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Enzyme activities and chemical changes in wet olive cake after treatment with *Pleurotus ostreatus* or *Eisenia fetida*

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Abstract

A laboratory experiment was conducted to evaluate the enzyme activities and chemical changes recorded in a recalcitrant phenolic-rich waste after treatment with *Pleurotus ostreatus* or *Eisenia fetida*. The waste used was wet olive cake (*alperujo* in Spanish), a waste produced in huge amounts by the olive oil industry. Both *P. ostreatus* and *E. fetida* were very effective in removing phenolic compounds, the initial concentration in the wet olive cake being reduced in both cases by around 90%. Laccase and manganese peroxidase activities were measured in the growth medium of *P. ostreatus*, and catechol 2,3 dioxygenase activity was only detected in the waste treated with *Eisenia*; these could be the main factors responsible for the oxidation of phenolic compounds. Increases of dehydrogenase and β-glucosidase activities were detected in the degraded wet olive cake by fungi or earthworms. In comparison with the natural wet olive cake, the degraded products had lower total organic carbon and humic acid contents but were rich in nitrogen and other nutrients, having lower C:N ratios. In addition, the toxicity of the wet olive cake against the seeds of *Lepidium sativum* significantly decreased after degradation. The low toxicity as well as moderate stability and maturity recorded in the wet olive cake treated with *P. ostreatus* or *E. fetida* imply that these products could be used as soil amendments.

Introduction

Olive cultivation and olive-oil production are important agroindustries in the Mediterranean countries, where approximately 98% of the world's commercial olive trees are grown. The huge quantities of wastes remaining after olive pressing constitute a major source of organic waste in olive-producing regions.

In the past, olive oil was extracted mechanically by pressure or by a three-stage centrifugation process. The major organic wastes of these processes were olive-mill wastewater and a semi-dry olive cake. Olive-mill wastewater is a black liquid effluent that contains highly polluting organic substances, including sugars, tannins, polyphenols, and lipids (Borja et al. 2002), and disposal of this waste is regarded as a serious environmental hazard throughout the Mediterranean region (Rozzi & Mapei 1996). In recent years, a new continuous centrifugation system with a two-phase decanter has been increasingly used as it extracts more oil from the olives, consumes less water and energy, and avoids the olive-mill wastewater (Moreno et al. 2000). The main organic waste from this process is a wet olive cake, known in Spanish as *alperujo*. Commonly, the wet olive cake is dried and treated with hexane to obtain olive-cake oil. The major waste from this second extraction, dry olive cake, has a solid consistency which enables easier handling and storage than the other wastes generated by

the olive-oil industry. In addition, other solutions have been proposed for the efficient management of the wet olive cake as its use as fuel in power-generating plants (Jurado et al. 2003) and its use in agricultural purposes as organic amendments. The toxicity of the wet olive cake related to the presence of some different types of polyphenols (Alburquerque et al. 2004) and its high C:N relation would prevent its direct application to agricultural soils.

The fungi causing white rot (basidiomycetes *Pleurotus* spp.) are known to colonize different types of agricultural and industrial wastes (Ragunathan & Swaminathan 2003; Yildiz et al. 2002). After harvesting of the mushroom, the substrates can be reused as organic amendments or soil conditioner for growing plants (Zhan et al. 2002). These fungal species produce a wide range of extracellular enzymes than enable them to degrade lignin, cellulose and hemicellulose into soluble substances which can be taken up by the mushroom (Morais et al. 2002). In addition, these fungi have been demonstrated to degrade phenols, detoxifying the phenolic wastes used as substrates (Aggelis et al. 2002; Maritani et al. 1996). Finally, the enzymatic system of white rot fungi is reportedly activated by the presence of polyphenolic compounds in olive-mill wastewater (Fountoulakis et al. 2002) and other wastes with high content in these toxic compounds (Rodriguez et al. 2004).

Eisenia fetida is the most common epigeic earthworm used in the vermicomposting processes. These low-cost biotechnological agents are used for processing and transforming a great number of organic wastes (Edwards et al, 1988). Likewise, vermicomposting could be used for produce stable organic amendments from wet olive cake, given that this process has been used efficiently for the stabilization, maturation and detoxification of other olive wastes, such as dry olive cake and olive-mill wastewater (Benitez et al. 2002; Mari et al. 2003; Nogales et al. 1998).

In the present study, we compare the changes recorded in a wet olive cake after treatment with *Pleurotus ostreatus* or *Eisenia fetida*. These changes were assessed by determination of some enzymatic activities, organic matter and elemental composition in this olive waste at the beginning and at the end of both biodegradation processes.

Material and methods

Starting material

The wet olive cake (WOC) was obtained from a commercial olive-oil manufacturer (Romeroliva, Deifontes, Granada, Spain). This lignocellulose olive waste contained 62% of water, a C:N ratio of 64, a content of phenols of 43 g kg^{-1}, and a content of hemicellulose, cellulose, and lignin of 150, 172, and 215 g kg^{-1}, respectively.

Treatment of wet olive cake with Pleurotus ostreatus

Wet olive cake was transformed by *Pleurotus ostreatus* as follows. A strain of *P. ostreatus* (3020 DSV7240), obtained from the Amycel Company, was grown in Petri dishes from the stock culture on potato dextrose agar (PDA) at 25 °C for 15 days. To obtain the appropriate inoculum of *P. ostreatus*, we used wheat grains as the growth substrate. A total of 500 g of wheat grain were washed in water and then boiled 15 min in deionized water. After the water was drained, the wheat grains were supplemented with 1.75 g of $CaCO_3$ and 6.3 g of $CaSO_4$ and packed in a sterilized polyethylene bag. After sterilization in an autoclave at 121 °C for 30 min, the mixture of wheat grains in the bag was inoculated with a piece of agar containing mycelium and incubated at 25 °C, without light, for 2 weeks.

Next, 1 kg (dw), per triplicate, of WOC was packed in a transparent polyethylene autoclavable bag of 40 × 30 cm. Later, each plastic bag containing WOC was supplemented with 1% $CaCO_3$ and 2% corn flour. The plastic bags containing the substrate were then homogenized, sterilized in an autoclave at 121 °C for 30 min, cooled to room temperature and inoculated in a continuous-flow chamber using 6% (on a d.w. basis) of inoculum. After homogenization, the substrate was moistened with phosphate buffer solution pH 6.0 to 80% moisture content. Inoculated bags were incubated in darkness in a controlled chamber at 25 °C. After incubation for 4 months, when the wet olive cake was completely colonized by P. ostreatus, the final degraded product (WOC + P) was removed from the bag. One portion of WOC + P was air dried prior chemical and phytotoxicity analysis. The second

portion was stored at 4 °C until enzymes assays were carried out.

Treatment of wet olive cake with Eisenia fetida

Wet olive cake was transformed by *Eisenia fetida* in the following manner. First, 1 kg (dw) of WOC was placed, per triplicate, in a PVC cylinder. A layer of 4 cm of vermicomposted cattle manure was placed on the WOC to provide an initial habitat for the earthworms and also to act as a source of microbial inoculum. Also, 100 g of clitellated and non-clitellated earthworms were placed in this layer. The substrate was then incubated for 6 months, in darkness, at 25 °C. During the vermicomposting period, the moisture content of the substrate was maintained at 85% ± 2%. Vermicompost dehydration was measured with a soil moisture sensor (Watermark®) and therefore compensated by adding distilled water. After 6 months, earthworms and cocoons were removed from the final degraded product (WOC + E), which was subsequently analyzed. During the vermicomposting period, the highest biomass in earthworms was recorded at month 3 (144 ± 7 g), decreasing afterwards, and a low biomass in non-clitellated earthworms was recorded at the end of the experimental period. This scant biomass implies that all the fresh substrate had been consumed and that the WOC was needed to maintain earthworm growth and reproduction. One portion of WOC + E was air dried prior chemical and phytotoxicity analysis. The second portion was stored at 4 °C until enzymes assays were carried out.

Chemical and phytotoxicity analysis in the natural and degraded WOC

In the different substrates the following parameters were analyzed: pH was measured with a glass electrode using a 1:25 sample: water ratio. Total organic C (TOC) and total N (TKN) were determined by the dichromate oxidation and Kjeldahl methods, respectively (M.A.P.A. 1986). Total humic substances were extracted with a 0.1 M $Na_4P_2O_7$–0.1 M NaOH solution (TEC). The extract was acidified to pH 1.0 with H_2SO_4, and centrifuged to obtain humic acids (HA) and fulvic acids (FA) (Dabin 1971). The water-soluble carbon (WSC) was extracted at 60 °C for 1 h with distilled water (1:10, w:v) and then determined with potassium dichromate and sulphuric acid digestion at 160 °C for 30 min. A spectrophotometric method was used to evaluate the Cr^{+3} produced by the reduction of Cr^{+6} (590 nm) (Sims & Haby 1971). Hemicellulose, cellulose, and lignin were analyzed by the method of Goering & van Soest (1970). The total phenolic compounds were determined by a slight modification of the method described by Khaazal et al. (1994). Total P was measured by the nitrovanadomolybdate method, and total K was measured by photometry (C.I.I. 1969), after digestion of the samples with H_2SO_4 + H_2O_2. Total micronutrients (Fe, Mn, Cu and Zn) were determined by atomic absorption spectrometry (AAS) after digestion of the samples with HNO_3:$HClO_4$ (2:1). The phytotoxicity bioassay was a slight modification of the method described by Zucconi et al. (1981). Water extracts (1:5) from the natural WOC and the degraded WOC were incubated (25 °C) in darkness for 24 h with cress seeds (*Lepidium sativum* L.). Distilled water was used as a control. Ten seeds were placed, by quintuplicate, in Petri dishes (7 cm diameter) lined with filter paper containing 1 ml of each extract or distilled water. Germinated seeds (G) were counted and radicle growth (L) measured. The germination index (GI) was calculated according to the formula $GI = (G/Go) \times (L/Lo) \times 100$, where G and L are, respectively, the germination percentage and radicle growth of organic substrates while Go and Lo are, respectively, the germination percentage and radicle growth of the control.

Enzyme assays in the natural and degraded WOC

Laccase and manganese peroxidase were examined in a WOC aqueous extract (1:5 w/v WOC: 50 mM phosphate buffer pH 6.0) taken from the initial (WOC) or degraded products by *P. ostreatus* (WOC + P) or *E. fetida* (WOC + E). The laccase activity was determined using syringaldazine (Acros Organics, New Jersey, USA) as a chromogenic substrate (Chefetz et al. 1998). The reaction mixture (3 ml) contained 200 μl of the enzyme sample, 1 ml of 0.22 mM syringaldazine and 1.8 ml of 50 mM buffer sodium phosphate (pH 6.0). Syrigaldazine oxidation was measured by monitoring absorbance at 530 nm. In addition, laccase activity was also determined at pH 5.0 by monitoring the oxidation

of ABTS (2,2′-azinobis-3-ethylbenzothialozin-6-sulfonic acid, Acros Organics, New Jersey, USA) at 420 nm (Childs & Bardsley, 1975). The mixture reaction (3 ml) contained 1.5 ml of 0.5 mN ABTS, 1.3 ml of sodium acetate (pH 5.0) and 200 μl of the sample. Manganese peroxidase activity and laccase activity were measured based on the oxidative dimerization of 2,6-DMP (Heinzkill et al. 1998). To determine laccase activity, 200 μl of the sample were added to a 2.8 ml of a solution containing 2.3 ml of 50 mM sodium malonate buffer (pH 4.5) and 0.5 ml of 1 mM 2.6-DMP (pH 4.5). To determine manganese peroxidase, a 200 μl of the sample was added to a solution of 2.8 ml containing 1.8 ml of 50 mM sodium malonate buffer (pH 4.5), 490 μl of 0.7 mM MnSO4, 10 μl of 10 mN H_2O_2 and 0.5 ml of 1 mM 2.6-DMP (pH 4.5). Both enzyme activities were measured by monitoring absorbance at 469 nm for 2 min. The manganese peroxidase activity was consistently corrected for laccase activity. Laccases and manganese peroxidase activities were expressed as 1 μmol of product formed min^{-1} in 1 g of organic sample (U g^{-1}).

For the determination of the dehydrogenase activity, 0.2 g of natural or degraded WOC was incubated for 20 h at 25 °C with 0.2 ml of 0.4% 2-p-iodophenyl-3 p-nitrophenyl-5 tetrazolium chloride (INT) as a substrate. Iodonitrotetrazolium formazan (INTF) produced in the reduction of INT was extracted with a mixture of acetone: tetrachloroethylene (1.5:1) and measured in a spectrophotometer at 490 nm (Garcia et al. 1997). To determine β-glucosidase and phosphatase activity, 0.5 ml of 0.05 M 4-nitrophenyl-β-D-glucanopyranoside (PNG) and 0.115 M 4-nitrophenyl phosphate (PNPP) were used as the substrate, respectively (Tabatabai, 1994). 0.2 g of natural or degraded WOC was incubated at 37 °C for 2 h with 2 ml of maleate buffer at pH 6.5. The samples were then kept at 2 °C for 15 min to stop the reaction and the p-nitrophenol (PNP) produced in the enzymatic reactions was extracted and determined at 398 nm (Nannipieri et al. 1982). Assays without WOC and without PNG or PNPP were made at the same time as controls.

For the determination of the catechol 2,3 dioxygenase (EC 1.13.11.2) activity, the following procedure was carried out. Five grams of natural or degraded WOC were mixed with 25 ml of 0.1 M phosphate buffer (pH 7), the solution was shaken at 28 °C for 72 h in the dark. Then, 5 ml of the extracts were incubated in 100 ml of minimum mineral medium (MMM) for growth and enzyme induction purposes. Catechol was added to MMM to a final concentration of 0.27 g l^{-1} (Cenci et al., 1999) and shake cultures were incubated at 28 °C for 7 days in the dark. Thereafter, cell-extracts were centrifuged at 8000 g for 15 min at 4 °C. Cell pellets were washed three times in phosphate-buffered saline (0.1 M NaH2PO4 in 0.9% NaCl at pH 7.5) and centrifuged at 8000 g for 15 min at 4 °C. The pellets were resuspended in lysozyme buffer (100 mM EDTA, 50 mM NaCl pH 6.9 and 1 mg ml^{-1} lysozyme (Sigma) and then harvested at 30 °C for 90 min to get cells disruption. Cell debris was removed by centrifugation (12000 g, 25 min) to give a clarified extract. The supernatant was transferred to a new tube and conserved at −20 °C until enzyme and protein analyses were carried out.

Catechol 2,3 dioxygenase activity was determined on cell-extract supernatants using the procedures described by Kojima et al. (1961). Samples (0.3 ml) were added to the reaction mixture containing 1 ml of 0.3 mM catechol (final volume: 5 ml). The 2,3-dioxygenase was assayed in 50 mM phosphate (pH = 7.5) and the formation of 2-hydroxymuconic semialdehyde (λ = 375 nm; ϵ_m = 33400 M^{-1} cm^{-1}) monitored. Assays without WOC (natural or degraded) and without catechol were carried out simultaneously as controls. Specific activity was defined as μmol products min^{-1} mg $protein^{-1}$. The protein content of suspensions was determined using a BioRad assay based on the Bradford (1976) procedure.

Statistical analysis

All results are the means of three replicates. Data were subjected to an analysis of variance (ANOVA) using STATGRAPHICS Plus 5.1 statistical software (Statistical Graphics Corp., Princeton, NJ), and Duncan's Multiple Range Test was used to separate the means.

Results and discussion

Changes in enzyme activities and phenolic-compound contents

Different olive wastes are known to contain high concentrations of phenolic compounds (Aggelis

et al. 2002; Lessage-Maesen et al. 2001). The wet olive cake used in the present study had a concentration in phenolic compounds of 42 g kg^{-1}, which would be the responsible for the high toxicity and antimicrobial activity of this waste (Casa et al. 2003; Perez et al. 1992). As in other olive wastes, such as olive mill wastewater, the phenolic compounds contained in the WOC would have a structure similar to lignin, which makes them difficult to biodegrade (Fountoulakis et al. 2002). However, the inoculation of WOC with *P. ostreatus* or *E. fetida* proved highly effective in degrading these compounds, given that the initial phenolic concentration in both cases was reduced by some 90% (Figure 1). *P. ostreatus* removed these toxic compounds by synthesizing and excreting laccases into the growth media containing phenolic substances. Laccases are remarkably non-specific extracellular multicopper enzymes that use molecular oxygen as an electron acceptor and that can oxidize polyphenols as well as other compounds (Davis & Burns 1990; Robles et al. 2000). In our study, laccase activities, measured using syringaldazine as chromogenic substrate or by oxidation of ABTS, were recorded in the WOC degraded by *P. ostreatus* (Figure 1), as has been observed in other studies involving phenolic-rich substrates (Linares et al. 2003; Tomati et al. 1991). Although laccases in fungi are often associated with sporulation, the formation of rhizomorphs, and fruiting bodies (Tour et al. 1995), the enzymatic activity recorded in our study implies that this enzyme can also be produced during the primary metabolic growth of *P. ostreatus*. This fact

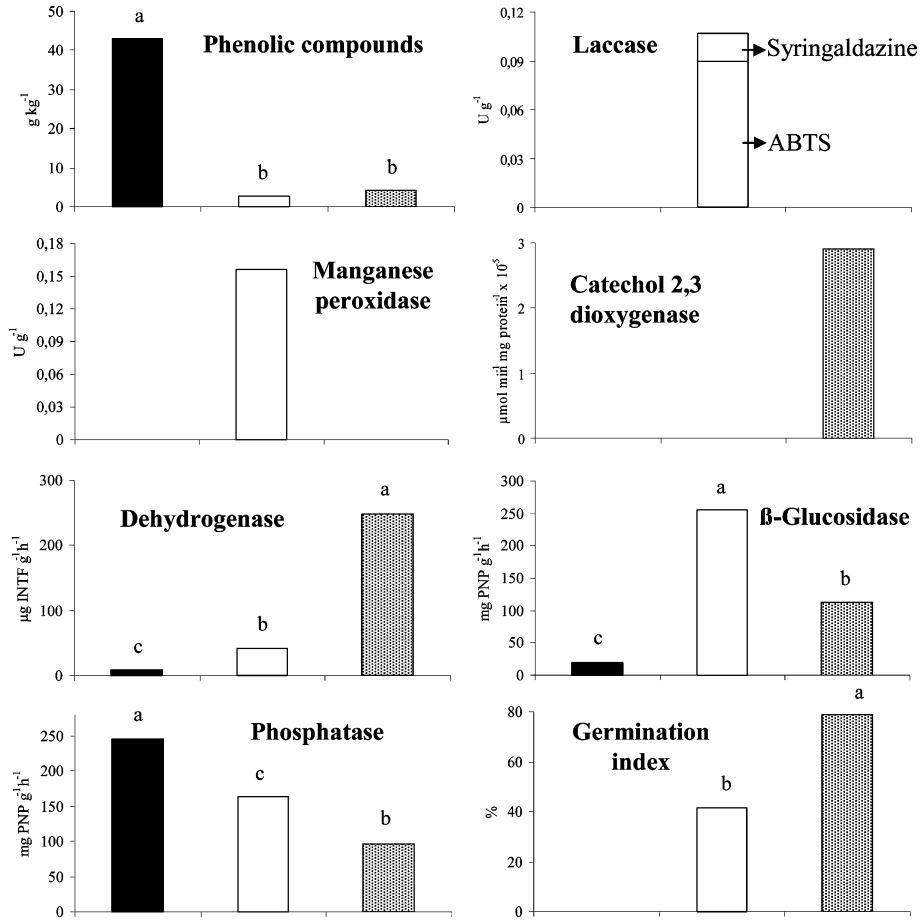

Figure 1. Phenolic compounds content, enzyme activities and germination index in the different treatments assayed. ■: wet olive cake; □: wet olive cake degraded by *P. ostreatus*;▒:wet olive cake degraded by *E. fetida*. In each parameter, columns with same letter are not significantly different according to Duncan's multiple-range test ($p < 0.05$).

would suggest, as mentioned by Tomati et al. (1991) and Tsioulpas et al. (2002) that this white-rot fungus benefits from the laccase reaction, which is involved in a detoxification procedure, preparing the environment or microbial growth. In addition, manganese peroxidase, a ligninolytic enzyme which oxidizes phenolic compounds via one-electron oxidation, was also detected in the WOC degraded by *P. ostreatus* (Figure 1). The production of manganese peroxidase by *P. ostreatus* is not frequent, and, in general, only laccases have been detected during growth of this white-rot fungus in phenolic-rich organic substrates (Aggelis et al. 2002, 2003). Finally, the reduction of phenolic compounds observed in the WOC inoculated by *E. fetida* appears to be due to the high microbial activity recorded in this substrate, which, together with the maintenance of aerobic conditions during the vermicomposting process, would favor the enzymatic oxidation of these toxic compounds. Activation of specific microorganisms with the capability of degrading phenolic compounds may also explain the phenolic reduction observed, although this hypothesis needs to be confirmed with further research. In this sense, the catechol dioxygenase activity was detected only when a vermicomposting process took place on the wet olive cake (Figure 1). This enzyme catalyzes the oxidative cleavage of the aromatic ring of catechol and could therefore be used as a measure of the capacity of the vermicompost microflora to break down aromatic compounds.

Dehydrogenase has been considered an indicator of overall microbial activity because it occurs intracellularly in all living microbial cells, and it is linked with microbial respiratory processes (Bolton et al. 1985). Dehydrogenase activity was very low in the WOC due to the high content of polyphenols, which can inhibit this activity (Benitez et al., 2004). Removal of these compounds allowed this enzymatic activity, and, therefore, higher dehydrogenase activity was recorded in the end products (Figure 1). Comparatively, higher dehydrogenase activity, and therefore, higher microbial activity (Tabatabai, 1994) was recorded in the WOC degraded by *E. fetida* than in the wet olive cake degraded by *P. ostreatus*. The activity of β-glucosidase, an enzyme which catalyses the hydrolysis of alkyl- andaryl-β-glucosides, diglucosides and oligosaccharides, was likewise very low in the WOC. Treatment of this olive waste with *E. fetida* or *P. ostreatus* boosted this activity, prompting the highest enzyme activity when the WOC was degraded by the white-rot fungus (Figure 1). The production of extracellular β-glucosidases and other cellulose- and hemicellulose-degrading-enzymes during the complete life cycle of *P. ostreatus* has been reported in other studies when this fungus grows on lignocellullose wastes, these being needed for the steady supply of carbon and energy from the substrate to the growing fungus (Kanan et al. 1999; Morais et al. 2002). Similarly, during the vermicomposting process, increases in β-glucosidase have been reported, especially during the first months, as a result of the release of glucosides by the combined action of microorganisms and earthworms (Benitez et al. 2002). Finally, phosphatase activity was higher in the initial WOC than in the final WOC, presumably for the consumption of the organic phosphate compounds by the indigenous microorganisms, *P. ostreatus* or *E. fetida* contained in or added to the olive waste (Figure 1).

Changes in organic matter and elemental composition

A significant reduction of the total organic carbon (TOC) was observed in the WOC after treatment with *P. ostreatus* (22%) or *E. fetida* (33%) (Table 1). These reductions were similar or greater than those recorded in other organic wastes decomposed by earthworms (Elvira et al. 1998; Nogales et al. 1999) or lignin degrader's fungi (Sing & Sharma 2002). The lower TOC recorded in the WOC decomposed by *E. fetida* appears to be a result of the presence of specific intestine microorganisms and gut enzymes in the earthworms (Nogales et al. 1999), which enhanced the mineralization of the organic matter contained in the substrate. The WOC contains high level of water soluble carbon (WSC; Table 1). The WSC contains the most easily metabolisable fraction of organic matter (Ceccanti et al. 1997; Garcia et al. 1994), including several types of low-molecular-weight organic compounds, including polyphenols, simple aliphatic acids, organic acids, amino acids, and sugar acids (Fox & Cormfield 1990). *P. ostreatus* or *E. fetida* degraded WOC or significantly disminished the WSC content by consuming low-molecular-weight organic compounds (Table 1). Combined action of microorganims and

Table 1. Organic matter fractions in the different substrates. WOC: wet olive cake; WOC + E: wet olive cake degraded with *E. fetida* and WOC + P: wet olive cake degraded with *P. ostreatus*

	TOC g kg^{-1}	WSC g kg^{-1}	TEC g kg^{-1}	HA g kg^{-1}	TKN g kg^{-1}	C/N
WOC	520a	148a	165a	98a	8.1b	64a
WOC + P	407b	76b	115b	57b	13.9a	29b
WOC + E	348c	12c	42c	19c	14.6a	24b

Means within a column followed by the same letter do not differ significantly according to Duncan's multiple-range test ($p < 0.05$).

earthworms enhanced the reduction of WSC contained in the WOC. Amounts of total extractable carbon (TEC) and humic acids (HA) were higher in the initial WOC than in the end products (WOC + E and WOC + P) (Table 1). This may be because the alkaline solution used for extracting the humic substances can also extract other organic components, such as the most easily metabolisable organic compounds (Boyd et al. 1980, Ciavatta et al. 1993), which appear in high amounts in the WOC. The disappearance of these organic compounds during the degradation of the WOC, especially when this olive waste was degraded with *E. fetida*, would explain the decrease of TEC and HA recorded in the end products. The total Kjeldahl nitrogen (TKN) concentration increased after the degradation of WOC (Table 1). This increase would be due primarily to an effect of concentration due to the mineralization of organic matter. On the other hand, although no information is available on how *P. ostreatus* affects the nitrogen concentration in organic substrates, some species of these basidiomycetes, such as *P. sajor-caju*, reportedly has the ability to fix atmospheric nitrogen (Ginterova & Maxianova, 1975; Rangaswami et al. 1975). In relation to *E. fetida*, increases, decreases, and no changes (Melgar, 2003) have been found, depending mainly on the initial C:N ratio of the organic wastes used in the vermicomposting processes. As a result of the increase in total nitrogen and reduction of total organic carbon, the C:N ratios diminished appreciably (Table 1), although the degraded WOC products had C:N ratios of greater than 20, indicating a moderate degree of stabilization and maturity of them.

The concentration of the other nutrients (P, Ca, Mg, Fe, Mn, Cu and Zn) tended to increase significantly after degradation of WOC after treatment with *P. ostreatus* or *E. fetida* (Table 2), probably because of mineralization of organic matter (Table 2). The only exception was the total potassium concentration, which decreased, although not significantly in the WOC degraded by *E. fetida* (Table 2). This decrease, also correlated with a lower conductivity in this degraded product, may be attributed to leaching of soluble salts by excess water that drained through substrate inoculated with earthworms. Finally, the treatment with *P. ostreatus* or *E. fetida* proved effective in raising the acidic pH of the WOC, which at the end reached neutral or alkaline values.

The drastic reduction in phenolic compounds, together with the larger amounts of nitrogen, phosphorus and other minerals of the end substrates, increased significantly the germination index of *Lepidium sativum* seeds (Fig. 1). Phenols have been suggested as the main determinants of the toxicity of olive mill wastewater (Aliotta et al. 2000; Casa et al. 2003). The allelopathic effect (herbicidal effect) observed when these wastes are used as amendments are also attributed to the

Table 2. Elemental composition, pH and conductivity in the different substrates. WOC: wet olive cake; WOC + E: wet olive cake degraded with *E. fetida* and WOC + P: wet olive cake degraded with *P. ostreatus*

WOC	P g kg^{-1}	K g kg^{-1}	Ca g kg^{-1}	Mg g kg^{-1}	Fe mg kg^{-1}	Mn mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	pH mg kg^{-1}	EC dS m^{-1}
	0.8c	10.5b	4.5c	1.2b	419c	12c	9b	10c	5.8c	7.2a
WOC + P	10.6a	20.7c	27.7a	4.2a	1820b	43b	21a	33b	6.8b	7.6a
WOC + E	2.2b	8.9b	21.5b	5.6a	2457a	60a	22a	56a	8.6a	3.3b

Means within a column followed by the same letter do not differ significantly according to Duncan's multiple-range test ($p < 0.05$).

introduction of some phenolic compounds to soil, as well as an increased in soil pH and salinity (Boz et al., 2003). A comparatively higher germination index was found in the WOC degraded by *E. fetida* than by *P. ostreatus*, although the phenolic-compound contents in both substrates were similar. These differences may be due, as has been suggested by Martinari et al. (1996) and Tsioulpas et al. (2002), to the possible presence of oxidation products of the laccase reaction (e.g. quinonoids, phenoxy radicals) in the WOC degraded by *P. ostreatus*, which did not precipitate and would be more toxic than the original phenolic.

Conclusions

Wet olive cake constitutes the main waste produced during the olive-oil extraction by the new two-phase centrifugation system. It is a recalcitrant phenolic-rich waste with known phytotoxic and antimicrobial effects. Treatment of wet olive cake with *Pleurotus ostreatus* or *Eisenia fetida* can constitute two alternative economical methods in order to convert wet olive cake into stable and mature amendments for agricultural soils. In both methods, the end products had low phenolic compounds content. *P. ostreatus* removed these toxic compounds by synthesizing and excreting ligninolytic enzymes (laccase and manganese peroxidase) into the wet olive cake. In comparison with the initial wet olive cake, the degraded products by *P. ostreatus* or *E. fetida* had lower total organic carbon and humic acid content, lower C:N ratios, higher dehydrogenase and β-glucosidase activities, greater nitrogen and nutrients contents, and lower toxicity, measured through *Lepidium sativum* test. The experimented changes undergone by the wet olive cake after degradation would allow its reuse for agricultural purposes.

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