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Bioconversion of wastes from olive oil industries by vermicomposting process using the epigeic earthworm *Eisenia andrei*

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The present work evaluates the possible bioconversion of wet olive cake by low-cost biostabilization (vermicomposting process). Wet olive cake fresh (WOC), precomposted (WOCP), or mixed with biosolids (WOCB), were vermicomposted for 6 months to obtain organic amendments for agricultural and remediation purposes. The results showed initial differences depending on previous treatment. WOCP was initially more stable, presented a low C:N ratio, and showed more dehydrogenase and urease activity. By contrast, there was no dehydrogenase activity initially in WOC and WOCB, due to the presence of some different types of polyphenols. Finally, the end product showed relatively higher amounts of total nitrogen and humic acid and met the standard of quality for composts and vermicomposts for use both in conventional and organic agriculture and soil-restoration programs.

Keywords: Olive oil industry; wet olive cake; vermicomposting process; by-products; biochemical parameters; standard quality.

Introduction

Spain leads the world in olive oil production, but this industry generates huge quantities of wastes through the extraction processes. The current major by-product is called wet olive cake (WOC) or *alperujo* (4×10^6 tons in Andalusia in 2004), a material with a high proportion of organic matter, phenols, volatile fatty acids, and poly-alcohols.[1–3] These compounds have phytotoxic and antimicrobial effects[4–6] which limit direct soil application, and thus its current disposal represents a great economic and technical problem for producers. The problem of *alperujo* disposal, therefore, has not been fully resolved and research is needed on new technological procedures that would enable profitable use of this material.

The alperujo can potentially be used as organic fertilizers or amendments due to their high contents of organic matter and plant nutrients, but these materials must be treated or re-used if their environmental impact is to be reduced, at the same time enabling some of their primary components to be recovered (organic matter, nutrients, etc.).

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The processing of organic wastes into organic fertilizer via composting and vermicomposting are adequate and profitable techniques for waste management.[7–10] Both techniques reduced the toxicity and negative effects in plant and soil, allowing end products to be reused for land application without risk to soil-plant systems.[11–14]

The feasibility of using vermicomposting to stabilize other olive wastes (eg. dry olive cake), has been demonstrated by showing that the by-products of the process can support earthworm growth and reproduction, especially when mixed with N-rich wastes, in both small- and large-scale experiments.[9,8] The subsequent application of vermicompost to soil prove that they can be used as organic amendments, promoting plant growth and regenerating degraded soils.[15–17]

There are numerous chemical, physical and biological parameters used to evaluate compost and vermicompost maturity. Due to the abundance of chemical and biological changes that occur during the stabilization process, the enzymes released by microorganisms during composting and vermicomposting also play a key part in the biological and biochemical transformations of the matrix. For this reason the study of intra- and extracellular enzymes are of particular interest.[18–20]

The current study was conduced to verify the suitability of three different types of wet olive cake for

vermicomposting. The chemical composition and the phytotoxicity of both initial substrates and end products were also determined, and the biochemical composition (hydrolases and dehydrogenase) was measured during the vermicomposting process.

Materials and methods

Experimetal designing

Three different wet olive cakes were evaluated: WOC, fresh wet olive cake; WOCP, wet olive cake mixed precomposted for two months with olive leaves and manure; and WOCB, wet olive cake and biosolids mixed at a ratio 8:1 (dw:dw). The olive waste was obtained from a commercial olive oil manufacturer (ROMEROLIVA, Deifontes, Granada, Spain). Dewatered, anaerobically digested biosolids derived from sewage sludge were from a municipal wastewater-treatment plant (EMASAGRA, Granada, Spain). Clitellated and non-clitellated earthworms of the species *Eisenia andrei* were acquired from a culture bank at the Estación Experimental del Zaidín (CSIC), Granada, Spain.

The different wet olive cakes were each placed in a wooden box (0.75 m^2) sloped 5% for drainage. First, 14 kg (dw) of the cake was placed in the box and then a layer of vermicomposted cattle manure was placed around this layer to provide an initial habitat for the earthworms and also to act as source of microbial inoculum. The manure layer was inoculated with earthworms having a total biomass equivalent to 10% of the material dry weight. In this way, the three types of wet olive cake described above were vermicomposted for six months. Samples were collected monthly during the vermicomposting process and stored in plastic vials at 4°C until both chemical and biochemical analyses were carried out.

Organic-carbon extraction and chemical analysis

Electrical conductivity and pH was measured in 1:5 (dw/v) organic material/deionized water ratio. Total organic carbon was determined by dichromate oxidation and subsequent titration with ferrous ammonium sulphate. Total N content was obtained by the Kjeldahl method. Pyrophosphate-extractable C (PEC) was extracted with $Na_2P_4O_7$ (0.1 M pH 7.1) in a 1:3 solid-liquid ratio by mechanical shaking at 37°C for 24h. The suspension was then centrifuged at 8000 g and filtered through a 0.45μm Millipore membrane. To obtain humic acids (HA) the PEC extract was acidified to pH 1.0 with HCl and centrifuged, the pellets were resuspended in NaOH 0.5 M. The water-soluble carbon (WSC) was extracted at 60°C for 1h with distilled water (1:10, w:v). The carbon contents in PEC, HA and WSC were determined by acid digestion with potassium dichromate and sulphuric acid at 160°C for 30 min. A spectrophotometric method was used to quantify the Cr^{3+} by reduction of Cr^{+6} ($\lambda = 590$ nm).[21] The total phenolic compounds were determined by a slight modification of the method described by Khazaal et al.[22]

Enzyme assays

For the determination of β-glucosidase and phosphatase activity, 0.5 mL of 0.05 M 4-nitrophenyl-β-D-glucanopyranoside (PNG) or 0.115 M 4-nitrophenyl phosphate (PNPP) were used as the substrates.[23] Organic matter portions (0.5 g) were 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 was extracted and determined at 398 nm.[24] Assays without soil and without PNG or PNPP were made at the same time as controls. For the assessment of dehydrogenase activity, organic matter (0.5 g) 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: tetrachloroetene (3:2) and measured in a spectrophotometer at 490 nm.[25] Assays without INT were carried out simultaneously as controls. For the determination of urease activity, 2 mL of pH 7 phosphate buffer and 0.5 mL 1.066 M urea were added to 0.5 g of olive waste or vermicomposted olive waste and then the mixture was incubated at 30°C for 90 min and the volume was made up to 10 mL with distilled water. The ammonium released was measured using an ammonium selective electrode (ORION Research Inc., Beverly, MA) mod. 95-12; a control without urea was used with each sample.[26]

Statistical analysis

Each treatment was considered as the independent variable. All the results reported were means of three replicates. The effect of the vermicomposting process in materials were submitted to analysis of variance and Duncan's multiple-range test ($P < 0.05$) using STATGRAPHICS Plus (Statistical Graphics Corp., Princenton, NJ).

Results and discussion

Earthworm biomass and number

The total biomass and earthworm numbers during the vermicomposting process are presented in the Figure 1. In WOC, earthworm biomass was low (20–28 g kg^{-1}) during initial months, and the earthworms' numbers did not exceed 106 earthworm kg^{-1}. Subsequently there was a marked increased in both earthworm biomass and numbers, reaching the highest value in the fifth month. This change was related to the loss of soluble toxic substances and release of nutritive compounds necessary to the development of the earthworms. A similar trend was

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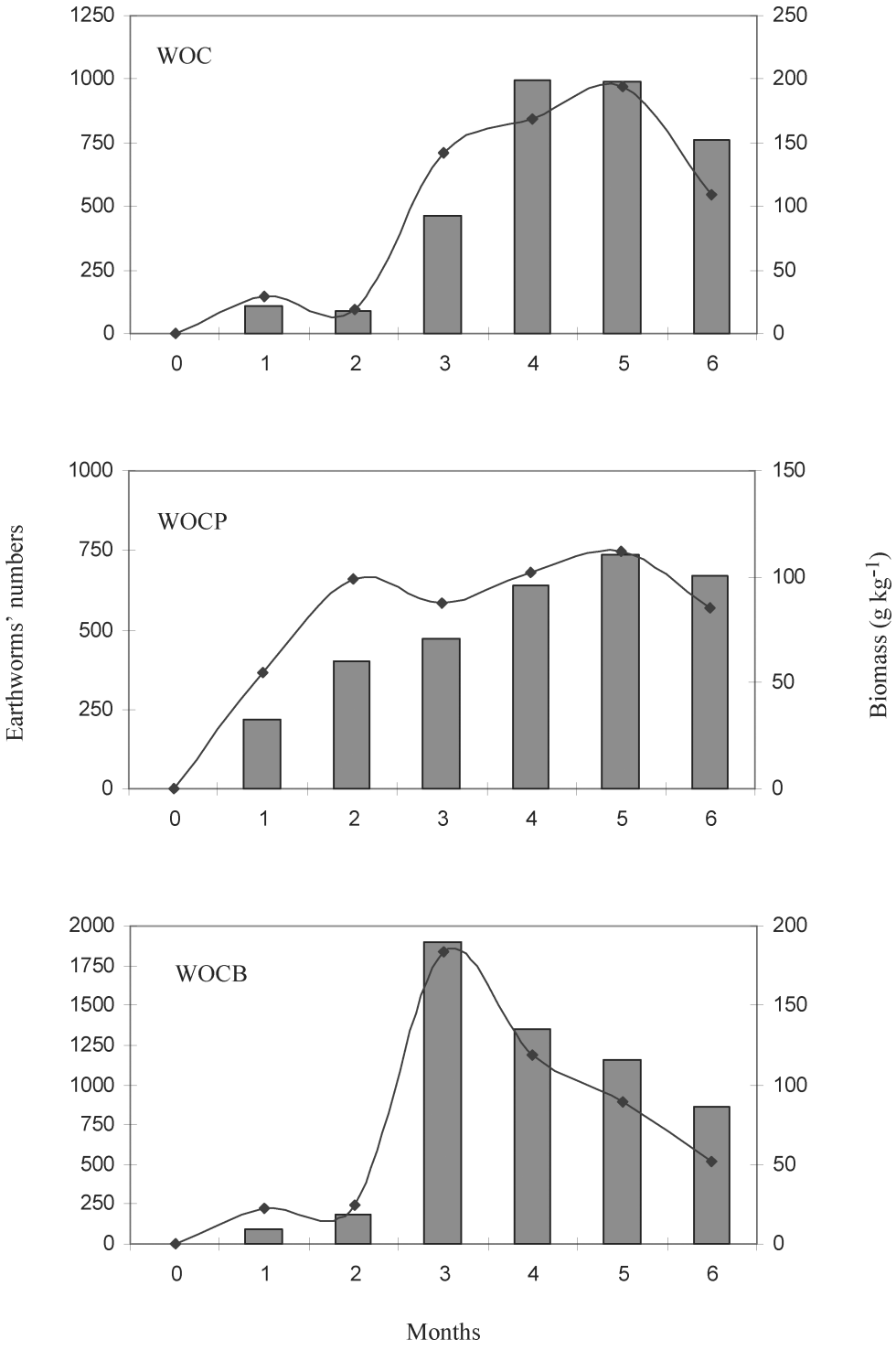

Fig. 1. Evolution of earthworms' numbers —■— and biomass (g kg^{-1}).

shown in WOCB during the first few months, but the highest value was noted in the third month (183 g kg^{-1} y 1895 earthworms kg^{-1}). At the end of the experimental time, there were differences in the biomass among the three treatments. In WOC, the total biomass and number of earthworms was high, indicating that the WOC still not was degraded completely due to its recalcitrant nature. On other hand, the total biomass and earthworm numbers in WOCB, decreased from the third month (54% reduction), implying that the addition of N with biosolid favored adequate degradation of the WOC. On the contrary, in WOCP, a significant increase from the first month appeared to be related to the previous precomposting, which favored nutrient availability to earthworms, although total biomass did not surpass the maximum reached in WOC and WOCB. This effect was also observed by Frederickson

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Table 1. Chemical analysis of the initial substrates (I) and final products (F) in WOC (wet olive cake), WOCP (wet olive cake precomposted) and WOCB (wet olive cake mixed with biosolid).

	WOC		*WOCP*		*WOCB*	
	I	*F*	*I*	*F*	*I*	*F*
Total organic carbon (g kg^{-1})	480	364*	405	344	462	351*
Total nitrogen (g kg^{-1})	6.4	10*	11	16*	10.6	14*
C/N	74	35*	37	22*	43	24*
PEC (g kg^{-1})	62	18*	46	35*	46	20*
Humic acids (g kg^{-1})	6.2	10	19	23*	2.5	11*
pH	5.3	7.9*	7.5	8.0*	5.8	7.4*
EC (dS m^{-1})	6.3	1.7*	4.1	1.0*	6.1	1.3*

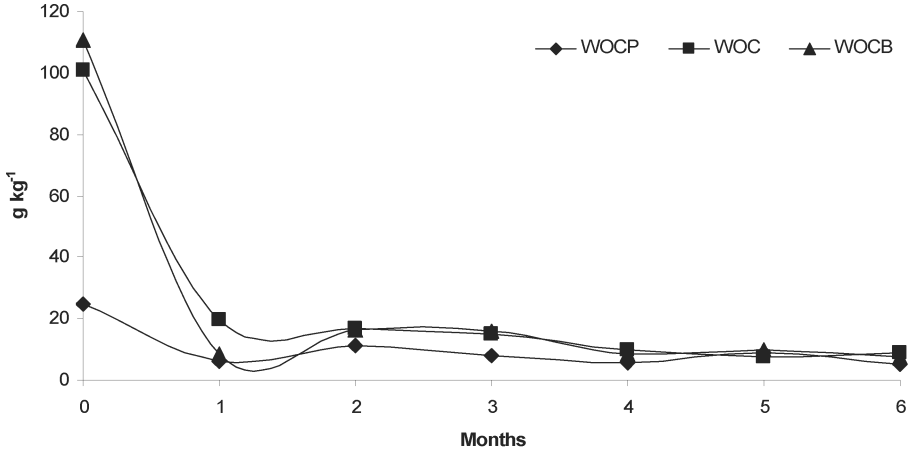
PEC: Pyrophosphate extractable carbon; EC: electric conductivity.
*Indicates a significant difference ($P < 0.05$) between substrates (I) and final products (F).

et al.[27], who concluded that with pre-composting an initial organic material reduced its total biomass during the vermicomposting process.

Chemical parameters

The total organic carbon (TOC) significantly decreased in the 3 treatments (Table 1), the higher losses being found in WOC and WOCB alone or mixed with biosolid than in the precomposted wet olive cake (WOCP). PEC (pyrophosphate extractable carbon) decreased at the end of the process in all substrates (Table 1), the highest decline being recorded in WOC. This was due to the lipid content and some readily metabolizable compounds in wet olive cake, which were also extracted with the alkaline solution, and disappeared during the vermicomposting process.[28–29] Despite the observed reductions, the humic-acid content increased in the final products.

WSC (water-soluble carbon) contains some readily metabolizable compounds, acting as an energy source for microorganisms and earthworms. In olive wastes, the main compounds in this fraction are carbohydrates, with glucose representing a significant part of the total monosaccharide content.[30] Microorganisms easily degrade carbohydrates during composting or vermicomposting processes.[31–32] Figure 2 showed how the amount of this fraction in WOCP was smaller than other substrates (WOC and WOCB), because it was probably consumed by microorganisms involved in the previous precomposting process.[33] The change in water-soluble carbon in the fresh wet olive cake alone (WOC) or mixed with biosolids (WOCB) could be divided into 3 stages: (i) a sharp decline during first month due to degradation of easily metabolizable organic matter by earthworms and microorganisms; (ii) a slight increase, related to the gradual release of simple carbon compounds by hydrolysis of more complex organic molecules; and (iii) stabilization during the last few months.



LSD ($p<0.05$)							
Months	0	1	2	3	4	5	6
	37.3	3.5	5.1	6.9	1.5	7.3	2.7
Treatment	WOCP		WOC		WOCB		
	4.03		23.6		4.3		

Fig. 2. Evolution of water soluble carbon (WSC) during vermicomposting process. WOCP: wet olive cake precomposted; WOC: wet olive cake; WOCB: wet olive cake mixed with biosolid (8:1). LSD denotes the least significant difference [ANOVA (analysis of variance) $P < 0.05$] between months and treatments.

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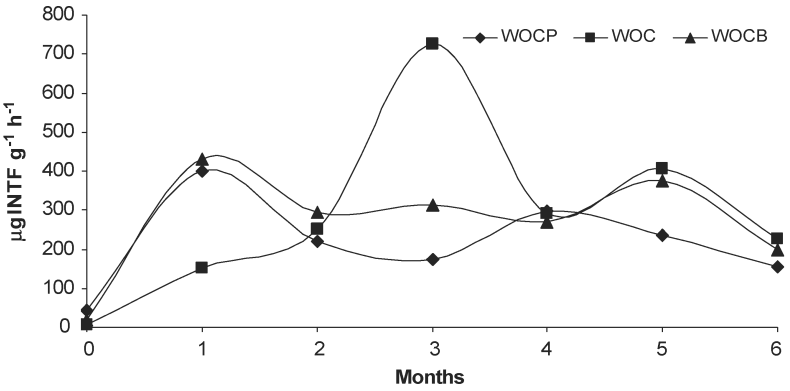

LSD ($p < 0.05$)							
Months	0	1	2	3	4	5	6
	12.8	114.8	60.5	171.8	123.5	74.8	24.8
Treatment	WOC		WOCP		WOCB		
	49.8		95.2		104.1		

Fig. 3. Evolution of dehydrogenase activity over the vermicomposting process. WOCP: wet olive cake precomposted; WOC: wet olive cake; WOCB: wet olive cake mixed with biosolid (8:1). LSD denotes the least significant difference [ANOVA (analysis of variance) $P < 0.05$] between months and treatments.

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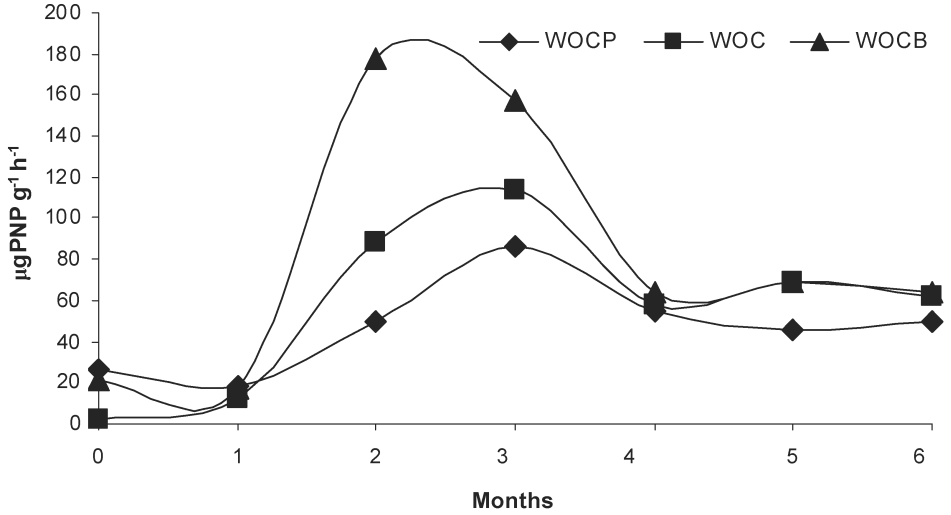
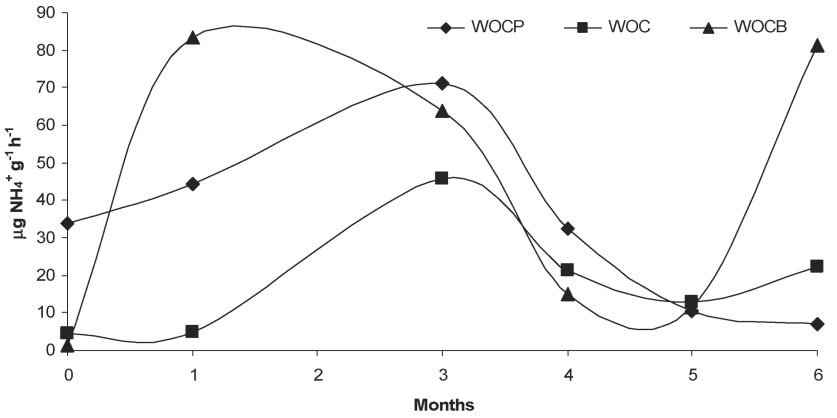
Enzyme activities

The dehydrogenase activity was used as a measure of the overall microbial activity since it is involved in the respiratory chain of all microorganisms.[34] In the WOCP, the dehydrogenase activity increased, during the first month, reaching a maximum of 400 μg g^{-1} h^{-1} (Fig. 3) as result of the activation of microorganisms and the action of earthworms on this partially degraded olive waste. Later, dehydrogenase activity decreased and from 2 to 6 months remained stable.



LSD ($p < 0.05$)							
Months	0	1	2	3	4	5	6
	6374	10219	74889	91733	80590	43750	56565
Treatment	WOC		WOCP		WOCB		
	45246		55889		57592		

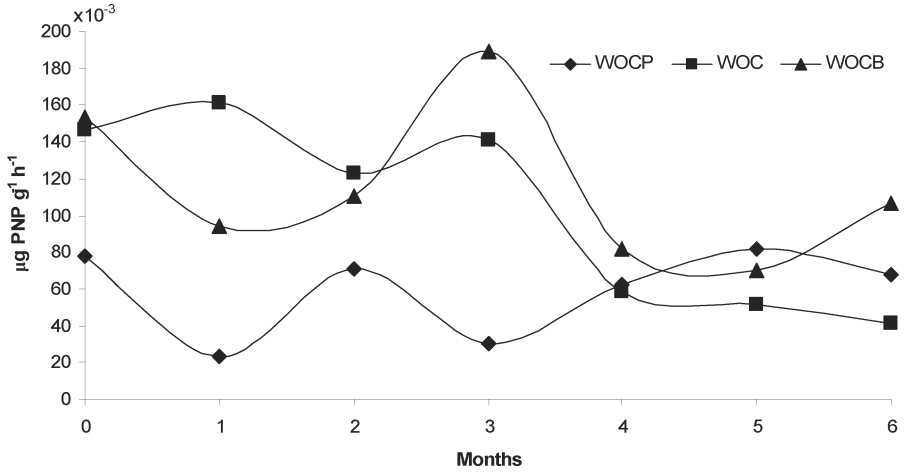
Fig. 4. Evolution of β-glucosidase activity over the vermicomposting process. WOCP: wet olive cake precomposted; WOC: wet olive cake; WOCB: wet olive cake mixed with biosolid (8:1). LSD denotes the least significant difference [ANOVA (analysis of variance) $P < 0.05$] between months and treatments.



LSD ($p<0.05$)							
Months	0	1	2	3	4	5	6
	14.6	20.2		11.9	7.6	14.2	9.6
Treatment	WOC		WOCP		WOCB		
	7.7		13.5		9.9		

Fig. 5. Evolution of urease activity over the vermicomposting process. WOCP: wet olive cake precomposted; WOC: wet olive cake; WOCB: wet olive cake mixed with biosolid (8:1). LSD denotes the least significant difference [ANOVA (analysis of variance) $P < 0.05$] between months and treatments.

In WOC and WOCB the initial activity of this enzyme was practically null, these values being related to the degradation resistance of this olive waste, basically due to the presence of polyphenols (≈24 g kg^{-1}), which are particularly toxics to microorganisms.[4] The action of the earthworms proved fundamental, increasing the surface:volume ratio and exhibiting a large substrate area that could be degraded by microorganisms.[35] The addition of



LSD ($p<0.05$)							
Months	0	1	2	3	4	5	6
	11375	33879	29776	41749	25235	29941	24598
Treatment	WOC		WOCP		WOCB		
	26061		20868		24355		

Fig. 6. Evolution of phosphatase activity over the vermicomposting process. WOCP: wet olive cake precomposted; WOC: wet olive cake; WOCB: wet olive cake mixed with biosolid (8:1). LSD denotes the least significant difference [ANOVA (analysis of variance) $P < 0.05$] between months and treatments.

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biosolids accelerated the toxic compound disappearance; these biosolids presenting a high metabolic capacity[36] and high levels of easily assimilable carbon[37,38]that helped the degradation of the wet olive cake.

The enzyme β-glucosidase hydrolyses terminal non-reducing β-D-glucose residues and releases β-glucose. Mineralization of the wet olive cake during the precomposting period would destroy part of these labile chains, and as a result lower activity was recorded in WOCP than in WOC and WOCB (Fig. 4). In all substrates, the evolution of β-glucosidase was similar, showing a slow release of glucosides that induced the synthesis of this enzyme.[39] The β-glucosidase levels recorded at the end of the vermicomposting process would indicate that there was still glucoside to be degraded.

Urease is an enzyme which depends largely on microbial biomass, and therefore the low values in the initial WOC and WOCB (Fig. 5) might result from inhibition possibly caused by polyphenols and the acidic nature of this product.[39,40] The high urease activity in WOCP was due to a lower content in polyphenols (19 g kg^{-1}), and other toxic compounds which could be degraded during precomposting. Addition of easily metabolizable N via biosolids to wet olive cake produced higher values of this activity than in the wet olive cake, fresh or precomposted. Nevertheless, in WOCB the urease activity showed no definite trend for 6 months, with values fluctuating from 0 at initial time to 80 μg NH_4 $g^{-1}h^{-1}$ at the end of the experiment. The increases recorded in the different substrates agree with findings by Nannipieri and others[41], who indicated that when urease activity reaches a minimum, a re-synthesis usually begins. This re-synthesis may be due to the presence of some bacterial species that respond directly to substrate urea or to the constitutive ureases.[42]

Phosphatases catalyse the hydrolysis reactions of organic phosphate compounds or polyphosphates and organic metaphosphates. Initially in WOCP, there was lower activity than in WOC and WOCB, which would confirm previous studies related to the lower value of this enzyme activity in stable organic wastes produced by composting than in fresh organic wastes.[18] In general, phosphatase activity was, with fluctuations, stable in all substrates with similar values at the beginning and final of the vermicomposting process (Fig. 6).

Conclusions

Our study demonstrates that the bioconversion of wet olive cake is possible using a vermicomposting process, although precomposting of wet olive cake reduced toxic compounds (polyphenols) and showed more initial stability than fresh wet olive cake. Despite these differences, it is possible to establish and develop earthworm populations by mixing a biosolid enhanced by the biodegradation of wet olive cake. This accelerates the disappearance of the toxic compounds due mainly to the high metabolic capacity and high levels of easily assimilable carbon. The end product had a relatively lower C:N ratio and was comparatively more stabilized, and also showed higher amounts of total nitrogen and humic acid. Finally, the end products (vermicomposts) were derived from standard composts and vermicomposts, for which there may be use both in conventional and organic agriculture.

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