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Research article

Biodegradation of high doses of commercial pesticide products in pilot-scale *biobeds* using olive-oil agroindustry wastes

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ABSTRACT

Biobeds systems containing soil, peat and straw (SPS) are used worldwide to eliminate pesticide point-source contamination, but implantation is difficult when peat and/or straw are not available. Novel *biobeds* composed of soil, olive pruning and wet olive mill cake (SCPr) or its vermicompost (SVPr) were assayed at pilot scale for its use in olive grove areas. Their removal efficiency for five pesticides applied at high concentration was compared with the *biobed* with SPS. The effect of a grass layer on the efficiency of these *biobeds* was also evaluated. Pesticides were retained mainly in the upper layer. In non-planted *biobeds* with SCPr and SVPr, pesticides dissipation was higher than in SPS, except for diuron. In the *biobed* with SVPr, with the highest pesticide dissipation capacity, the removed amount of dimethoate, imidacloprid, tebuconazole, diuron and oxyfluorfen was 100, 80, 73, 75 and 50%, respectively. The grass layer enhanced dehydrogenase and diphenol-oxidase activities, modified the pesticides dissipation kinetics and favored the pesticide downward movement. One metabolite of imidacloprid, 3 of oxyfluorfen and 4 of diuron were identified by GC-MS. These novel *biobeds* represent an alternative to the traditional one and a contribution to promote a circular economy for the olive-oil production.

1. Introduction

Biobed bioremediation systems originated in Sweden (Castillo et al., 2008) provide an efficient and secure environment for treating on-farm pesticide contaminated wastewater. *Biobeds* can reduce more than 760 times the input of pesticides from the application machinery washings (Castillo et al., 2008; Cooper et al., 2016; Karanasios et al., 2012; Sniegowski and Springael, 2015). The efficiency of these systems lies on the biomixture, traditionally composed with soil, peat and straw (25:25:50, v: v:v) (De Wilde et al., 2007), ensuring a strong adsorption of pesticides, while keeping them bioavailable and creating optimal conditions for pesticides biodegradation (Vischetti et al., 2004). *Biobeds* has been widely extended around the world (Castillo et al., 2008) as a reliable and affordable strategy to prevent water deterioration and to achieve compliance with water quality standards. However, implementation of this system to other countries involves research to adapt it to their local conditions and agricultural practices (Castillo et al., 2008). Recent efforts have been done in our research group to use local organic wastes for reducing the *biobed* cost and, most important, for favoring its sustainability by contributing to a proper waste management (Castillo-Díaz et al., 2016). Wet olive mill cake or its vermicompost may be an alternative for peat since these materials increase the sorption and degradation of pesticides and stimulated microbial activity when used as soil amendments (Delgado-Moreno and Peña, 2009). Moreover, the olive tree pruning may be used as a cheap texturizing material (Castillo-Díaz et al., 2016).

The current work investigates the efficiency of new *biobeds* composed of organic wastes from the olive oil agroindustry for degrading high pesticides loads at pilot-scale as a preliminary step for their implementation in olives crop areas. The original *biobed* with soil, peat and straw was used as reference. The role of a grass layer on pesticide dissipation was also assayed. Dehydrogenase and orthodiphenol oxidase enzymes as an indicator of the total microbial biomass activity and of the *biobed* capacity for degrading organic compounds, respectively, were analyzed. Pesticide metabolites were determined to confirm pesticide biodegradation and the possible accumulation of compounds more toxic than the discharge pesticides. This study reveals a new use of the byproducts from the olive-oil production that allows an effective implementation of

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novel and low-cost *biobeds* in olive crops areas integrating the economic activity with the environmental sustainability.

2. Material and methods

2.1. Chemicals

The registered formulations of five pesticides used in olive culture were used, i.e., Confidor® 20 LS (imidacloprid 20% w/v, Bayer, Leverkusen, Germany), Dimetoato® 40 Progress (dimethoate 40% w/v, Kenogard, Barcelona, Spain), Diurokey (Diuron 80% w/w, Industrial Química Key S.A, Tarragona, Spain), Goal Supreme (oxyfluorfen 41% w/w, Dow AgroScience, IN, USA) and Song (tebuconazole 25% w/v, Sipcam Iberia, Valencia, Spain). The chemical structure and some physicochemical properties of pesticides are included in Fig. S1A. All other solvents and chemicals used were of HPLC grade.

2.2. Biobeds components and set up at pilot scale

Three biomixtures were made to construct the *biobeds*, two with soil mixed with pruning and raw wet olive cake (SCPr) (25:50:25, v:v:v) or previously vermicomposted (SVPr) (25:50:25, v:v:v), and one biomixture, used in the reference *biobed*, made with soil, straw and peat (SPS) (25:50:25, v:v:v). All these materials were air-dried, ground and passed through 4 mm sieved before use. A description of each component of the biomixture is included in the Supplementary Material. Physicochemical properties of the biomixtures are shown in Table 1.

Six *biobeds* were constructed using PVC boxes (38 cm wide × 48 cm long x 28 cm high), provided at the bottom of a 1.5 cm sand layer (<4 mm), a mesh and a faucet to collect the leachates when necessary. Inside the container, a cylindrical PVC tube of 7 cm i.d. was installed to control the presence of water at the bottom. These boxes were filled with the three biomixtures in duplicate. Water was added to maintain the biomixture humidity at 80% of their field capacity. Six humidity sensors (Theta Probe type ML2x, Delta T Devices, Cambridge, England) were installed to control the irrigation in each *biobed* (Figs. S1B and S1C). The six *biobeds* were stabilized during 3 months under greenhouse conditions at 22±1 °C and 41 ± 7 % air humidity. After the stabilization periods, one duplicate of each *biobed* was sown with 60 g of seeds of prairie grass (ROCALBA®, Girona, Spain), which contains 25% *Bromus parodii*, 25% *Lolium perenne* and 50% *Lolium multiflorum* (Fig. S1C). The three *biobeds* with grass cover were named SCPr-G, SVPr-G and SPS-G, respectively. After two months the grass was cut up to 2 cm above the biomixture and the six *biobeds* were treated with the pesticides as indicated below.

2.3. Dissipation study

Two applications of different mixtures of pesticides were added to the *biobeds* at different times as often occur in olive tree crop. The first application was with aqueous solutions containing formulations of imidacloprid, dimethoate and tebuconazole. After a first incubation periods of 5 months (0–151 days), the *biobeds* with grass were retired and the other three *biobeds* without grass were treated with the commercial formulation of the herbicides diuron and oxyfluorfen and incubated for a second period of 5 months. Thus, total incubation period for *biobeds* without grass cover was 10 months (0–299 days). The pesticide solutions were homogeneously distributed over the *biobed* surfaces to reach a final concentration of 50 mg of each pesticide per kg of biomixture. Then, the *biobeds* were sprayed with water to incorporate the pesticides and to keep the biomixtures humidity at 80% of their field capacity. Sampling was carried out using a metal auger (5.3 wide x 5 cm high) (Fig. S1B) and in triplicate from the upper layer (0–5 cm) and at 5–20 cm depth before pesticide application and at different incubation times after pesticide application (1, 22, 47, 78, 106, 151, 161, 168, 179, 207, 236, 264, 271 and 299 days).

No percolation waters were detected at the bottom of the containers during the incubation.

2.4. Analytical methods

The extraction of the pesticides residues from the biomixtures was carried out, with some variations, following the method described in Castillo-Díaz et al. (2016).

Recoveries of the extraction method ranged between 90% and 102%, depending on the pesticide and the biomixture with relative standard deviation never exceeding 8%.

The pesticides were analyzed by HPLC-DAD (series 1100, Agilent Technologies, Santa Clara, CA) on a Zorbax RX-C8 column (5 μm, 2.1 × 150 mm) (Agilent Technologies, Santa Clara, CA) connected to an Eclipse XDB-C8 (5 μm, 2.1 × 12.5 mm) precolumn (Agilent Technologies, Santa Clara, CA). The chromatography conditions were described in Castillo-Díaz et al. (2016). The limit of quantification was 0.1 mg kg^{-1}.

The metabolites were identified by GC-MS analyses using a gas chromatograph Varian Model 480 GC coupled to a 240 MS detector (Agilent Technologies, CA, USA) following the method described in Castillo-Díaz et al. (2016). The structural assignment was based on the retention times of the compounds and fragment ions of standards injected under the same conditions and analyzed in either full-scan or SIM mode. For the identification of the metabolites, a NIST08 library spectra included in the MS Workstation software 6.9.1 was used.

Table 1
Physicochemical properties of the biomixtures.

Biomixtures	WHC[a] (%)	pH	EC[b] (mS cm^{-1})	OC[c] g Kg^{-1}	C/N ratio	WSC[d] g Kg^{-1}	HA[e] g Kg^{-1}	TEC[f] g Kg^{-1}	Lig./Cel./Hemic.[g] (%)
SCPr	86.3	8.1	1.9	213	24.4	13.7	19.7	41.9	7.5/8.2/7.8
SVPr	64.7	7.9	1.1	216	20.1	7.3	17.4	31.9	8.6/6.3/8.2
SPS	79.3	7.1	3.4	161	29.1	1.5	39.7	55.5	2.8/2.8/3.4

WHC was determined following the method described in Castillo and Torstensson (2007); EC, pH, WSC, OC, N, TEC and HA were determined according to established methods (Fernández-Gómez et al., 2011); Lig./Cel./Hemic. were analyzed using the Goering and van Soest method (Goering and Van Soest, 1970).

[a] WHC- water holding capacity.
[b] EC- electrical conductivity.
[c] OC- organic carbon content.
[d] WSC- water soluble carbon.
[e] HA- humic acid.
[f] TEC- total extractable carbon.
[g] Lig./Cel./Hemic.- Lignin/Cellulose/Hemicellulose.