characteristics of a series of plant waste materials, most of them suitable for mixing with OMW samples as carriers for subsequent composting. Except in the case of one of the carriers (spent mushroom compost) all contain 75% or more OM, a relatively low percentage of lignin (except for grape and olive marcs) with a consequently high level of both α -cellulose and hemicellulose and generally show low electrical conductivity (EC) values. These results mean that these

Table 3. OMW-EC, EC and Other Characteristics of the Carriers (d.m.)

Carriers	HC (l kg ⁻¹)	EC (mS cm ⁻¹)	pH H ₂ O	Organic matter	Lignin (%)	α -Cellulose	Hemicellulose
Sweet sorghum bagasse	6.42	0.39	6.3	96.66	23.9	37.0	31.8
Sugar cane bagasse	6.33	1.04	6.1	95.92	18.5	32.6	38.3
Barley straw	5.41	1.62	7.2	93.32	20.0	40.3	36.6
Maize straw	5.27	1.64	6.8	93.87	27.1	25.5	30.9
Cotton waste	3.28	1.61	7.3	87.99	23.3	40.4	23.9
Spent mushroom compost	1.89	8.65	7.0	37.44	22.2	16.3	14.0
Grape marc	1.92	1.02	7.2	79.38	55.3	37.4	18.3
Olive marc	1.12	1.52	5.4	97.85	32.7	29.5	42.7
Rice hull	2.56	0.84	4.5	78.64	22.4	36.1	30.0
Sugar cane sludge	2.02	4.66	6.2	74.55	17.0	15.4	27.4

Table 4. Macro and Micronutrient Content of the Carriers (d.m.)

Carrier	N	P (%)	K	Fe mg kg ⁻¹	Cu mg kg ⁻¹	Mn mg kg ⁻¹	Zn mg kg ⁻¹
Sweet sorghum bagasse	0.42	0.02	0.12	1032	20	12	31
Sugar cane bagasse	0.54	0.06	0.53	1090	13	20	93
Barley straw	0.53	0.08	1.57	280	12	13	30
Maize straw	0.52	0.16	1.14	410	11	30	72
Cotton waste	1.64	0.18	1.48	1911	10	80	60
Spent mushroom compost	2.12	0.57	1.92	4920	33	210	111
Grape marc	1.69	0.17	1.35	2573	40	60	34
Olive marc	0.64	0.04	0.50	351	10	11	21
Rice hull	0.35	0.07	0.44	2705	14	205	32
Sugar cane sludge	3.25	0.26	0.88	7320	40	202	160

materials may be considered suitable for preparing organic fertilizers, because a high content of organic compounds rich in cellulose and of low salinity enhances microbial growth during composting and improves compost quality. As regards the OMW-holding capacity (HC) of the carriers, sweet sorghum and sugar cane bagasses, barley and maize straws, and cotton waste show the highest values among all the materials studied. Quite high levels of N (sugar cane sludge, spent mushroom compost, cotton waste, grape marc), K (spent mushroom compost, barley straw and cotton waste) and also Fe (sugar and mushroom wastes) are present in the carriers.

When carrier and OMW are mixed, it is necessary to ensure that appropriate values of the C/N ratio are reached in the starting mixtures and so it may be considered convenient to add organic wastes containing heavy loads of N, such as poultry manure and sewage sludges, to the above mixtures. Also, neutral or acid pH values are to be recommended for the starting raw mixtures, since this minimizes N losses during OMW composting. In addition, care should be taken to keep EC values as low as possible, as composts with high salt loads are detrimental to soil fertility.

EFFECT OF ADDING FRESH OMW ON THE FERMENTATION OF THE CARRIER

In order to ascertain the influence of adding fresh OMW on the aerobic fermentation of the solid carriers used to hold these liquid wastes during composting, an experiment was carried out by making two identical mixtures of sewage sludge (S) and cotton waste (C), which were prepared by mixing 32.1% of S and 67.9% of C on a fresh weight basis (9.2 and 90.8% dry weight, respectively), and composting in the pilot plant.

One (SCO) was watered with fresh OMW (943 l/tonne of wet solid mixture) and the other (SC) with only water, their characteristics at the beginning of the process are shown in Table 5. The moisture of both mixtures was brought up to 70% during composting.

About 1500 kg of each mixture was placed in trapezoidal piles of 1–1.5 m high with a 2×3 m base. The Rutgers static pile composting system was used, involving on-demand ventilation through temperature feedback control. The air was blown from the base of the pile through the holes of three PVC tubes of 3 m length and 12 cm diameter. The timer was set for 30 s ventilation every 15 min, and the ceiling temperature for continuous air blowing was kept to 55°C, except during the first week of composting when the temperature reached nearly 60°C due to the excess of heat produced by the vigorous biodegradation of OM. Piles SCO and SC were turned after 35 and 21 days, respectively, in order to improve the homogeneity of the material and the fermentation process. The biooxidative phase of composting

Table 5. Analysis of the Sewage Sludge and Cotton Waste Mixtures Watered (SCO) and Not Watered (SC) with Fresh OMW at the Starting (1) and Maturity (2) Steps of Composting (d.m.)

	SCO (1)	SCO (2)	SC (1)	SC (2)
pH (H ₂ O)	7.10	7.84	7.61	7.31
EC (1:10) (mS cm ⁻¹)	4.39	7.66	3.91	6.70
Organic matter	80.74	56.43	81.53	64.85
Organic C	42.50	29.37	43.86	35.55
Nitrogen (%)	1.92	3.11	2.08	3.79
C/N ratio	22.13	9.44	21.09	9.38
HA-C	2.83	1.36	7.18	5.07
FA-C	2.95	1.84	3.75	2.57
HA/FA	0.96	0.74	1.91	1.97
HR (%)	13.60	10.90	24.92	21.49
CEC (me per 100 g)	59.1	109.8	53.5	124.4

was considered finished when the temperature of the pile was stable and near to that of the atmosphere, this stage being reached after 84 and 49 days of composting for piles SCO and SC, respectively. The airblowing was then stopped to allow the compost to mature over a period of two months.

Results of the experiment show that the addition of OMW led to a longer composting time, the thermophilic phase of the process lasting about 8 weeks, compared with the 4–5 weeks this step lasted in the pile containing no OMW. As a result of the longer composting time, the biodegradation undergone by the OM was greater in the SCO mixture than in the SC and, at the end of the process, the OM contents in the mature composts were 56.43 and 64.85%, respectively (Table 5). The addition of OMW resulted in higher EC values (7.66 as opposed to 6.70 mScm⁻¹ in the mature composts). The initial cation exchange capacity (CEC) value was lower in SCO than in SC, but not significantly, given the limitations of the technique used to measure this parameter in fresh organic wastes. However, the CEC was notably lower in the mature compost containing OMW. The characteristics of the humic-like substances were also affected by the addition of OMW, and the data referring to the humification rate (HR) and the HA/FA ratio were particularly striking, as the values of both parameters in mature composts were clearly lower in that prepared with OMW.

ORIGIN AND CHARACTERISTICS OF THE OMW COMPOSTS

In addition to the SCO compost made with fresh OMW, two other composts were made by using the above mentioned C waste and maize straw (M) as carriers, and mixing them with OMW sludges. The starting mixtures were prepared by mixing (fresh weight) 20% of C with 80% of OMW sludge (COS) and 11.1% of M with 88.9% of the same OMW sludge (MOS). The same technical conditions as were employed for fresh OMW composting were used.

Composts from OMW sludges, clearly showed different characteristics to that obtained from fresh OMW (Table 6). The former had considerably lower contents of OM, total N, nitrates and P, and had a lower EC value compared with the latter. On the other hand, they

Table 6. Characteristics of the Mature OMW Composts (d.m.)

	SCO	COS	MOS
pH (H ₂ O)	7.84	8.99	8.73
EC (1:10) (mScm ⁻¹)	7.66	5.01	5.03
Organic matter (%)	56.43	34.84	37.40
Organic C (%)	29.37	19.95	18.78
Nitrogen (%)	3.11	1.87	1.44
NO ₃ -N (%)	0.36	Traces	Traces
C/N ratio	9.44	10.67	13.04
Phosphorus %	0.87	0.26	0.22
Potassium %	2.61	3.73	3.25
Iron (%)	0.50	1.46	1.30
Copper (mg kg ⁻¹)	52	62	62
Manganese (mg kg ⁻¹)	241	310	268
Zinc (mg kg ⁻¹)	245	117	73
HA-C (%)	1.36	6.29	7.49
FA-C (%)	1.84	1.72	2.28
HA/FA	0.74	3.66	3.29
HR (%)	10.90	40.15	52.02
CEC (me per 100g)	109.8	111.9	96.2
GI	94.4	82.4	69.8

exhibited higher pH values, a greater content of K and particularly Fe and had humic-like substances which were more alkali-soluble (higher HR) and polymerized (higher HA/FA ratio) than compost prepared using the fresh OMW. The compositions of the mature OMW composts are consistent with that of the starting mixtures. Thus, both the COS and MOS starting mixtures had the highest amounts of OMW, which was a determining factor in producing the longest biooxidative periods during composting and the greatest biodegradation of the OM of all the OMW composts. Also, the high degree of water evaporation due to the excessive heat generated during composting of OMW sludges, meant that the piles had to be watered frequently during the process, which increased salt percolation and probably losses of highly soluble compounds such as nitrates and low molecular weight humic substances (FA). Lastly, the clearly higher HR values and those of the CEC in COS and MOS (321 and 257 me per 100g of OM, respectively in comparison to 167 me per 100g of OM in SCO) meant that organic fertilizers obtained with OMW sludges were more humified and showed better stability and maturity characteristics than that made with fresh OMW.

USE OF OMW COMPOST FOR CROP PRODUCTION

Below we detail the results of field and pot experiments with three horticultural crops, cereal

and rye-grass using the SCO compost made with fresh OMW and that prepared with the same raw materials but not watered with OMW (SC), as well as the two OMW sludge composts (COS and MOS).

Field experiments with horticultural crops using compost of fresh OMW

In an attempt to ascertain comparatively the effectiveness of SC and SCO composts on plant yield and quality, field experiments were carried out with two horticultural plants. The results obtained using the composts in a calcareous and sandy clay loam textured soil (Typic Calcicorthids) were compared with those obtained using a balanced mineral fertilizer in the same soil (control treatment). Chard (*Beta vulgaris*) was cultivated for 77 days and then the soil was tilled and allowed to rest for 3 weeks. Lettuce (*Lactuca sativa*) was then cultivated for 44 days. For irrigation purposes the soil was divided into lots measuring 2×3 m with 1 m left between each plot. Three treatments (control with mineral fertilizer, and soil amended either with SC or SCO composts) were then carried out, using two different doses for the composts (30 and 60 tonnes ha⁻¹). Four repetitions of each dose and treatment were made. *B. vulgaris* was collected in three successive harvests (total yield being the sum of the three), while *L. sativa* was harvested once.

The yields of both crops obtained by means of organic fertilization did not differ significantly from the yields obtained in the control either as a consequence of the compost type or of the doses used [Fig. 1(a)]. The only difference was in the length of *B. vulgaris* leaves, which were slightly shorter with low doses of both composts, while the same parameter did not differ from the control when the composts were added at high concentrations.

The macronutrient content of *B. vulgaris* plant material [Fig. 1(b)] was significantly affected by both compost types and application rates, with higher concentrations of all elements except K being observed after treatment. Thus, the N content was higher in compost-fertilized plants at high application rates than in the control, although this increase was less evident when the compost prepared from fresh OMW was used (SCO). A similar type of behaviour was observed for P, although in the case of this nutrient both application rates resulted in significantly higher

concentrations than was obtained with the control. The levels of the other macronutrients, Ca and Mg, were also higher in soil treated with both composts than in the control, but only at low doses. As regards the micronutrient content (Fe, Cu, Mn and Zn) of the *B. vulgaris* plants, only the concentrations of Fe and Mn were significantly affected by application rate and treatment [Fig. 1(c)]. The levels of the former were always higher in plants cultivated with compost than those in the control particularly at low doses and in those plants cultivated with compost SC. The levels of Mn were greater in organically fertilized plants, except in those cultivated with the high dose of the SC compost, which was similar to that of the control.

The macronutrient content of *L. sativa* plants was not significantly affected by either treatment or application rate [Fig. 1(d)] and the micronutrient content was only slightly affected [Fig. 1(e)]. In the case of Mn, the concentration in this plant material was almost the same as in the control when SCO compost was added but clearly less when the soil was fertilized with SC. Copper behaved in a similar way to Mn, although the differences between both composts and between those and the control were very slight.

Cultivation of barley with compost of fresh OMW

Barley (*Hordeum vulgare*) was cultivated in the same plots as those employed for the cultivation of both *B. vulgaris* and *L. sativa* and only the control was again fertilized with an appropriate mineral fertilizer. The barley grew for exactly 5 months before it was harvested, the grain being separated from the straw for yield determination and macro and micronutrient analyses.

The total biomass yield (11.96 tonnes ha⁻¹) was clearly highest in the crop harvested from mineral fertilized plots [control, Fig. 2(a)], whereas barley grown with composts yielded less biomass than the control. At low doses, the compost prepared with OMW (SCO) yielded less than the SC compost, but higher and equivalent results were recorded in plots which had been fertilized with high doses of the two organic fertilizers. Grain yield followed an almost identical and approximately equal pattern to that of total biomass, although it is worth noting that the grain harvested from organically fertilized plots accounted for about 50% of the total biomass, particularly at the high dose, this rate decreasing

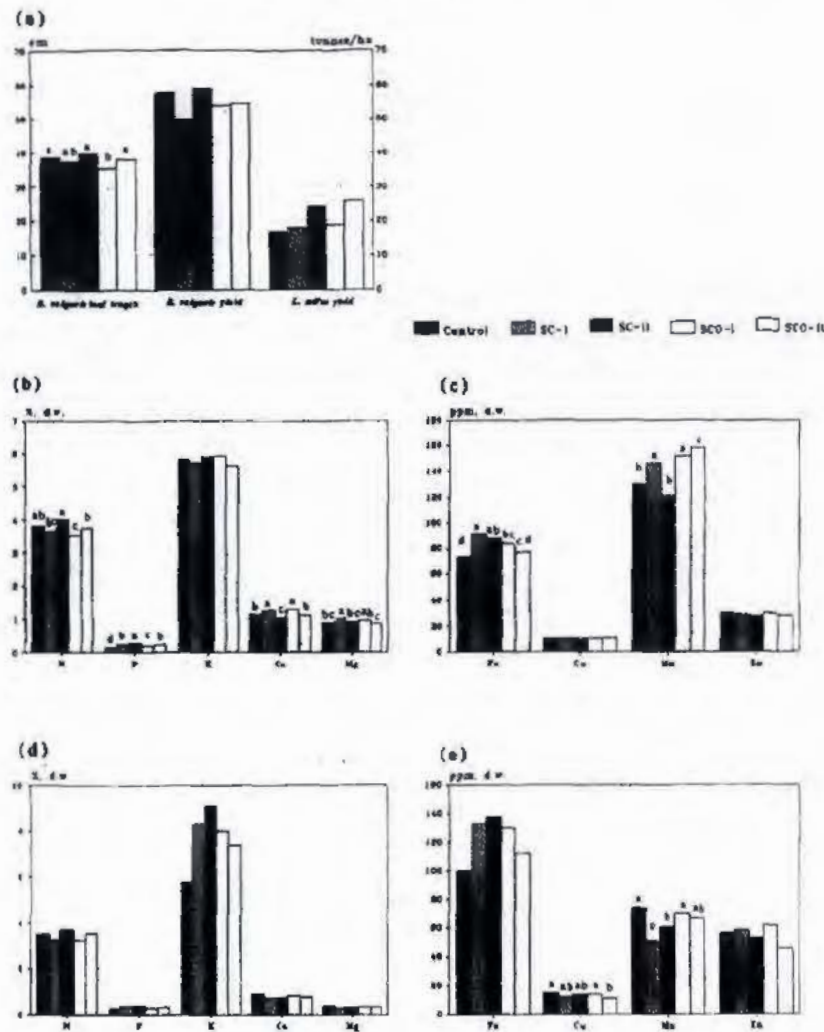


Fig. 1. Effect of the SC and SCO compost treatments and doses (I, II) on leaf length and yield of (a) *B. vulgaris* and *L. sativa*, (b) macro and (c) micronutrients of *B. vulgaris* and (d) macro and (e) micronutrients of *L. sativa*. I, II, Doses of 30 and 60 tonnes ha^{-1} , respectively. Columns with the same letter are not significantly different at the 0.05 probability level (Duncan test).

to nearly 40% for the crop obtained with mineral fertilization.

As regards macronutrients, barley grain concentrated more N and P than did straw, whereas the latter concentrated more K, Ca and Mg [Fig. 2(b)]. Among the micronutrients, the concentration of Fe was much higher in straw than in grain, the difference being lower for Mn. However, the Cu and Zn contents, were nearly the same in grain and straw. The N content of both plant components [Fig. 2(b and d)] from crops grown on mineral fertilized plots was greater than in crops cultivated with the composts, slightly greater values being found for SCO-cultivated barley. The P concentration of both grain and straw was generally higher in compost-amended plots than in the control, the effect being more evident in the grain of

the SCO fertilized crop. Results for K showed that composts were generally more effective in supplying this macronutrient to barley than was the mineral fertilizer (in the case of straw), the effectiveness being nearly equal for grain. As for the Ca content of the straw, the differences were not significant but they were slightly so in grain, whereas Mg was found to be more concentrated in SCO-cultivated barley, this being particularly evident for straw.

As regards the micronutrient content, it must be stressed that no important differences were observed between treatments in grain [Fig. 2(c)]. In the case of straw, the concentrations of Fe, Cu and Zn in plants cultivated with the SC compost were generally higher than in the control, and the results for this latter treatment were often similar to those recorded from the SCO-fertilized plots

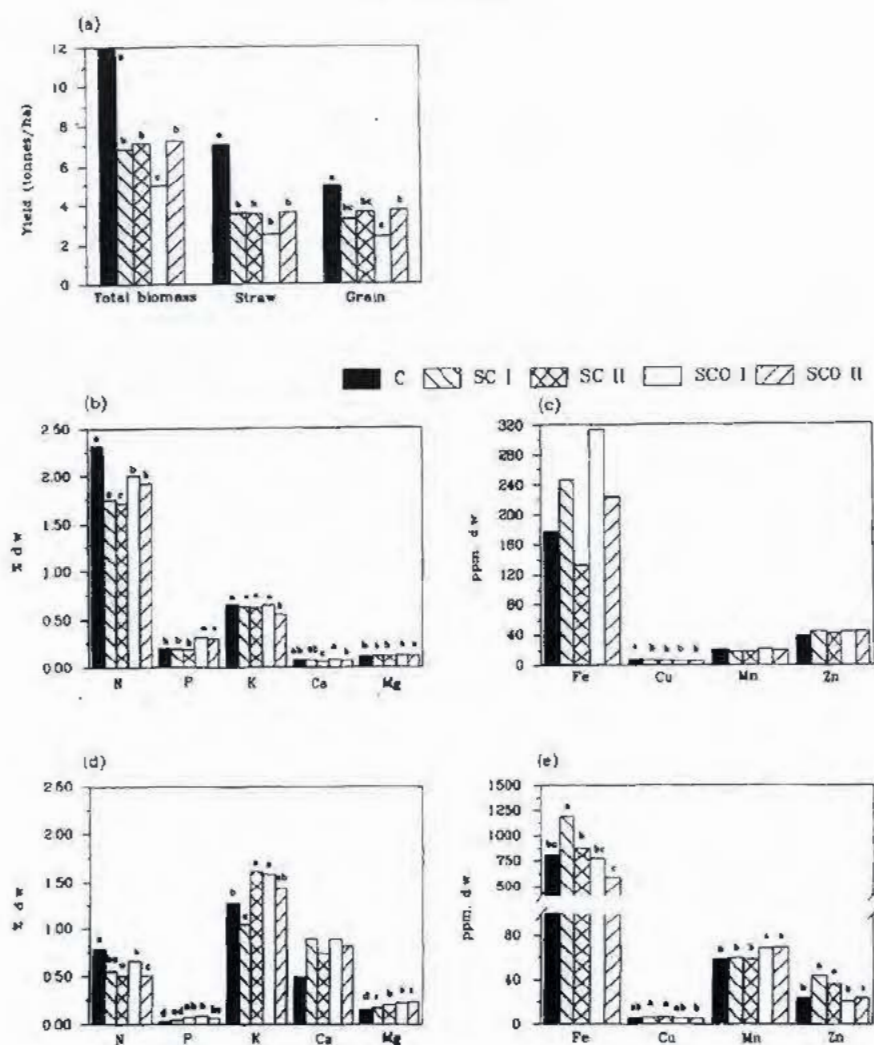


Fig. 2. Effect of the SC and SCO compost treatments and doses (I, II) on (a) total biomass, grain and straw yields, (b) macro and (c) micronutrients of the grain and (d) macro and (e) micronutrients of the straw of *H. vulgare*. I, II, Doses of 30 and 60 tonnes ha^{-1} , respectively. Columns with the same letter are not significantly different at the 0.05 probability level (Duncan test).

[Fig. 2(e)]. The pattern for Mn was opposite, as the highest content of this element was found in straw from barley fertilized with the SCO compost.

Ryegrass pot experiments using compost of fresh OMW

English ryegrass (*Lolium perenne*) was cultivated in pots containing the above mentioned calcareous soil which was fertilized with the SCO compost and the results compared with those obtained either using a balanced mineral fertilizer or with no fertilizer added. Three repetitions of each treatment were carried out. The unfertilized pots were filled with 3000 g of soil while those fertilized with the organic SCO received 2700 g of soil and 100 g of compost (equivalent to 30 tonnes ha^{-1}). The mineral fertilizer

was added weekly as a nutrient solution, which contributed 284 mg of N, 129 mg of P and 236 mg of K during the cultivation period. The grass was harvested three times at 30 day intervals and the yields of plant material along with the N, P, K, Ca, Mg and Fe contents in plant tissues were measured for each harvest.

The yield of *L. perenne* fertilized with the OMW compost was in all cases higher than the yields recorded for the experiments using mineral fertilizer and with no added fertilizer [Fig. 3(a)], the greatest yield corresponding to the second harvest and the smallest to the third.

The N content in plant tissues obtained by means of organic fertilization was greater than in the other two treatments at the first harvesting, with an even greater difference being recorded at the second

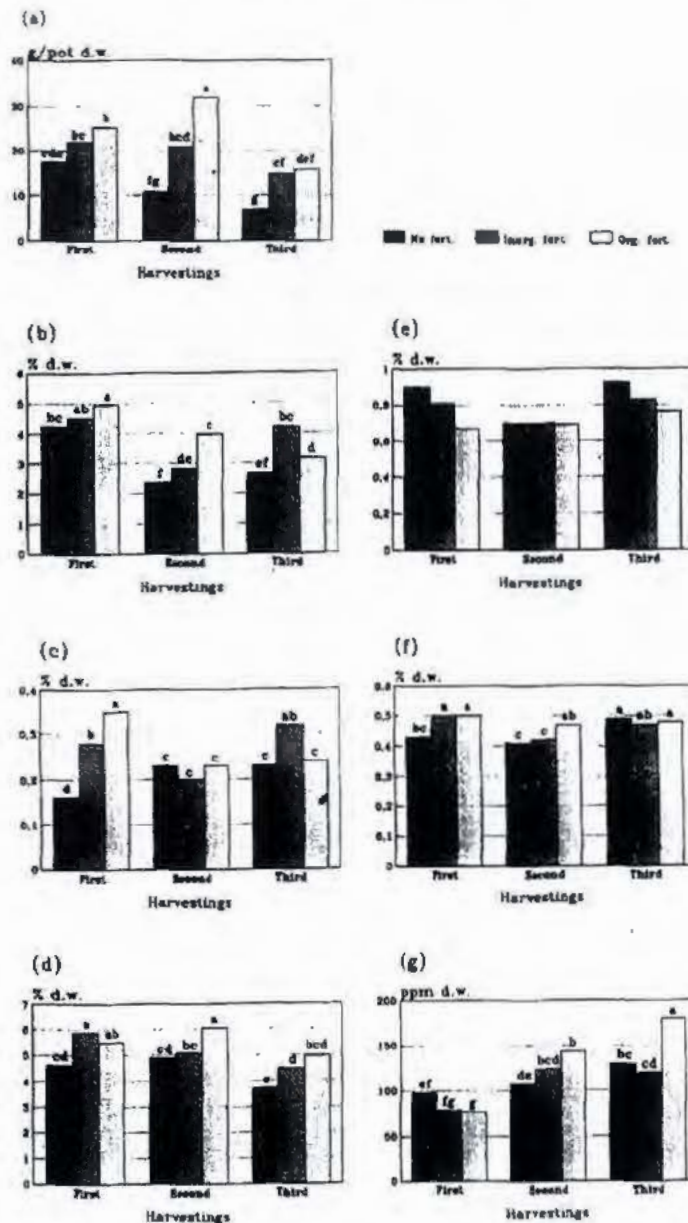


Fig. 3. Effect of the SCO compost treatment on (a) yield and (b) nitrogen, (c) phosphorus, (d) potassium, (e) calcium, (f) magnesium and (g) iron contents of *L. perenne*. Columns with the same letter are not significantly different at the 0.05 probability level (Duncan test).

harvesting [Fig. 3(b)]. However, at the third harvesting the N level had fallen to below that recorded for the plants grown in soil receiving mineral fertilizer. The P content of *L. perenne* [Fig. 3(c)] was also higher in the organically fertilized experiment than in the other two treatments, although only at the first harvesting. At the second harvesting, the P content was similar in all three treatments while at the third harvesting it was clearly higher in the plants grown with mineral fertilizer. Considering the results for N and P

together, it is clear that the compost rapidly made available to the plants the most easily assimilable fraction of both nutrients, but by the end of the experiment the concentrations of bioavailable N and P were well below the easily available levels of the mineral fertilizer. As regards K [Fig. 3(d)], the organic fertilizer supplied a sufficient quantity of this mineral throughout the experiment, since at the first harvesting the levels were almost equal in the plants cultivated with both fertilizers but much higher in those grown with organic fertilizer at the

second harvesting, this same pattern continuing at the third harvesting. As was to be expected given the ready availability of Ca in experiments using a calcareous soil, there were no significant differences between the Ca levels recorded in the three treatments at any time [Fig. 3(e)]. As regards Mg, it is clear that this nutrient was easily available in the compost-treated soil, where its levels were highest at all three harvestings, although the mineral fertilized soil at the first harvesting and the unfertilized soil at the third harvesting supplied similar levels of Mg to plants [Fig. 3(f)].

The case of Fe is worth mentioning [Fig. 3(g)] since the levels of this micronutrient were clearly lowest in the organically fertilized plants at the first harvesting, while by the second harvesting and even more so by the third, its concentration in the plant material had increased substantially and was higher than in the other two treatments. This suggests that the increasing humification of the SCO compost in the soil facilitated the bioavailability of Fe, probably through the formation of humic complexes which would have contributed to the increased mobility of Fe in the soil and to improved cationic exchange in the root zone.

Cultivation of cauliflower with composts made with OMW sludges

The effectiveness of both the organic fertilizers made from OMW sludges was tested by cultivating cauliflower (*Brassica oleracea*) in other plots of the same calcareous soil, which was fertilized with the two composts at 30 tonnes ha⁻¹. The field experiments lasted for exactly 4 months in similar conditions to those used for the organic fertilizers made from fresh OMW. Three treatments (control with mineral fertilizer, plots fertilized with COS and those treated with MOS) and four replicates of each were made. In order to monitor the nutritional status of plants, samples of leaf material were analysed at the start of the twelfth week and at the last harvesting, which took place two weeks after the first harvesting. Total yield of fruit and total biomass was measured and analyses of fruit tissues were performed.

Taking into account the important variability of plots (high standard deviations were observed particularly for control and MOS treatments), the results of Fig. 4(a) indicate that the total biomass of cauliflower yielded by plots fertilized with composts was not significantly different to that

produced by the control, and the same was true for the yield of the edible flower (mean value for biomass and flower of the three treatments being around 50 and 25 tonnes ha⁻¹, respectively).

Data of both the macro and micronutrient contents of the leaves are shown in Fig. 4(b and c), respectively. The N content was similar in young (I) and adult (II) leaves but compost-fertilized plants had slightly lower levels of N than the control at both growing steps, those cultivated with MOS showing the lowest N content. The P content was higher in step II than in step I and no differences were found between treatments, whereas organic fertilization increased the K content in young leaves compared to the levels found in plants grown by means of mineral fertilization. As regards, Ca and Mg, it can be added that leaves from adult plants generally had the greater content of both elements, particularly of Mg, only slight differences between treatments being found. As regards micronutrients, Fe is worth mentioning as this was clearly more available to adult plants in plots fertilized with the organic fertilizers, particularly when MOS was employed. These differences were not detected during step I of plant growth because at this time the plants probably had not great need of Fe. It was only later, when the Fe demand increased, that organically-linked Fe was absorbed by the plants. The complete opposite was found for Mn, suggesting that linking of this metal by the composts' organic compounds restrained its uptake by plants, at both I and II steps of the cropping cycle. Results recorded for Cu and Zn were not particularly relevant, and no definite trends were observed concerning their uptake by the plants.

The nutrient contents of cauliflower fruits were generally similar, irrespective of treatment and only very slight significant differences were found for K, Fe, Cu and Zn [Fig. 4(d and e)], the concentration of the first nutrient being higher in plants grown in compost fertilized plots and, that of the other mentioned micronutrients in plants cultivated with the mineral fertilizer. Among organically-fertilized plants, those cultivated with COS generally exhibited greater concentration of Fe, Cu and Zn in the flower material than plants grown in MOS fertilized soil.

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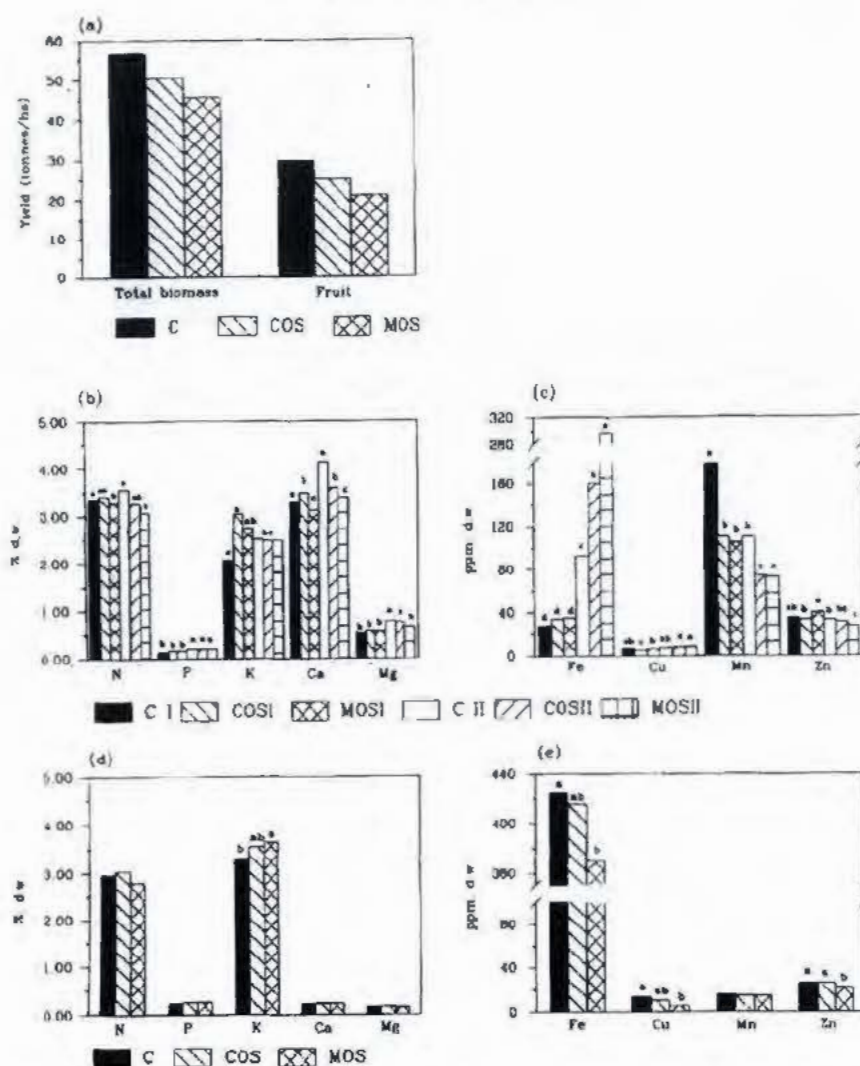


Fig. 4. Effect of the COS and MOS compost treatments on (a) total biomass and flower yields, (b) macro and (c) micronutrients of leaf and (d) macro and (e) micronutrients of flower of *B. oleracea* I, II, young and adult leaves, respectively. Columns with the same letter are not significantly different at the 0.05 probability level (Duncan test).

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Composting of the solid fraction of olive mill wastewater with olive leaves: organic matter degradation and biological activity

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Abstract

The flocculated solid fraction of olive mill wastewaters, obtained from two different olive oil extraction systems (FOMW1 and FOMW2) was composted, with olive leaves (OL) as bulking agent, by the static pile system (Rutgers). The dynamic of organic matter (OM) degradation during composting and its relationship with the basal respiration and fluorescein diacetate (FDA) hydrolytic activity, as indicators of biological activity, were studied. Two mixtures were prepared: C1, from 65% FOMW1 plus 35% OL; and C2, from 74% FOMW2 plus 25% OL and 1% urea. The biooxidative phase of composting in C1, which had a high initial C/N ratio, was long, leading to a high OM degradation, mainly of the lignocellulosic compounds. The water-soluble organic carbon content, C/N ratio and the urea supplied as a N source for the C2 compost make this mixture more adequate for composting, as it had a shorter composting time than C1, and developed a microbial population with a high metabolic activity. The results for basal respiration in C1 and C2 were correlated at a high probability level with those of FDA hydrolysis, and both parameters can be used for establishing the degree of biological stability of the composting material.

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Keywords: Composting; Organic matter degradation; Biological activity; Basal respiration; Fluorescein diacetate hydrolysis

1. Introduction

The importance of the olive mill industry in Mediterranean countries is well known, as is the serious problem that the olive mill factories have in disposing of their by-products, mainly the olive mill wastewater (OMW) (Cabrera, 1994; Paredes et al., 1999). There are two main olive oil production systems: the three-phase centrifugation system, which produces OMW as the waste product, and a solid byproduct which is used for further oil extraction; and the two-phase centrifugation system, in which olive husks are left, together with a small amount of OMW from oil washing. OMW is a liquid waste highly contaminating and phytotoxic, due to the presence of polyphenols, salts and fats (Saviozzi et al., 1993; Paredes et al., 1999). Different methods have been used for its elimination or transformation. A new technology devel-

oped by Trainalba S.L. consists of a physico-chemical treatment to flocculate the organic matter (OM) of OMW with an organic commercial polyelectrolyte. This produces water, which can be used for irrigation, and a sludge as a waste, which is difficult to dispense of. This flocculated fraction of OMW (FOMW) is characterised by a slightly acid pH, due to the presence of fatty acids and alcohols from the OMW (Lanzani and Fedeli, 1986).

Composting has been shown to be a suitable method for recycling olive mill wastes, mainly OMW and olive husk, which need to be mixed with lignocellulosic materials to obtain adequate physical conditions for composting, due to their sticky texture (Madejón et al., 1998; Paredes et al., 2000). Preliminary studies of FOMW composting with different bulking agents, in a composting simulator, produced high OM degradation and a decrease in phytotoxicity when using straw or vine shoots as bulking agents (Negro and Solano, 1996). Composting produces degradation and stabilisation of OM, and degrades the phytotoxic organic substances. The composting process requires adequate conditions of

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pH, temperature, moisture, oxygenation and nutrients, to allow the adequate development of the microbial population (De Bertoldi, 1992). Therefore, changes in these conditions during the process will affect the proliferation of certain microflora, having different enzymatic activities, which control the OM degradation.

A knowledge of the dynamic of OM degradation and biological activity aspects is required to further understand FOMW composting, but literature concerning this particular topic is scarce. Indices of microbial activity include measurement of basal respiration rate ($C-CO_2$ evolved), which indicates the total metabolic activity of all microbiological processes that occur during OM degradation. This has been used as a measure of potential OM degradation in soil and during composting (Haynes, 1999; Wu et al., 2000). Measurement of fluorescein diacetate (FDA) hydrolysis has been used frequently to evaluate the total enzymatic activity of the microbial population present in soil and in organic substrates (Schnurer and Rosswall, 1982; Craft and Nelson, 1996; Haynes, 1999). This determination can be useful for studying the evolution of the biological activity during composting, however it has been seldom used for this purpose.

In the present work, the composting of FOMW was studied by mixing it with olive leaves (OL), which are usually harvested with the olives and separated from them in the mills before starting the oil extraction procedure. The aim was to study the mineralisation process of the OM during composting of FOMW with OL as a bulking agent, and its relationship to the basal respiration and FDA hydrolysis, used as indicators of the biological activity.

2. Methods

2.1. Composting procedure

By-products from the olive mill industry were supplied by Trainalba S.L. the solid fraction of the OMW

from two mills (FOMW1 and FOMW2), obtained by flocculation of OMW through the process developed by Trainalba S.L. (patent no. P9401934), and OL were selected for composting (Table 1). FOMW1 came from OMW produced during the olive oil cleaning procedure in a two-phase extraction system, while FOMW2 came from OMW of the three-phase system, and contained olive pulp, giving it a more solid texture than FOMW1. The fresh OL (free of branches) used were separated from the harvested olives on arrival at the mill. The mixtures were prepared as follows:

C1: 65% FOMW1 + 35% OL (dry weight), equivalent to 80% FOMW1 + 20% OL (fresh weight).

C2: 74% FOMW2 + 25% OL + 1% urea (dry weight), equivalent to 91.5% FOMW2 + 8.0% OL + 0.5% urea (fresh weight).

The proportion of FOMW in the mixture C2 was increased with respect to C1 in order to recycle as much waste as possible, also an additional source of urea was used to increase the initial N concentration and lower the C/N ratio of the mixture to a more adequate value for composting than in C1 (i.e. about 30).

About 2500 kg of each mixture were composted in trapezoidal piles 1.5 m high with a 2×3 m base, by the Rutgers static composting system (Finstein et al., 1985). Air was blown from the base of the pile through the bores of three PVC tubes, 3 m in length and 12 cm in diameter. The timer was set for 30 s ventilation every 15 min, which meant alternative periods of air blowing (30 s), and 14 min 30 s without ventilation. Moreover, this system maintained a temperature ceiling in the pile at 55 °C, through the on-demand removal of heat by pressure-forced aeration (feedback). When $T > 55$ °C the forced ventilation system was connected automatically for continuous air blowing until $T \leq 55$ °C (Paredes et al., 2000). Ventilation demand was measured as the time of ventilation necessary to maintain the temperature below 55 °C. This encourages a high decomposition rate, since high temperatures inhibit and slow down the OM de-

Table 1
Main chemical characteristics of the original wastes used, the solid fraction of OMW (FOMW1 and FOMW2), the OL, the initial mixtures C1 and C2 (i), and the mature composts (m) (dry weight basis)

	pH	OM (%)	TOC (g kg ⁻¹)	HOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	Lignin ^a (%)	Cellulose ^a (%)	Hemicellulose ^a (%)	Fat (%)
FOMW1	5.63	85.24	626.3	nd ^b	11.6	54.2	44.9	9.7	39.3	13.8
FOMW2	5.80	95.02	551.5	nd	15.1	36.5	40.0	22.6	40.0	10.2
OL	6.27	73.39	391.6	nd	11.9	33.0	30.4	19.3	25.4	6.2
C1-i	5.89	65.14	434.0	19.1	10.8	40.1	36.9	13.7	26.3	15.2
C2-i	6.15	92.69	528.5	33.9	19.0	27.8	38.6	26.0	36.3	7.1
C1-m	8.46	42.33	248.6	15.8	17.6	14.1	14.7	5.5	6.9	1.1
C2-m	7.75	88.31	468.4	22.5	30.2	15.5	23.6	16.2	13.4	1.1

^a Percentages with respect to initial dry weight of pile, considering constant the ash content during the composting process.

^b nd: not determined

gradation. Temperature probes were situated inside the pile, halfway up, where the highest temperatures developed. When the temperature started to decrease, the pile was turned once in order to homogenise the mixture and stimulate the process. The biooxidative phase of composting (active phase) was considered finished when the temperature of the pile stabilised near to the ambient temperature (final stage). Air blowing was stopped and the mixtures were then allowed to mature over a period of two months (maturation phase). The moisture level of the pile was controlled weekly during the biooxidative phase of composting and adjusted by adding the necessary amount of water, using overhead sprinklers, to obtain values between 40% and 60% moisture content. One representative sample was taken at each sampling time by mixing six sub-samples from six sites of the pile, spanning the whole profile (from the top to the bottom of the pile). A sub-sample was air-dried and ground to 0.5 mm for chemical analysis, the rest of the sample was kept frozen (-20°C) until being used for biological determinations.

2.2. Analytical methods

The samples were analysed for pH in a 1:10 (w/v) water-soluble extract, dry matter by drying at 105°C for 12 h, and for OM content by loss on ignition at 430°C for 24 h (Navarro et al., 1993). Total nitrogen (TN) and total organic carbon (TOC) were determined by automatic microanalysis (Navarro et al., 1991), as was the water-soluble organic carbon (HOC), after a 1:20 (w/v) extraction. Lignin and cellulose concentrations were determined by the American National Standard methods (ANSI/ASTM, 1977) and the hemicellulose concentration by subtracting the cellulose concentration from the total value for the delignified sample (holocellulose) obtained by Browning's method (Browning, 1967). The fat content was determined by diethyl-ether extraction (Soxhlet system). All analyses were done at least in duplicate. The relevant parameters of the original wastes and the initial and mature samples of the composting are shown in Table 1.

The proportion of OM-loss by mineralisation was calculated using the initial (X_1) and final (X_2) ash concentrations, according to the equation:

$$\text{OM-loss (\%)} = 100 - 100[X_1(100 - X_2)]/[X_2(100 - X_1)]$$

The results were fitted to a first-order kinetic equation by a non-linear least-square procedure (Marquardt–Levenberg algorithm, Table 2), described by the equation:

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

where OM-loss represents the OM degraded (% of initial OM), t is the composting time (d), A the maximum

Table 2

Parameters obtained from the fitting of the OM-loss curves to a first-order kinetic model: maximum OM degradation (A), rate constant (k) and mineralisation rate ($A \times k$)

Mixture	A (% OM)	k (d^{-1})	$A \times k$ (% OM d^{-1})	RMS	F
C1	77.1 (4.48)	0.010 (0.0012)	0.771	20.6	556.6***
C2	46.0 (8.67)	0.011 (0.0033)	0.506	24.8	85.9***

Statistical parameters: residual means square (RMS) and F factor. Standard deviation in brackets.

*** $P < 0.001$.

degradation (% of initial OM) and k is the rate constant (d^{-1}).

Basal respiration and FDA hydrolysis activity were determined on the frozen sub-samples, after defrosting, homogenisation and chopping in a mill. Samples were incubated at 20°C for 24 h in order to reactivate the microbial biomass. Fresh samples, equivalent to 8 g dry matter, were incubated in 250 ml plastic flasks, at 26°C for 10 d. The CO_2 produced was adsorbed into 10 ml of 2 M NaOH solution placed in a vial on the compost sample inside the plastic incubation flask. The CO_2 –C evolved was determined by titration of the NaOH solution with 2 M HCl in an excess of BaCl_2 . Basal respiration rate was expressed as mg C– CO_2 evolved per gram of dry material d^{-1} . The FDA hydrolysis was determined, by the modified method of Inbar et al. (1991) and You and Sivasithamparam (1994), on a sample equivalent to 1 g dry matter. The calibration curve was performed for each sample, using 1 g dry matter, to avoid any interference of the humified OM (Swisher and Carroll, 1980; Inbar et al., 1991).

3. Results and discussion

3.1. Organic matter degradation

Both piles were turned once during the full composting process, when the temperature first started to decrease (after 56 and 28 d in C1 and C2, respectively, Fig. 1b). After turning, the process was re-activated as shown by the increased temperature and ventilation demand (Fig. 1a and b). During turning, material at the exterior of the pile was incorporated into the pile, providing degradable substrate for the microbial biomass. The presence of urea in the C2 mixture could have retarded the development of high thermophilic temperatures, producing a long initial mesophilic phase, as shown by Sánchez-Monedero et al. (2001). The ventilation demand indicates the maximum microbial activity reached during composting (Jeris and Regan, 1973). Therefore, microbial activity was higher in both piles during the

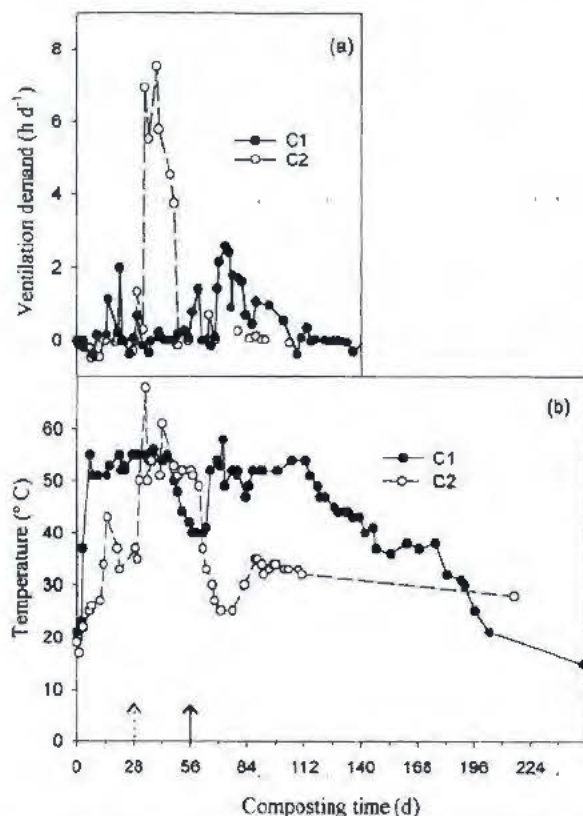


Fig. 1. (a) Ventilation demand required in piles C1 and C2 during the biooxidative phase of composting, to maintain the maximum temperature at 55 °C and (b) temperature profile during composting of the piles C1 and C2, (arrows indicate whirling).

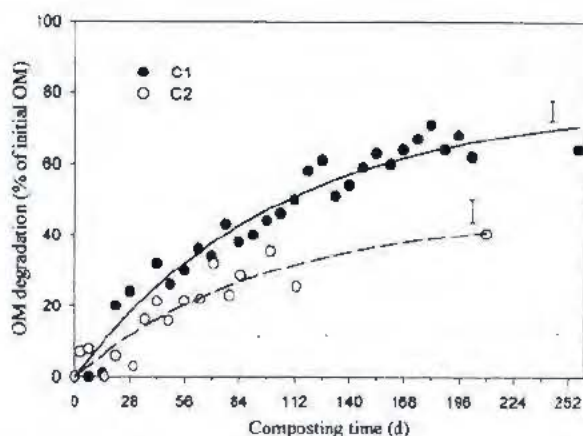


Fig. 2. Degradation of OM during composting of piles C1 and C2. Symbols represent the experimental results and lines the fitted curves. Bars indicate the least significant difference.

thermophilic phase. The high initial C/N ratio in C1 (Table 1) caused an excessively long composting time,

with low ventilation demand in the thermophilic phase (Fig. 1).

However, the longer biooxidative phase in C1 led to a greater proportion of the OM fraction being degraded during composting, the values being 64% in C1 and 40% in C2 (Fig. 2). The curve of OM degradation fitted a first-order kinetic function (Table 2), as generally found during composting (Bernal et al., 1996; Paredes et al., 2000). The value for maximum OM degradation (A) of C1 was close to the range found for the composting of sewage sludge, animal manure, OMW and OMW sludge with different lignocellulosic wastes as bulking agents (55–68% OM) (Bernal et al., 1996; Paredes et al., 1996, 2000). But the value found for C2 was lower than the cited range, indicating that its OM was more resistant to degradation. This was due to the nature of the FOMW2, as the same OL was used in both piles. FOMW2 came from three-phase OMW, which contains olive pulp, mucilage, pectin, etc. (Paredes et al., 1999), while FOMW1 came from the two-phase OMW produced during the washings of the olive oil (free of olive pulp).

About 60% of the initial contents of lignin and cellulose were degraded during composting in C1 (Table 1), compared to 39% and 38% respectively in C2. Moreover, the high concentration of ammonium formed during hydrolysis of the added urea in C2 could have inhibited the activity of fungi responsible for degradation of lignocellulosic compounds (Ko et al., 1974). The degradation of fats is of special interest due to their high concentration in FOMW samples (Table 1). Fats give a particular texture to the composting mass that inhibit the oxygenation (gas exchange) of the pile, whilst their hydrophobic character makes difficult the absorption of water added to the pile in order to maintain an adequate moisture content. Most of the fat was degraded during composting (Table 1), as has been found also during composting of all olive mill wastes, such as olive marc (Estaún et al., 1985), olive husks with wheat straw (Madejón et al., 1998), and FOMW with grape marc and straw in composting simulator experiments (Negro and Solano, 1996).

3.2. Microbial activity

In C1 the basal respiration did not change significantly during composting, and only a slight increase in the FDA hydrolysis activity occurred after 84 d of composting (Fig. 3a and b). In C2, basal respiration and FDA activity increased during the thermophilic phase, reaching their maximum values on day 49, when both maximum temperature and ventilation demand occurred (Fig. 1a and b). The development of the microbial biomass needs a large quantity of available nutrients, particularly nitrogen, which was mainly supplied by urea hydrolysis in this pile. Later, microbial respiration activity and FDA hydrolysis decreased in

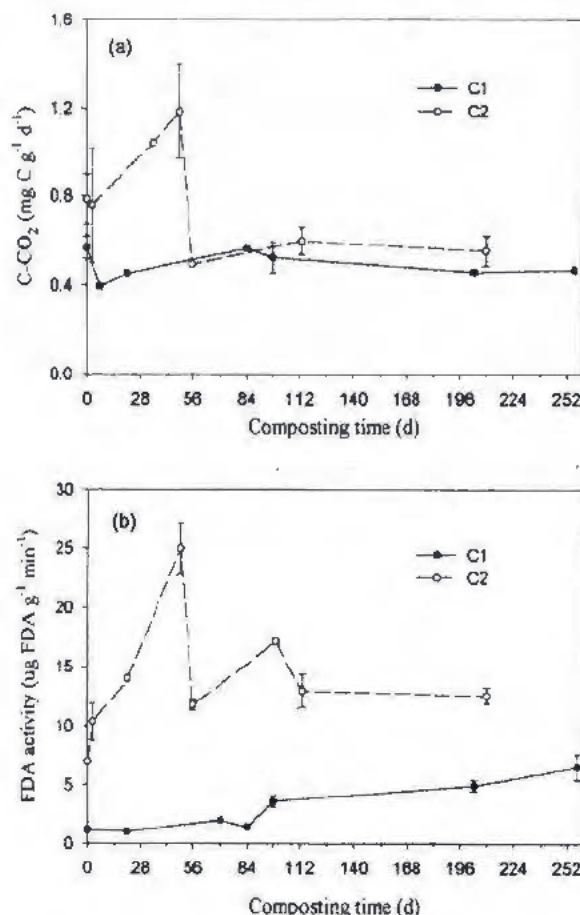


Fig. 3. (a) Evolution of basal respiration and (b) FDA hydrolytic activity during the composting process.

C2, as did the temperature, indicating a decrease in the organic substrate degradable by the microflora. This was also found by Schwab et al. (1994), in a simulated solid waste using a pilot plant-scale composter. Both basal respiration and FDA activity remained constant during the maturation phase. The values of basal respiration after two months of maturation were very close to those found by Forster et al. (1993) ($0.25\text{--}0.62\ mg\ C-CO_2\ g^{-1}\ d^{-1}$) and Hue and Liu (1995) ($0.06\text{--}0.63\ mg\ C-CO_2\ g^{-1}\ d^{-1}$) in mature composts of different origin, which indicates the microbial stability reached after the composting process.

The results for basal respiration in C1 and C2 were correlated at a high probability level with those of FDA hydrolysis ($r = 0.749$, $P < 0.01$), thus both parameters indicated the total microbial activity during composting. Some studies have shown that the absorbance value of FDA can indicate the microbial biomass in organic substrates and soils amended with organic materials (Swisher and Carroll, 1980; You and Sivasithamparam, 1994; Sánchez-Monedero, 1997). According to Swisher

and Carroll (1980), and Inbar et al. (1991), not all microorganisms show FDA activity. In fact, Schwab et al. (1994) found a good relationship between microbial biomass, measured by direct bacteria counting using microscopy, and FDA activity during composting of simulated solid waste, but the relationship was not found for fungi and actinomycetes. Similarly, You and Sivasithamparam (1994) did not find a significant correlation between total fungi counts and FDA activity in mixtures of manure and straw. Therefore, in C1, fungi may have been the dominant microflora developed during composting, which was able to degrade mainly polymers, such as cellulose and lignin, as sources of C and N, because of the initial conditions of the C1 mixture (high C/N ratio, low HOC and N concentrations, and high fats). However, further research is needed to confirm this.

Therefore, the FOMW can be composted, with OL as bulking agent, into a stabilized product. However, the mixture of wastes for C2 was better for composting than C1, as shown by the shorter composting time with higher microbial activity than C1. The high initial HOC and N concentrations, together with an adequate C/N ratio of C2 were responsible for the development of a microbial population with a high metabolic activity. The basal respiration and FDA hydrolysis activity are valid parameters for establishing the degree of biological stability of the composting material. As a general conclusion, it can be said that both the two- and three-phase olive oil extraction systems can become environmentally friendly technologies by integrating a composting system for transformation of the wastes into stabilised compost.

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OTROS USOS DE RESIDUOS DE ALMAZARA



Olive mill solid residues as heavy metal sorbent material: a preliminary study

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Abstract

Biosorption of heavy metals is an innovative and alternative technology to remove these pollutants from aqueous solutions using inactive and dead biomasses such as agricultural and industrial wastes, algae and bacteria. In this study olive mill solid residue was used as heavy metal adsorbent material for its wide availability as agricultural waste and also for its cellulosic matrix, rich of potential metal binding active sites. Preliminary studies concerned with the removal of different heavy metals (Hg, Pb, Cu, Zn and Cd), the effect of pre-treatments by water and *n*-hexane and the regeneration possibility. Olive mill solid residue resulted able to remove heavy metals from aqueous solutions with an affinity series reflecting the hydrolytic properties of the metallic ions, but also a particular affinity for copper. It can be supposed that biosorption phenomenon occur by a general ion exchange mechanism combined with a specific complexation reaction for copper ions. Water pre-treatment is sufficient to reduce COD release in the effluent according to the law limit, while *n*-hexane pre-treatment strongly reduces also the adsorption properties of this material. Experimental isotherms obtained under different operating conditions were fitted using a non linear regression method for the estimation of the Langmuir parameters. Moreover a simple Scatchard plot analysis was performed for a preliminary investigation of the active sites, showing the presence of two different site affinities depending on the metal concentration, according to the previous hypothesis of two kinds of uptake mechanisms for copper biosorption. Regeneration tests gave good results in terms of yield of regeneration and also concentration ratios.

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1. Introduction

Biosorption of heavy metals from aqueous solution can be considered as an alternative technology in industrial wastewaters treatments (Vegliò and Beolchini, 1997). This innovative depurative process uses biomaterials which are either abundant in nature such as marine algae (Schiewer and Volesky, 1995) or wastes coming from industrial production (Gallagher and Moo-Young, 1998) and biological processes such as fermentation (Tianwei and Peng, 2000) and water treatments (Leung et al., 2000). Several agricultural wastes were already been tested to remove heavy metals such as apple residues (Chong et al., 1998), olive mill

solid residue (Gharaibeh et al., 1998), plant root tissues (Chen et al., 1998) and so on.

This study presents the first outcoming results about the possible use of the solid wastes of an olive mill as heavy metal biosorbents. This material was chosen considering its large amount availability and the basic cellulosic structure, rich of possible binding active sites (Irwin et al., 1984; Platt and Clydesdale, 1987).

In the Mediterranean area where the largest worldwide olive oil producers are concentrated (Spain, Italy, Greece, Portugal, Tunisia, Turkey and Syria), treatment and disposal of residues from oil processing are a serious problem the industries have to solve.

The oil production is based on a series of steps (cleaning the olives, grinding the olive to paste, mixing the olive paste, separating the oil and water from the fruit—pomace—, separating the oil from the water and processing the olive oil) that yields an oily phase (20%), a solid residue (30%) and an aqueous phase (50%)

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A part from vegetation waters treatments (Vlyssides et al., 1996), the solid wastes are partially used as natural fertilizer (Saviozzi et al., 2001), nutrient additive for animal food, source of heat energy (Tekin and Dalgic, 2000) and soil stabilizer (Attom and Al-Sharif, 1998), but the larger amount is still left in place causing a big environmental problem for the acute odour and the possible contamination of soil and ground water. Many researchers and environmental technicians suggest incinerating these wastes to solve part of the problem. An alternative use could be as low cost adsorbent in the treatment processes and operations of contaminated air and water (Gharaibeh et al., 1998; Abu-El-Shar and Gharaibeh, 1999). This last application could be reasonably effective considering the pomace composition. In general the plant cell wall is a macromolecular matrix that consists of different kinds of polymer chains packed together in a three dimensional structure. Olive pomace consists of fibre (as cellulose), lignin and uronic acids along with oily wastes and polyphenolic compounds (Saviozzi et al., 2001). This complex matrix contains numerous fixed polyvalent functional groups (such as carboxylic, hydroxylic and methoxy groups) and a high amount of fixed anionic and cationic functional groups. This particular composition enables olive pomace to bind metallic ions in solutions by different mechanisms: complexation, chelation, physical adsorption, ion exchange and electrostatic interaction (Veglio and Beolchini, 1997).

In this work some aspects concerning with possible technological application of olive mill solid wastes as heavy metal sorbent material were reported. In particular the attention was focused on the potential heavy metals that the solid waste can remove, the effect of the pre-treatment and the possible regeneration of the material.

2. Materials and methods

2.1. Olive mill solid residue

The olive mill solid wastes used in this study were collected from an olive oil production plant near Teramo (Italy). The solid residue, as pressed and sunny dried disks, was ground and the size distribution was determined by using an automatic sieve (Fig. 1).

2.2. Kinetic tests

A biomass suspension (10 g/l) was rehydrated under agitation for 1 h, then a multi-metal bearing solution (Hg, Pb, Cu, Zn, Cd as divalent ions nitrate salts) was added. The metal bearing biomass suspension was kept under magnetic stirring and the pH was monitored and maintained constant. During the test different samples

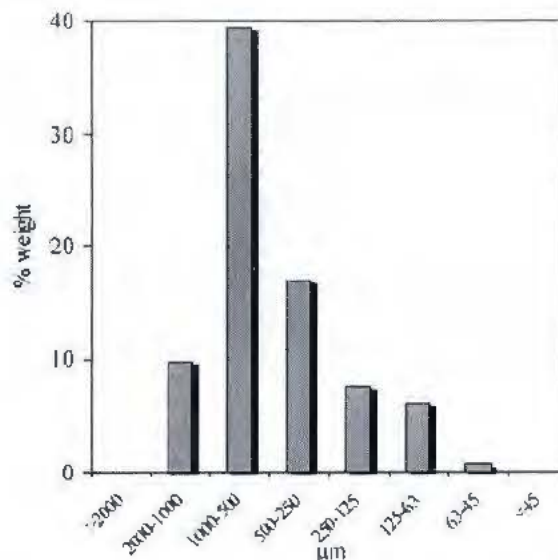


Fig. 1. Size distribution diagram as weight percent for a representative ground sample of the olive mill solid residue used as heavy metal adsorbent material.

were collected, centrifuged to separate the solid and the metal residual concentration of the different metals in the liquid was determined using an Inductively Coupled Plasma Spectrophotometer (ICP).

2.3. Equilibrium tests

The ground solid was preliminary washed twice with distilled water (or *n*-hexane), dried and stored in closed recipients.

Equilibrium biosorption was determined by using 10 g/l re-hydrated biomass suspension samples in which different initial metal amounts were added. The samples were kept under magnetic stirring at constant pH and temperature for the time necessary to reach the equilibrium conditions. Solid-liquid separation was performed by centrifugation and the liquid equilibrium concentration was determined by an ICP. For each sample a blank test without biomass was also performed to avoid confusion between biosorption and possible metal precipitation.

The adsorption equilibrium tests after regeneration were carried out using regenerated pomace samples washed by distilled water and dried, according to the experimental procedure described above.

2.4. Regeneration

Exhausted biomass samples were washed by distilled water and dried. Then different regenerator solutions of CaCl_2 , HNO_3 and HCl were tested at various concentrations mixing diverse volumes of regenerator with dried metal bearing pomace samples.

3. Results and discussion

3.1. Biosorption kinetic tests

Preliminary kinetic tests were carried out to determine the time necessary to reach the equilibrium conditions and the affinity of different heavy metals for the solid residue. In Fig. 2 the kinetic profiles of the biosorption of the different heavy metals were reported. The experimental results show that the equilibrium conditions were reached in a time ranging from 2 to 4 h depending on the metal.

Copper, zinc and cadmium biosorption takes place within 2 h, while for mercury and lead, the heaviest tested metals, a 4 h time is necessary to reach the equilibrium conditions probably because of the large ionic radius and the related problems of diffusion in the biological matrix.

Considering the metal equilibrium residual concentrations, the metal specific uptake (q) of the solid waste can be calculated as:

$$q = \frac{V \cdot (C_i - C_f)}{g} \quad (1)$$

where V is the total suspension volume, g is the amount of biomass, C_i is the initial metal concentration in solution determined on blank sample and C_f is the residual metal concentration in solution at the equilibrium conditions.

The metal specific uptakes were reported in Fig. 3 (as mmol/g of dried solid) showing that the most efficiently removed ion is copper, while the affinity order for the other heavy metals is strictly related to their acidic

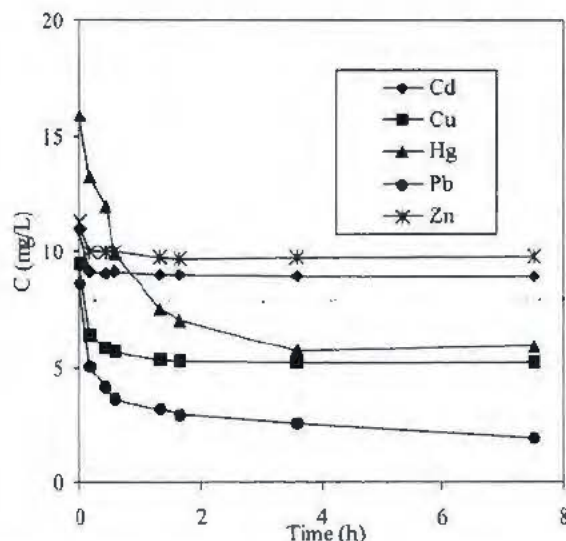


Fig. 2. Kinetic profiles related to the biosorption of different heavy metals using 10 g/l of rehydrated ground solid adsorbent at pH 5.

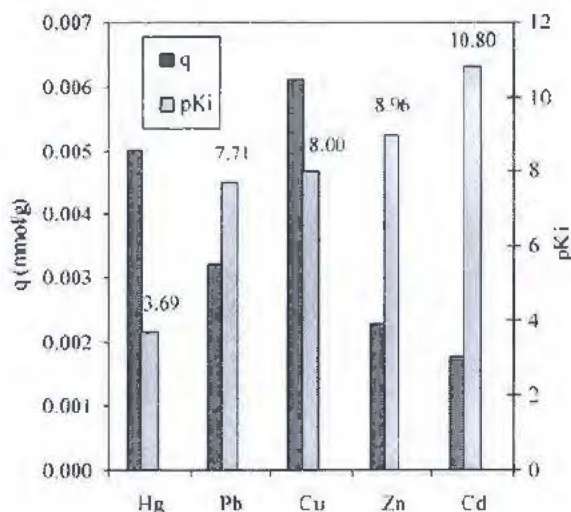


Fig. 3. Metal specific uptake (q) of different heavy metals (pH 5; 10 g/l biomass concentration) related to the hydrolytic properties of the same metallic ions (pK_a).

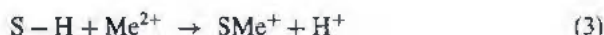
properties as it is possible to see by the pK_a values of the first hydrolysis reaction (2).



This experimental finding was already observed for heavy metal adsorption both onto biological matrices (Esposito et al., 2000; Pagnanelli et al., 1995, in press) and inorganic materials (Spark et al., 1995; Elliot et al., 1986) confirming the general trend that an adsorbent generally prefers to bind acidic ions and that speciation predominates on adsorbent characteristics.

The outlined general trend can be interpreted supposing that the affinity of the surface for the hydrolysed metal species $Me(OH)$ is significantly greater than that for the unhydrolysed ones (Spark et al., 1995) also considering that in the usual pH range $[Me(OH)] < [Me]$.

Otherwise this experimental result can also found an explanation by considering the analogy between the hydrolysis reaction (2) and the metal uptake by the active sites (3):



where Me is the heavy metal and SH is the active site in the protonated form.

In both reactions a metal ion breaks a bond and a hydrogen ion is released. When an heavy metal present a large first hydrolysis constant (because of its particular charge to mass ratio and electronic configuration) it can react more easily with a protonated site respect to a weaker heavy metal. Of course if a different mechanism (i.e. complexation) takes place instead of ion exchange (3), the discriminating factors among heavy metals can change. So that the existence of an affinity series in

agreement with the hydrolysis constant series can help in the identification of the operating mechanisms.

In the case of heavy metal removal by olive mill solid residues, the particular affinity for copper could be probably due to the presence of a further operating mechanism besides the basic ion exchange, such as specific complexation by carboxylic and hydroxylic groups present in the basic fibrous cellulosic structure of this material. The presence of different chemico-physical mechanisms operating simultaneously is rather in biosorption also considering the heterogeneous nature of the biological matrixes (Veglio and Beolchini, 1997). Anyway, a part from these first mechanism hypotheses, this agricultural waste is able to remove different heavy metals from aqueous solution with removal percentages ranging from 50–70% for mercury, lead and copper. These first positive results encourage further experimental work to determine maximal uptake capacity and optimum operative conditions.

3.2. Biosorption equilibrium tests

Copper biosorption was performed using differently pre-treated pomace as adsorbent at various equilibrium pH. The olive mill solid waste was preliminary washed in order to have effluents with Chemical Oxygen Demand (COD) values according to the law limits (Italian law no. 152/1999). The pre-washings were carried out with distilled water or *n*-hexane and the experimental isotherms were reported in Fig. 4.

The use of *n*-hexane reduces the organic release in the treated effluent, but the metal uptake is also diminished in comparison with the water pre-washed sorbent material that anyway gives a COD release under the

legal limits: 500 mg/l is the maximum allowable COD concentration for wastewaters discharge into a sewer system.

In Fig. 4 the experimental isotherms obtained at two different equilibrium pH (4 and 5) using the solid sorbent material washed by water show the strong effect of pH on the adsorption properties due not only to the competition between copper and hydrogen ions for the active sites in the ion exchange mechanism, but especially to the complexation properties of the dissociated forms of hydroxyl and carboxylic groups.

Experimental data were represented using the Langmuir (1918) isotherm which is the empirical model (4) generally used for the representation of biosorption data obtained under constant operating conditions:

$$q_{eq} = \frac{q_{max} \cdot b \cdot C_{eq}}{1 + b \cdot C_{eq}} \quad (4)$$

where:

q_{eq} is the adsorption capacity at the equilibrium solute concentration C_{eq} (mg of solute adsorbed per g of adsorbent or mol/g);

C_{eq} is the concentration of adsorbate in solution (mg/l or mmol/l);

q_{max} is the maximum adsorption capacity corresponding to complete monolayer coverage (mg of solute adsorbed per g of adsorbent or mmol/g);

b is a Langmuir constant related to the energy of adsorption (l/mg or l/mmol).

In this study a non linear least square regression was performed using the Scientist package (Anon., 1994) in which the least squares fitting is performed using a modified Powell algorithm to find a local minimum, possibly the global minimum, of the sum of squared deviations (Φ) between observed data and model calculations (5).

$$\Phi = \sum_{i=1}^N (q_{cal} - q_{exp})^2 \quad (5)$$

In Table 1 the Langmuir coefficients were reported along with the relative standard deviations, the regression coefficients and the model residual variances (Himmelblau, 1978).

These experimental results are very interesting showing a copper maximum uptake ranging from 5.0 to 13.5 mg/g in the different operating conditions. In the literature there are some recent papers about the possible use of this waste as adsorbent after a preliminary thermo- or electro-activation (Gharaibeh et al., 1998; Abu-El-Sha's and Gharaibeh, 1999) to produce granular activated carbon. In particular Gharaibeh et al. (1998) tested a granular activated carbon obtained by solid wastes of an oil plant to remove Zn and Pb obtaining maximal uptake of 5.4 and 21.56 respectively at pH 5.6–5.7.

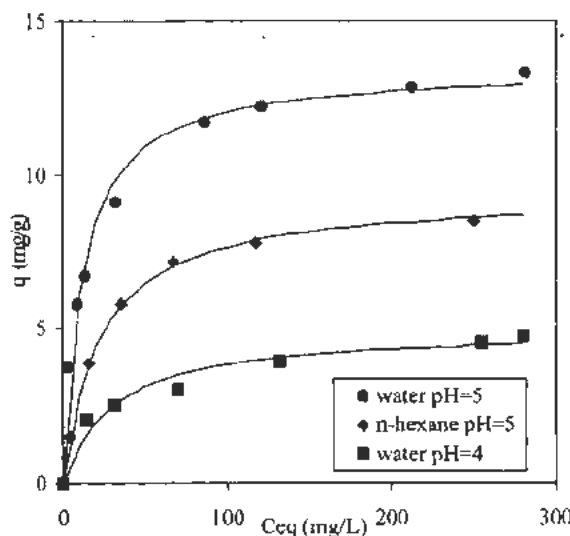


Fig. 4. Copper isotherms obtained under different operating conditions of pre-treatments and equilibrium pH; experimental data and Langmuir isotherms.

Table 1
Langmuir adjustable parameters (q_{\max} and b), relative standard deviations (σ), model residual variances (S_k^2) and regression coefficients (R^2)

Langmuir Isotherm					
Operating conditions		Non linear fitting parameters			
Pre-treatment	pH	$q_{\max} \pm \sigma_{q_{\max}}$ (mg/g)	$B \pm \sigma_B$ (L/mg)	S_k^2 (mg/g) ²	R^2
Water	4	5.0 ± 0.3	0.033 ± 0.008	0.0750	0.9942
Water	5	13.5 ± 0.4	0.08 ± 0.01	0.2703	0.9973
n-Hexane	5	9.4 ± 0.2	0.043 ± 0.002	0.0073	0.9998

The results obtained in this paper acquire importance by this comparison considering that they are obtained working with not activated materials without a preliminary thermo- or electro-activation that requires high energy consumption. Moreover the results reported in Gharaibeh et al. (1998) were obtained at higher pH values and pH is one of the most influencing factors affecting biosorption phenomenon: increasing the equilibrium pH, the metal uptake rises also for the simultaneous metal hydroxide precipitation.

A Scatchard linearized form (6) of the Langmuir equation is used here not to estimate the adjustable parameters (q_{\max} and b), but to have a preliminary analysis about the number of site types and their relative affinity for copper.

$$\frac{q}{C} = b \cdot q_{\max} - b \cdot q \quad (6)$$

When the Scatchard plot is a straight line it means that there is no change in the affinity of the binding sites for the metal over the range of concentration used. Otherwise if a curved plot is observed it indicates that binding sites are present with a metal affinity dependent by the metal concentration (generally stronger affinity sites for low metal concentration and lower affinity sites for equilibrium metal concentration exceeding a certain adsorbing capacity) (Scatchard, 1949).

The Scatchard plots for the isotherms obtained by using water washed biomass (Fig. 5) present double linear profiles probably due to the presence of two main kinds of operating mechanisms for copper as already deduced looking at the correlation between metal uptake and hydrolytic metal properties (Fig. 3). It could be possible that at low metal equilibrium concentrations (below about 20 mg/l) copper ions were probably removed by a first kind of mechanism such as a specific complexation. For larger equilibrium metal concentrations a second mechanism (such as ionic exchange) should occur with a minor ion affinity as it's possible to see by the slope decrease. The theoretical development of Langmuir isotherm is based on the combination of

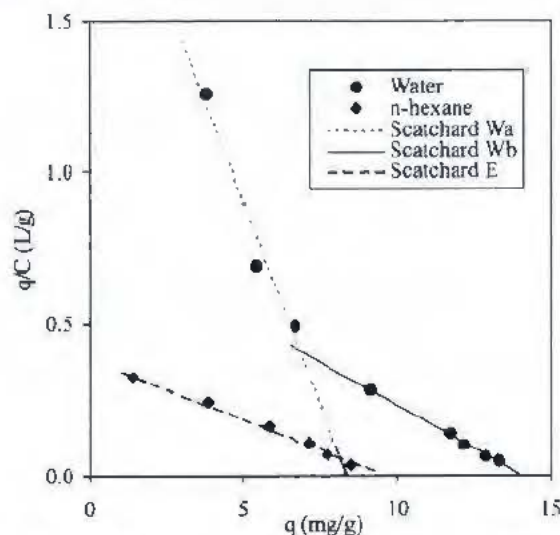


Fig. 5. Scatchard plot analysis of copper biosorption data obtained under different pre-treatment conditions: n-hexane washed biomass presents a linear trend (Scatchard E) over all the tested range of metal concentration, while water washed biomass presents a double linear profile depending on the equilibrium metal concentration value (Scatchard Wa for low metal concentration and Scatchard Wb for high metal concentrations).

equilibrium constant (8) of the reaction among active site (S) and metal (M) (7) and mass site (9):



$$K = \frac{[SM]}{[S][M]} \quad (8)$$

$$[S]_{\text{Tot}} = [S] + [SM] = [SM] \left(\frac{1}{K[M]} + 1 \right) \quad (9)$$

Comparing this last equation with the previous reported Langmuir isotherm $q_{\max} = [S]_{\text{Tot}}$, $b = K$. The adjustable parameter b is then related to the equilibrium formation constant of the metal-site complex. When the Langmuir isotherm is linearised (6) and a non linear trend is observed, the model assumptions fall because of the heterogeneity of the adsorbent and the possible presence of multiple sites on the adsorbent matrix and different operating mechanisms. In this case the value of the adjustable parameter b obtained by using a non linear regression method (Anon., 1994) can be considered as the average value of different metal-site complex formation constants for each site and/or mechanism.

On the other hand the Scatchard plot obtained for n-hexane washed biomass (Fig. 5) presents a linear monotonic profile indicating that one predominant active site is present acting according to one mechanism of metal uptake. It is possible that this pre-treatment eliminates the organic fraction responsible for specific copper complexation, while the non specific uptake by

ion exchange still occurs. The slope of the *n*-hexane washed biomass is very near to the slope of the linear trend at high copper concentration obtained by using water washed biomass, confirming the previous hypothesis about the nature and number of uptake mechanisms (Table 2).

3.3. Regeneration tests

The exhausted biomass can be burned and the metal reduced in leachable ashes, otherwise it could be regenerated giving concentrated effluents and reused in different cycles. According to the second opportunity ionic and acid extracting agents were tested using different concentration ratios (CR, volume of extracting agent used for the regeneration of exhausted pomace/volume of treated synthetic solution). The preliminary tests here reported were carried out in order to outline the possibility of regenerate the exhausted biomass using mild reagents not affecting pomace structure stability. In Fig. 6 the experimental results obtained in the different experimental conditions were reported as % of regeneration (maximal regenerable metal concentration/actual released metal concentration $\cdot 100$, where the

Table 2
Scatchard linear regressions ($y = q/c$; $x = q$) at pH 5; (a) for low and (b) for high metal concentration using water washed biomass

Scatchard plot		
Operating conditions	Equations	R^2
Water pH = 5 (a)	$y = -0.2669x + 2.2277$	0.9571
Water pH = 5 (b)	$y = -0.0569x + 0.7990$	0.9963
<i>n</i> -Hexane pH = 5	$y = -0.0408x + 0.3894$	0.9934

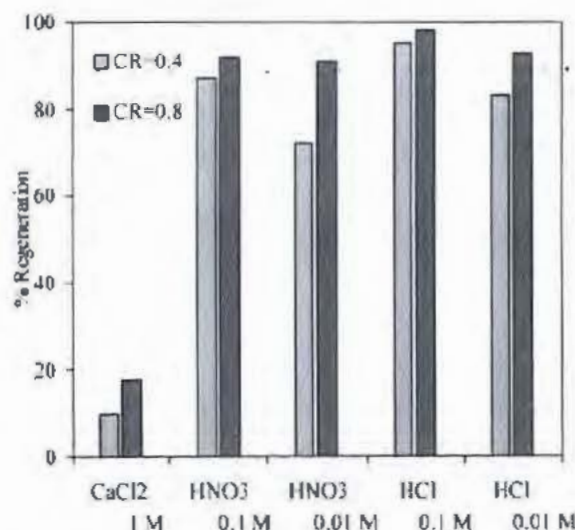


Fig. 6. Regeneration tests under different operating conditions using ionic and acid regenerators at two concentration ratios (CR = regenerating volume/initial contaminated treated volume).

maximal regenerable metal concentration is calculated for each sample from the metal uptake (q) determined during previous adsorption test and the volume of regenerant used). The acidic solutions resulted the most effective in regenerating pomace; in particular hydrochloric acid resulted better than nitric one probably for the additional complexing properties of the anion Cl^- . These preliminary tests were carried out using concentration ratios (CR = regenerating volume/initial contaminated treated volume) not very low, because batch tests require a minimum liquid level to ensure suspension mixing and final solid-liquid separation. Successive tests in column reactors could be performed also using lower regenerating volumes.

The adsorption capacity of olive pomace after regeneration tests (HCl 1N) was tested comparing the isothermal data at pH = 4 for lead and cadmium obtained before and after one regeneration (Fig. 7). Experimental data confirm the major affinity of the biomass for lead with respect to cadmium and show a partial diminution of pomace adsorbent capacity after the regeneration. Cadmium maximum uptake capacity [q_{max} , calculated according to Langmuir isotherm (Anon., 1994)] after regeneration is 56% of the initial value, while for lead is 81%. Further experimental work is required to optimised the regeneration conditions in terms of choice and concentration of the regenerator and CR, paying attention also to the decrease of adsorption performances after the regeneration phase. A final economical analysis could also consider a depuration process without regeneration because of the low economical value of the adsorbent: in this case the exhausted adsorbent could be burned at the end of each cycle of adsorption and the metals leached from the residual ashes.

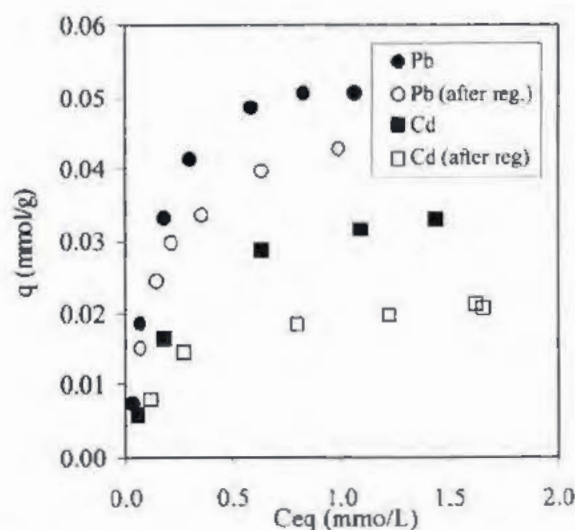


Fig. 7. Lead and cadmium biosorption at pH 4 onto olive pomace before and after regeneration (in the legend after reg.) with HCl 0.1N.

Recycling of Olive Oil By-Products: Possibilities of Utilization in Animal Nutrition

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Olive tree culture is especially important in the Mediterranean area, Spain being first as regards total culture surface (2121 181 ha) and number of productive trees (180000). Both olive tree culture and olive oil industry produce large amounts of by-products. It has been estimated that pruning produces 25kg of by-products (twigs and leaves) per tree per year. Leaves represent 5% of the weight of olives in oil extraction. On the other hand, the olive oil industry produces 35kg of solid waste (crude olive cake) and 100l of liquid waste (oil mill wastewaters) per 100kg of treated olives. Such substantial amounts of by-products may have harmful effects on the environment. Consequently, many alternative utilizations of by-products have been considered. One important alternative from the quantitative point of view is their utilization as a source of nutrients for animals. Information concerning chemical and nutritional characteristics of the various types of olive tree culture by-products and, particularly, of those by-products resulting from the new industrial procedures applied to olive oil extraction must be generated in order to achieve efficient uses of such by-products in animal feeding. The possibilities of different chemical and biological methods for the nutritive evaluation of olive tree culture and olive oil industry by-products have also to be investigated. Olive tree culture and olive oil industry by-products could play a crucial role as sources of local feeds for small ruminants. They may also contribute to the development of efficient and environmentally conservative extensive animal production systems within Mediterranean semi-arid ecosystems. © 1997 Elsevier Science Limited. All rights reserved

INTRODUCTION

There are presently 600million productive olive trees on the planet, which occupy a surface area of 7million ha. The worldwide productions of edible olives and olive oil have been calculated at 8 and 1.6 million metric tonnes, respectively. The Mediterranean area alone provides area 98% of the total surface for olive tree culture and total productive trees, and, 97% of the total olive production. Olive tree culture is especially important in Spain, Italy, Greece and Tunisia, Spain being first as regards total culture surface (2121 181ha) and number of productive trees (180000) (Nefzaoui, 1985, 1987; Delgado Pertitñez, 1994).

Both, olive tree culture and the olive oil industry, produce large amounts of by-products. It has been estimated that pruning alone produces 25kg of by-products (twigs and leaves) per tree annually. It must also be considered that leaves represent 5%

of the weight of olives in oil extraction. On the other hand, the olive oil industry produces 35kg of solid waste (crude olive cake) and 440l of liquid waste (oil mill wastewaters) per 100kg of treated olives.

It has been thought that such substantial amounts of by-products may have harmful effects on the environment. Consequently, many alternative utilizations of by-products have been considered. One important alternative from the quantitative point of view is the utilization of by-products from olive tree culture and olive oil industry as sources of nutrients for animals. The appropriate utilization of by-products in animal nutrition can improve the economy and the efficiency of agricultural, industrial and animal production. Furthermore, it has social and environmental benefits, especially important in the very fragile Mediterranean semi-arid ecosystems where available pastures and forages are scarce.

DEFINITION OF THE MAIN OLIVE TREE CULTURE AND OLIVE OIL INDUSTRY BY-PRODUCTS (FIG. 1)

Leaves and twigs whose diameter are less than 3cm

Obtained by tree pruning and separation of big branches. Leaves are also obtained after the washing and cleaning of olive fruits in the factory.

Liquid residues

Obtained by centrifugation or by sedimentation after pressing of olive tree fruits in the oil factory. Different names have been applied to the liquid residues: vegetation waters; olive mill wastewaters; alpechins; etc.

Solid residues

Obtained from olive oil industrial production: olive cakes.

Crude olive cakes

Obtained by pressure extraction of oil from the entire olive fruit. Their water and oil contents are relatively high (24 and 9%, respectively).

Solvent extracted olive cakes

Obtained after removing the oil from the crude olive cake with a solvent, generally hexane.

Solvent extracted screened olive cakes

Result from the partial removal of stone crumbs by screening or ventilation.

Olive pulp

Paste obtained when the stone is removed before oil extraction. It is rich in water (60%) and difficult to conserve.

CHEMICAL AND NUTRITIONAL CHARACTERISTICS OF OLIVE TREE CULTURE AND OLIVE OIL INDUSTRY BY-PRODUCTS

There is little information concerning the chemical and nutritional characteristics of by-products from olive tree pruning (Boza & Guerrero, 1981; Nigh, 1981; Alibés *et al.*, 1982; Gómez Cabrera *et al.*, 1982, 1992; Delgado Pertinéz, 1994). Information concerning olive cakes is much more abundant

(Boza *et al.*, 1970; Theriez & Boule, 1970; Nefzaoui, 1985, 1987; Aguilera & Molina, 1986; Molina & Aguilera, 1988; Aguilera *et al.*, 1992), whereas there is less on the characteristics of oil mill wastewater as animal feed (Martilloti, 1983; Aguilera *et al.*, 1992).

Chemical composition

Twigs and leaves

The chemical composition of leaves and twigs varies according to many factors such as olive variety, climate conditions, pruning period, wood proportion, tree age, etc. (Table 1).

Dry matter (DM) and crude protein (CP) contents are highly variable. The content of fibrous components (neutral detergent fibre, NDF) is moderate (33–56g/100g DM) but the lignin content (ADL) is generally high (16–21g/100g DM).

Information on the content of phenolic compounds is scarce (Delgado Pertinéz, 1994). Even so, its phenolic compound content appears to be very variable (1.4–6.4g/100g DM).

Olive cakes

The chemical composition of olive cakes varies within very large limits (Table 1) according to the industrial procedures of oil extraction. Crude fat (CF) and NDF are the most variable components. Lignin content is particularly high. Crude protein content is generally low, and a substantial part is linked to cell wall components. Aminoacid composition is similar to that of barley grain with a deficit in glutamic acid, proline and lysine (Nefzaoui, 1985).

Oil mill wastewaters

The main components of oil mill wastewater are water, organic substances and minerals (Table 1). Although the content of phenolic compounds is very variable, it can be very high.

Nutritional characteristics

Twigs and leaves

Intake and digestibility of twigs and leaves are considerably variable, depending on wood proportion, preservation procedure, the method for its determination, the animal species used etc. (Table 2). Crude protein digestibility is especially variable, although very low values have been reported (Boza & Guerrero, 1981; Alibés *et al.*,

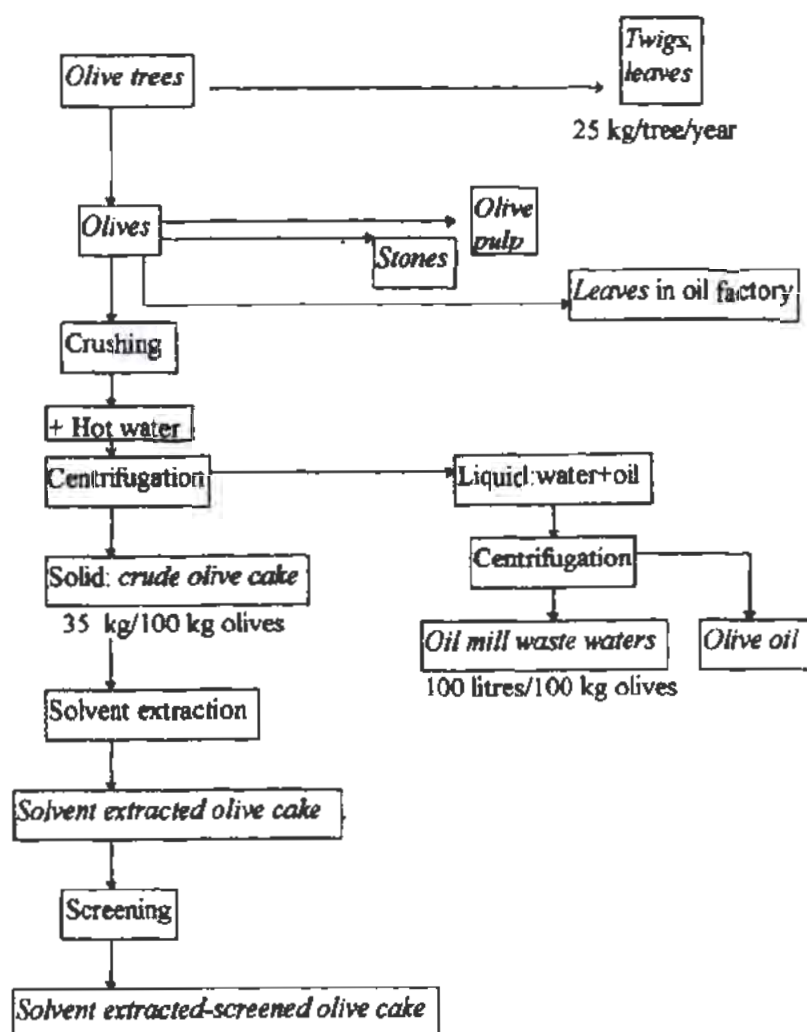


Fig. 1. By-products of the olive tree culture and olive oil industry.

1982). There is not much information on the digestibility of cell wall components.

Olive cakes

There are not many data concerning the voluntary intake of nutrients from olive cakes. Reported values are high and variable. Values for nutrient digestibility are also variable, depending on the type of olive cake evaluated, the amount of olive cake present in the experimental diets, the experimental procedure for determining the digestibility, etc.

The available information seems to indicate a generally low nutritive value for olive cakes (Boza *et al.*, 1970; Nefzaoui & Vanbelle, 1983; Nefzaoui, 1985, 1987; Aguilera & Molina, 1986; Molina & Aguilera, 1988). Various reasons have been explored to explain such results. The high CF content has been considered to limit the nutritive

utilization of olive cakes, but high intakes of crude olive cakes and other results obtained *in vitro* seem to indicate the absence of a harmful effect due to high CF content (Nefzaoui, 1985). The presence of phenolic compounds has also been associated with the low nutritive value of olive cakes. However, neither high amounts of phenolic compounds (Nefzaoui, 1987) nor toxic effects have been found either in ewes fed diets containing olive cakes or in lambs at birth (Nefzaoui & Koaiser, 1981; Aguilera *et al.*, 1992). Consequently, it does not seem that the presence of phenolics in olive cakes can be considered as an explanation of the low nutritive value. However, the absence of a toxic effect when the animals are fed diets including olive cakes could also indicate that phenols are highly polymerized or an integral part of parietal components and, consequently, not very reactive (Nefzaoui, 1987).

Table 1. Composition (g per 100g dry matter) of By-Products from Olive Tree Pruning and Olive Oil Industry

	DM, g per 100g fresh matter	Organic matter	Crude protein	CF	NDF	Acid detergent fibre	ADL	Crude energy (Mj kg ⁻¹ DM)	Phenolic compounds
Wood, twigs, leaves	45.7-96.4	89.1-94.9	6.1-13.1	3.7-11.2	32.5-55.7	18.6-43.6	16.0-21.0	4.8-5.11	1.42-6.37
Olive cakes	65.0-90.0	91.0-97.5	3.75-11.9	2.0-36.0	51.7-71.8	40.9-59.6	24.0-33.0	19-21	≤1.00
Oil millwaste water	16.8	14.8	—	—	—	—	—	—	0.13-6.5 ^a

^ag per 100g MO. Gómez Cabrera *et al.* (1982, 1992); Aguilera and Molina (1986); Nefzaoui (1985, 1987); Ramos-Cormenzana (1986); Molina and Aguilera (1988); Aguilera *et al.* (1992); Delgado Pertinéz (1994).

Table 2. Intake (g DM per kg^{0.75}) and Nutrients Digestibility (g per 100g ingested) of some Olive Oil By-Products

By-product	Animal	Intake	DMD	OMD	CPD	ADFD	Reference
Fresh leaves	Sheep	55	54-60	59-61	31-45	28	Alibés <i>et al.</i> (1982); Gómez Cabrera <i>et al.</i> (1992)
Dry leaves	Sheep	23-70	24-48	32-51	<0-24	2-20	Alibés <i>et al.</i> (1982); Gómez Cabrera <i>et al.</i> (1982, 1992)
Fresh twigs	Goats	80	57	60	32	—	Boza and Guerrero (1981)
Dry twigs	Goats	71	24-52	36-55	6-14	—	Boza and Guerrero (1981); Nigh (1981)
Ensilaged leaves	Sheep	—	40-46	30-48	<0-17	—	Alibés <i>et al.</i> (1982)
Crude olive cake	Sheep	85-128	33	26-46	10-29	—	Boza <i>et al.</i> (1970); Theriez and Boule (1970); Nefzaoui (1987)
Screened olive cake	Sheep	—	42	22-50	16-33	—	Maymone <i>et al.</i> (1961)
Screened extracted olive cake	Sheep	—	19-37	19-50	8-52	9	Nefzaoui (1985); Aguilera and Molina (1986); Molina and Aguilera (1988)
Olive pulp	Sheep	—	48-57	44-58	10-67	—	Theriez and Boule (1970)
Extracted olive pulp	Sheep	—	70	69	28	—	Theriez and Boule (1970)

DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; ADFD, acid detergent fibre digestibility.

EFFICIENT USE OF PLANT BY-PRODUCTS IN ANIMAL FEEDING

Cell walls are protective plant structures, but they are also resistant to degradation (van Soest, 1982). Herbivores have developed different adaptations in order to degrade cell walls and, as a consequence, obtain nutrients from plants.

Ruminants are probably the most efficient herbivores in terms of their utilization of materials which are rich in cell walls, as is the case for by-products. One part of the ruminant digestive tract (the reticulum-rumen) has increased in size. A symbiosis with microorganisms (bacteria, protozoa, fungi) which produce enzymes (cellulases, hemicellulases) that can degrade cell walls, has also been established in the reticulum-rumen, thus providing the ruminant with the ability to efficiently use lignocellulosic plant by-products. As a consequence of the increased size of the reticulum-rumen, feed is longer available for the action of degradative enzymes in the digestive tract. The main end-products of the ruminal degradation of cell wall components are microbial protein and volatile fatty acids (VFA) from which ruminants can meet their protein and

energy requirements. The fact that reticulum-rumen is pregastrically placed increases the efficiency of the microorganism-ruminant symbiosis (van Soest, 1982).

NUTRITIVE EVALUATION

Chemical methods

They provide information about the quantity of nutrients present in a by-product. When lignocellulosic by-products, such as olive by-products, are concerned, the most important chemical information is related to the cell wall components. Several methods can be used, but the one developed by van Soest (1967) is the most generally applied.

The van Soest (1967) method is based on the use of neutral and acid detergents for breaking up the plant cell components and it allows the different cell wall components to be quantified. Despite its generalized utilization, at present it has conceptual and analytical limitations and many modifications have been attempted for various by-products (Mika, 1981; Nefzaoui, 1985; Molina Alcaide, 1991).

Physicochemical methods

They provide information on quantity and structural organization of the cell wall components. The most presently applied technology is near infrared reflectance spectroscopy, which opened new perspectives for an accurate, fast and cheap quantitative and qualitative nutritive evaluation of diverse materials.

Biological methods

The nutrient intake and digestibility are the most important determinant factors for the nutritive value of the by-products. Their accurate determination have to be carried out *in vivo* using an appropriate number of animals. These *in vivo* determinations are difficult as by-products cannot be fed to the animals alone but must be added to other feeds (making part of a diet) which are able to meet the nutrient requirements of the rumen microorganisms and of the animal.

The difficulties involved in the *in vivo* determination of intake and digestibility of the nutrients from by-products make necessary a rigid standardization of the experimental conditions concerning both the diet and the animal.

Indirect methods

The difficulty associated with the *in vivo* nutritive evaluation of by-products has led to a greater focus on direct and simple, rapid, accurate methods.

In situ methods

Based on the incubation of the experimental by-product in the rumen of fistulated animals. The most frequently used method involves incubation of the experimental samples in small bags of porous materials like nylon. This type of method has to be standardized (Madsen & Hvelplund, 1994).

In vitro methods

Many of the applied *in vitro* methods are based on the incubation of samples in buffered solutions of rumen liquor. Variations which do or do not involve either preincubation or postincubation treatments have been applied. The most generally used method has been the one developed by Tilley and Terry (1963).

Both *in situ* and *in vitro* (Tilley & Terry, 1963) methods require rumen cannulated animals. There has, thus, been a strong focus on methods which use solutions of commercial enzymes, mainly cellulases (Aufrère, 1982).

In general, indirect methods are less time-consuming, require fewer animals and allow the nutritional evaluation of a large number of samples in comparison with *in vivo* methods.

There are also several specific equations which make possible predictions regarding the nutritive value of by-products from olive tree pruning and the olive oil industry. These equations relate data obtained by applying indirect chemical and biological methods with data obtained by applying *in vivo* methods (Table 3).

The use of continuous culture systems appears to be promising for the future nutritive evaluation of

Table 3. Linear Regressions ($y = a + bx$) for Prediction of *In Vivo* Digestibility and Energy Value of Different Olive Oil By-Products from Data Obtained with Laboratory Methods

By-product	$y(\%) =$	$a +$	bx	n	r	RSD	Reference
Leaves	DMD <i>in vivo</i>	101.76	-1.38 NDF	16	0.91	4.32	Delgado Pertinhez (1994)
	DMD <i>in vivo</i>	-12.40	1.06 DMD <i>in vitro</i>	16	0.69	7.61	Delgado Pertinhez (1994)
	DMD <i>in vivo</i>	-11.50	0.98 DMD cellulases	16	0.91	4.25	Delgado Pertinhez (1994)
Screened extracted olive cake	DMD <i>in vivo</i>	93.35	-1.03 NDF	8	0.88	3.46	Aguilera and Molina (1986)
	DMD <i>in vivo</i>	0.93	1.14 DMD <i>in vitro</i>	12	0.89	3.26	Aguilera and Molina (1986)
	DMD <i>in vivo</i>	1.73	0.85 DMD cellulases	12	0.91	2.94	Aguilera and Molina (1986)
	DMD <i>in vivo</i>	23.01	0.551 OD	8	0.99	0.591	Molina and Aguilera (1988)
	DE <i>in vivo</i>	3.25	0.96 DMD <i>in vitro</i>	12	0.87	2.89	Molina and Aguilera (1988)

n , Sample number; r , regression coefficient; RSD, residual standard deviation.

by-products, although very little information is available at present.

IMPROVEMENT OF THE NUTRITIVE VALUE OF OLIVE OIL BY-PRODUCTS

Many different treatments (grinding, crushing, sieving and chemical treatments) have been applied to both pruning by-products and olive cakes in order to improve their nutritive value. As has also been done with other lignocellulosic materials, chemical treatments, mainly with sodium hydroxide and ammonia, have been those most frequently applied to olive oil by-products (Alibés *et al.*, 1982; Martilloti, 1983; Nefzaoui, 1985; Aguilera & Molina, 1986; Molina & Aguilera, 1988).

Chemical treatments have been shown to affect the chemical composition, mainly cell wall components, of olive oil by-products (Table 4). The effect of chemical treatments on the nutritive value of such by-products seems to be variable (Table 5).

The results obtained concerning the effect of NaOH and ammonia on the nutritive value of olive oil by-products are not conclusive. As a consequence, many other alternatives are being tried: separation of branches and leaves in pruning by-products; separation of the stones before oil extraction, and supplementation in olive cakes. More information is needed in this respect.

POTENTIAL OF OLIVE OIL BY-PRODUCTS AS AN ENERGY SOURCE FOR SMALL RUMINANTS

Olive oil by-products represent a global production of 135 billion MJ of energy. The production in Andalusia alone represents 3081 million MJ per year. Taking into account the energy requirements of sheep (Aguilera *et al.*, 1986) and of goats (Prieto *et al.*, 1990), these by-products could potentially meet the requirements of small ruminants (2251 million heads of sheep and 1077 million heads of goats) during 117 days of the year.

ROLE OF OLIVE OIL BY-PRODUCTS IN THE PRESENT SITUATION OF ANIMAL PRODUCTION

Animal production, in general, must be economically efficient and environmentally conservative. Moreover, Mediterranean countries are in great need of developing extensive animal production systems with a sharp production in semi-arid lands and based on local sources of feeds and local breeds of animals (Le Houerou, 1993). Olive culture is especially important in Mediterranean countries (Nefzaoui, 1985). Thus, olive oil by-products could play a very important role as sources of local feeds in the present and future development of extensive Mediterranean animal production systems.

Table 4. Effect of Different Treatments on the Chemical Composition (% of dry matter) of Olive Oil By-Products

By-product	+ 0% alkali	+ 4.7% NaOH	+ 5.2% NaOH	+ 1.5% NH ₃	+ 2.5% NH ₃	Reference
Wood, twigs, leaves	DM	62.7	59.5	60.2	61.5	Martilloti (1983)
	CP	5.87	6.50	14.06	16.9	
	NDF	58.1	52.7	47.6	55.9	
	ADL	16.0	13.8	14.4	15.5	
Dry leaves	Ensilaged leaves + H ₂ O	Ensilaged leaves + 4% NaOH	+ NH ₃			
Leaves	DM	87.0	45.7	44.8	83.3	Alibés <i>et al.</i> (1982)
	CP	7.70	7.70	6.21	16.8	
	NDF	47.8	—	—	—	
	ADL	19.1	18.6	16.7	15.8	
Screened-extracted olive cake	+ 0% NaOH	+ 5% NaOH	+ 7.5% NaOH	+ 10% NaOH	Level of significance	Aguilera and Molina (1986)
	DM	68.2	67.4	66.0	68.8	NS
	CP	11.5	12.1	12.0	11.8	NS
	NDF	68.1 ^a	65.5 ^b	63.9 ^b	58.6 ^c	P < 0.001
	ADL	29.7	28.6	28.4	28.4	NS

^{a,b,c} Values in the same row without a common superscript differ significantly ($P < 0.001$).

Table 5. Effect of Different Treatments on the Nutritive Value of Olive Oil By-Products

By-product		+ 0% alkali	+ 4.7% NaOH	+ 5.2% NaOH	+ 1.5% NH ₃	+ 2.5% NH ₃	Reference
Wood, twigs, leaves	DMD (%)	35.3	40.6	49.5	47.5		Martilloti (1983)
	OMD (%)		36.7	44.2	52.3	48.7	
	ME (MJ kg ⁻¹ DM)		5.1	6.0	7.2	6.7	
Dry leaves	Ensilaged leaves + H ₂ O	Ensilaged leaves + 4% NaOH + NH ₃					
Dry leaves	DMD (%)	45.2	40.0	43.0	47.8		Alibés <i>et al.</i> (1982)
	OMD (%)	40.6	29.5	38.5	42.1		
	ME (MJ kg ⁻¹ DM)	5.6	4.1	5.3	5.8		
	Intake (g DM kg ^{-0.75} day ⁻¹)	41.7	48.5	47.7	48.9		
		+ 0% NaOH	+ 5% NaOH	+ 7.5% NaOH	+ 10% NaOH	Level of significance	Reference
Screened-extracted olive cake	DMD (%)	26.3 ^a	38.8 ^b	38.7 ^b	42.2 ^b	P < 0.001	Aguilera and Molina (1986)
	CPD (%)	4.3 ^a	23.8 ^b	16.0 ^b	14.8 ^b	P < 0.01	Aguilera and Molina (1986)
	NDFD (%)	15.5 ^a	34.2 ^b	32.2 ^b	31.4 ^b	P < 0.001	Molina and Aguilera (1988)
	ADLD (%)	8.6 ^a	27.8 ^b	25.1 ^b	27.9 ^b	P < 0.001	Molina and Aguilera (1988)
	ME (MJ kg ⁻¹ DM)	3.9	4.8	4.5	4.6		

ME, Metabolizable energy; NDFD, neutral detergent fibre digestibility.

^{a,b}Values in the same row without a common superscript differ significantly (P < 0.01 or P < 0.05).

FUTURE RESEARCH TOWARDS AN EFFICIENT USE OF OLIVE OIL BY-PRODUCTS IN RUMINANT FEEDING

Further research concerning both the by-product and the animal is needed in order to optimize the use of olive oil by-products as nutrient sources for ruminants.

As regards by-products, it is necessary to characterize their chemical and nutrient composition by means of the new industrial procedures which are being applied in olive oil extraction. The characterization and quantification of compounds like phenols are of special interest because they can limit the digestive and metabolic utilization of the by-products.

There should also be a stronger emphasis put on the research into methods for the preservation of pruning by-products, especially as well as cheap and non-polluting methods for improving nutritive value.

As regards further animal research on those

factors which determine the efficiency of rumen activity, particularly the synthesis of microbial protein could help to optimize fermentation and, consequently, nutrient utilization of olive oil by-products.

ECONOMICAL AND ENVIRONMENTAL IMPLICATIONS OF AN EFFICIENT USE OF OLIVE OIL BY-PRODUCTS IN ANIMAL FEEDING

The utilization of olive oil by-products as nutrient sources for animals could play an important role in the recycling of such by-products and could also contribute to the integrated use of available resources. There is, thus, an environmental gain. Furthermore, such a utilization may also increase the efficiency of vegetal, industrial and animal production and, consequently, their respective profitabilities.

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