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De-oiled two-phase olive mill waste may reduce water contamination by metribuzin

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The impact of de-oiled two-phase olive mill waste (DW) on the behavior of metribuzin in Mediterranean agricultural soils is evaluated, and the effects of the transformation of organic matter from this waste under field conditions are assessed. Four soils were selected and amended in the laboratory with DW at the rates of 2.5% and 5%. One of these soils was also amended in the field with 27 and 54 Mg ha^{-1} of DW for 9 years. Significant increases in metribuzin sorption were observed in all the amended soils. In the laboratory, the 5% DW application rate increased metribuzin's persistence from 22.9, 35.8, 29.1, and 20.0 d for the original soils to 59.2, 51.1, 45.7, and 29.4 d, respectively. This was attributable mainly to the inhibitory effect of the amendment on microbial activity. However, the addition of DW transformed naturally under field conditions decreased the persistence down to 3.93 d at the greater application rate. Both amendments (fresh and field-aged DW) significantly reduced the amount of metribuzin leached. This study showed that DW amendment may be an effective and sustainable management practice for controlling groundwater contamination by metribuzin.

Keywords: Adsorption; Dehydrogenase activity; Leaching; Metribuzin; Organic Amendment; Persistence

1. Introduction

Pesticides have been one of the major technological advances that allowed food production to increase spectacularly over the past half-century, along with breeding for high-yielding varieties, the use of fertilizers and irrigation (Gosme et al., 2010). However, pesticides pose a risk to human and environmental health, including toxicity to non-target organisms such as pollinators and wildlife, environmental contamination of soil, water, and air affecting ecosystem functions, selection of resistant pests, and acute and chronic toxicity to humans (Barceló, 1991; Oluwole and Cheke, 2009). The groundwater contaminated with pesticides not only affect the human health when it is directly used for drinking purpose but the food chain is also affected by these contaminated groundwater when used for irrigation purposes (Majumdar and Singh, 2007). Moreover, pesticide contamination of groundwater is of paramount importance because it is the most sensitive and the largest body of freshwater in the European Union (Köck-Schulmeyer et al., 2014). These environmental problems suggest that is necessary to develop effective management practices to control risks of the pesticides and transitions towards sustainable agricultural intensification should be sought (Pretty et al., 2011). Organic amendments are promoted to improve the sustainability of agriculture systems and reduced the environmental impact of agricultural activities (Settle and Garba, 2011)

Metribuzin [4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one], is a triazine herbicide applied pre- or post-emergence to intensive vegetable crops, including potatoes, tomatoes, and wheat. Its herbicidal efficiency and its relatively low toxicity are such that it is widely used around the world (Oukali-Haouchine et al., 2013). However, it presents very low sorption by soils and a relatively high water solubility (1050 mg L^{-1}), so that it is frequently detected in ground and surface waters (Pot et al., 2011).

Agronomic practices like the addition of organic amendments play an important role in the management of run-off and leaching losses of pesticides from agricultural fields (Singh, 2008). Usually, with the increase in total organic carbon (TOC), the sorption of herbicides onto soil particles also increases, and thus their mobility down the soil profile may be reduced (Si et al., 2011, Singh, 2003). Since alternatives such as animal manure or green covers are usually expensive or impractical, the application of residues rich in organic matter to agricultural soils is increasingly being recommended (Delgado-Moreno and Peña, 2008). Moreover, the other possible destinations of these residues (landfill, or incineration) can cause environmental problems, so that their agricultural re-use as organic amendment represents a sustainable opportunity to obviate such problems (Reis et al., 2014).

In many Mediterranean areas, the soils are generally characterized by low organic matter content. This contributes to their limited fertility and productivity together with problems of erosion and desertification (Nunes et al., 2007; Pleguezuelo et al., 2009). Therefore, the agricultural use of waste as soil amendment can be particularly advantageous in these areas because it not only recycles the waste, lessening problems of pollution, but also improves the physical, chemical, and biotic condition of their soils (Costa et al., 2008).

Currently, the most commonly used procedure in olive oil extraction is a two-phase continuous centrifuge process that generates a liquid phase (olive oil) and an organic slurry known as two-phase olive mill waste (OW). After drying, the OW is generally subjected to extraction with hexane to recover the remaining oil still present, leading to the formation of a solid residue known as de-oiled two-phase olive mill waste (DW). In the Mediterranean countries, more than $30 \cdot 10^6$ m^3 of these two wastes are produced during the harvest season, (Barbera et al., 2013) and thus constitute a major problem for the industry. The use of OW and DW as organic amendments has been proposed as an effective strategy in the control of herbicide leaching in Mediterranean agricultural soils (Cañero et al., 2015; López-Piñeiro et al., 2013; Peña et al., 2013). However, application of raw OW and DW can add a major amount of water soluble organic carbon (WSOC) to the soil which may enhance herbicide mobility down the soil profile (Cabrera et al., 2011; Cox et al., 2007; García-Jaramillo et al., 2014; Peña et al., 2015). Therefore, the effect of the organic amendment on herbicide behavior depends on the type of amendment and the dosage, as well as on the herbicide’s properties and on the type of soil (Ahangar et al., 2008; Albarrán et al., 2004). Moreover, the evolution and transformation of organic matter may also modify the further interactions of pesticides with the amended soils (López-Piñeiro et al., 2014), so that it would be of great interest to know the effect of aging, preferably under field conditions, on the herbicide's behavior.

Although metribuzin is widely used and represents a potent source of water pollution, only a very few studies have investigated the effects of organic amendment on its behavior (Majumdar and Singh, 2007; Singh, 2008; Singh et al., 2013), and only one study has investigated the impact of OW on its sorption-desorption, leaching, and persistence in the soil (López-Piñeiro et al., 2013). Also, to the best of our knowledge, there have been no published studies evaluating metribuzin's fate in DW-amended soils. Such information would be useful from the environmental perspective of pesticide management in soils receiving this waste. The objective of this study was to evaluate the effects of de-oiled two-phase olive mill waste on metribuzin's behavior in different Mediterranean agricultural soils, and to assess the effects of the transformation of organic matter from this waste under field conditions. We shall compare the results of the present study with those of a previous study in which the intermediate by-product (OW) was applied to the same soils (López-Piñeiro et al., 2013).

2. Materials and Methods

2.1. Herbicide

Metribuzin [4-amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one, purity 99.5%] was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Its water solubility was 1050 mg L^{-1} at 20 °C.

2.2. Soils and Organic Amendment

Four typical Mediterranean agricultural soils, with different physicochemical properties, were collected for this study (0-30 cm depth). Three soils were selected from Extremadura (south-western Spain): a loam soil (S1, 38°54′N–6°54′W), which consisted of 239 g kg^{-1} clay, 324 g kg^{-1} silt, and 437 g kg^{-1} sand; a sandy loam soil (S2, 38°54′N–6°53′W), which consisted of 142 g kg^{-1} clay, 323 g kg^{-1} silt, and 535 g kg^{-1} sand; and a clay soil (S3, 38°48′N–7°06′W), which consisted of 422 g kg^{-1} clay, 144 g kg^{-1} silt, and 434 g kg^{-1}sand. The fourth soil was collected from the Alentejo (south-eastern Portugal) a sandy clay loam soil (S4, 38°53′N–6°59′W), which consisted of 297 g kg^{-1} clay, 211 g kg^{-1} silt, and 492 g kg^{-1} sand. Prior to analyses, the soil samples were air-dried at room temperature, and the fraction that passed through a 2-mm sieve was stored at 4 °C until use.

The organic amendment used in this study (DW) was obtained from the UCASUL oil industry located in Beja (Portugal). It had the following properties: pH 5.30, 516 g kg^{-1} total organic carbon (TOC), 74.3 g kg^{-1} water soluble organic carbon (WSOC), 14.6 g kg^{-1} water soluble phenols (WSP), 5.40% moisture content, and 5.30 dS m^{-1} electrical conductivity (EC). The DW amendment was air-dried and homogenized to < 2 mm.

To investigate the effect of DW on metribuzin's behavior, the amendment was added to the original soils in the laboratory at 2.5% and 5% dosages by weight. The samples of the amended soils so obtained were labeled as S1DW2.5 and S1DW5, S2DW2.5 and S2DW5, S3DW2.5 and S3DW5, and S4DW2.5, and S4DW5 (2.5% and 5% of DW for each of the S1, S2, S3, and S4 soils, respectively). To evaluate the "aging" effects of DW organic matter transformation on metribuzin's behavior under field conditions, amended soil samples (0-30 cm) were also collected from a field experiment 15 months after the last DW addition to the same S4 soil mentioned above. The soil had received application of this waste for nine years. The two amendment treatments selected for this study consisted of 27 Mg DW ha^{-1} yr^{-1} (equivalent to 0.56% yr^{-1}, S4ADW5) and 54 Mg DW ha^{-1} yr^{-1} (equivalent to 1.12% yr^{-1}, S4ADW10), dry weight equivalents. Therefore, after nine years of repeated field DW application, the total amount of DW received by the field-amended soil is similar to the amounts received by the laboratory amended soils. For this reason, the S4 soil was also amended in the laboratory with 10% of DW, labeled as S4DW10, in order to give a similar final total organic matter content as in the S4ADW10 field-amended soil. Selected characteristics of the unamended and amended soils are given in Table 1.

Table 1. Selected characteristics of the unamended and de-oiled two-phase olive mill waste amended soils

Properties	TOC ($g\ kg^{-1}$)	WSOC ($mg\ kg^{-1}$)	HA ($g\ kg^{-1}$)	FA ($g\ kg^{-1}$)	HI	EC ($dS\ m^{-1}$)	pH	DH (μg INTF g^{-1} h^{-1})
S1	9.69a	131a	1.35a	0.769a	13.9c	2.47a	6.81c	0.968c
S1DW2.5	21.1b	2135b	2.03b	2.81b	9.64b	4.48b	6.45b	0.214b
S1DW5	32.9c	3975c	2.80c	4.20c	8.50a	6.67c	6.05a	0.131a
S2	6.67a	103a	0.962a	0.584a	14.4c	3.08a	5.70c	0.747c
S2DW2.5	19.4b	2647b	1.65b	2.60b	8.52b	5.31b	5.44b	0.203b
S2DW5	31.3c	4434c	2.07c	4.38c	6.60a	7.84c	5.18a	0.093a
S3	13.4a	195a	1.30a	0.727a	9.70b	0.660a	8.15c	1.32b
S3DW2.5	27.3b	2630b	2.02b	2.80b	7.40a	2.60b	7.25b	1.36b
S3DW5	39.7c	4032c	2.68c	4.33c	6.75a	4.43c	6.68a	0.692a
S4	9.86a	69.0a	1.02a	0.960a	10.3c	0.426a	7.80de	1.37c
S4DW2.5	24.9b	3149d	1.64b	2.75d	6.57a	3.78d	7.08c	1.31c
S4DW5	39.0c	5372e	2.40c	4.75e	6.15a	6.57e	6.54b	1.11b
S4DW10	58.6d	10297f	4.13e	7.58f	7.05a	10.6f	5.96a	0.541a
S4ADW5	20.4b	444b	2.27c	1.23b	11.1c	0.940b	7.97e	1.89d
S4ADW10	34.2c	686c	2.92d	1.67c	8.55b	1.56c	7.69d	2.17e

Values with the same letter within a column, for a given soil, are not significantly different at the $p < 0.05$ level of probability.
TOC total organic carbon, WSOC water-soluble organic carbon, HA humic acid, FA fulvic acid, HI humification index, PG polymerization grade, EC electrical conductivity, DA dehydrogenase activity

2.3. Analysis of the soils, and organic amendment

Texture was determined by sedimentation using the pipette method after organic carbon destruction with H_2O_2 and chemical dispersion using $Na_4P_2O_7$ (Gee and Or, 2002). The TOC was determined by dichromate oxidation. WSOC was extracted with de-ionized water at 3:1 (water to soil) and 100:1 (water to DW) ratios. Humic and fulvic acids (HA and FA, respectively) were extracted by a solution of 0.1 M $Na_4P_2O_7$ + NaOH using a ratio of extractant to sample of 10:1, and to precipitate humic acid the supernatant was acidified to pH 2 with H_2SO_4. The humification index (HI) was calculated as (HA/TOC) × 100. The water content of the DW was calculated from weight loss after oven drying to constant weight at 105 °C. The electrical conductivity (EC) was measured in a saturation extract for soil and 1:10 (w/v) DW water mixtures. The pH was measured in 1:1 (w/v) soil/water and 1:5 (w/v) DW water mixtures using a combination electrode. Water soluble phenolic (WSP) substances were determined by the Folin-Ciocalteu colorimetric method (Box, 1983).

2.4. Adsorption-desorption experiment

The isotherms were determined using a batch equilibration method. Soil samples (5 g) were treated with 10 mL of metribuzin. The initial concentration of metribuzin ranged between 5 and 50 μM in 0.01 M $CaCl_2$ and each concentration was replicated three times. Samples were equilibrated by shaking mechanically at 20 ± 2 °C for 24 h. Equilibrium concentrations in the supernatants were determined by high performance liquid chromatography (HPLC). The amount of metribuzin sorbed (Cs) was calculated from the difference between the initial (Ci) and equilibrium (Ce) solution concentrations.

Following the adsorption experiment, the desorption of metribuzin from the soils was measured by successive dilution from the 50 μM initial concentration points. The 5 mL of supernatant removed for sorption analysis was replaced with 5 mL of 0.01 M $CaCl_2$. The samples were re-suspended, shaken for 24 h, and centrifuged, and the equilibrium concentration in the supernatant was determined. This desorption procedure was repeated three times. All treatments had three replicates. The herbicide sorption and desorption results were fitted by the Freundlich model, $Cs = K_f\,Ce^{1/nf}$, where Cs (mM kg^{-1}) is the amount of herbicide sorbed at the equilibrium concentration Ce (mM L^{-1}), and K_f ($mM^{1-1/n}$ kg-1 $L^{1/n}$) and n_f are the Freundlich coefficient and linearity parameter, respectively. The values of K_d (the partition coefficient, L kg^{-1}) were calculated from the fit of the experimental sorption isotherms ($Cs = K_d\,Ce$) at a selected Ce (10 μM). The organic carbon soil sorption coefficient (K_{OC}, L kg^{-1}) was calculated from the K_d values as $K_{OC} = (K_d \times 100)/TOC\%$. Hysteresis coefficients, H, for the sorption-desorption isotherms were calculated as $H = n_a/n_d$, where n_a and n_d are the Freundlich constants obtained from the sorption and desorption isotherms, respectively.

2.5. Persistence studies

Triplicate unamended and amended soil samples (500 g) were spiked with 8 mL of an ethanol solution of metribuzin to give a concentration of 1 mg metribuzin kg^{-1} of dry soil. The moisture content was adjusted to 40% field capacity, and then the samples were thoroughly mixed by passing them several times through a 2 mm-sieve. Herbicide-treated soil samples were transferred to 1-L glass jars where they were incubated at 20 ± 2 °C for 62 d. The moisture content was maintained at a constant level throughout the experiment by adding distilled water as necessary, followed by 5 min vigorous manual shaking. The soils were sampled periodically, and finally frozen until assay. For the assay, 5 g of soil samples in duplicate were extracted with 10 mL of methanol by shaking mechanically on an end-over-end shaker at 20 ± 2 °C for 24 h followed by centrifugation, and the metribuzin concentration in the extracts was determined by HPLC. Metribuzin dissipation curves in soils were fitted to first-order kinetics ($C = Co\ e^{-kt}$) and the half-lives ($t_{1/2}$) were calculated. Here C is the herbicide concentration at time t (days), Co is the initial herbicide concentration, and k (day $^{-1}$) is the degradation constant.

2.6. Dehydrogenase activity

Dehydrogenase (DH) activity was determined in unamended and amended soil samples from the dissipation studies by the method of (García et al., 1993). One gram of soil was incubated for 20 h at 20 °C in the dark with 0.2 mL of 0.4% 2-(p-Iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) as substrate. At the end of the incubation, the iodonitrotetrazolium formazan (INTF) produced was extracted with 10 mL of methanol and the absorbance measured at 490 nm.

2.7. Column leaching tests

Leaching experiments were carried out using disturbed-soil columns (30 cm length × 5 cm i.d.) constructed of PVC. To minimize losses of soil during the experiment, the top 5 cm of the columns was filled with sea sand and the bottom 5 cm with sea sand plus glass wool. The remaining 20 cm was hand-packed with unamended or amended air-dried soil. The experiment was performed with triplicates of the unamended and amended soil samples. All columns were packed to field bulk densities. The soil columns were saturated with 0.01 M $CaCl_2$, allowed to drain for 24 h, and then the amount of metribuzin corresponding to an application rate of 1.0 kg ha^{-1} dissolved in water was applied to the top of the columns. Each day the columns were leached with 0.01 M $CaCl_2$ at a rate of 50 mL day $^{-1}$ until no herbicide was detected in the leachates. Leachates containing the herbicide were collected daily, filtered, and assayed by HPLC. At the end of the leaching experiment, the columns were sectioned into 5-cm deep portions. Soil samples (5 g) from different depths were extracted once with 10 mL of methanol by shaking mechanically at 20 ± 2 °C for 24 h. The suspensions were centrifuged, filtered, and assayed by HPLC in order to determine the residual amount of metribuzin at the different depths of the soil column.

2.8. Herbicide assay

The metribuzin was assayed by HPLC using a Waters 600E chromatograph coupled to a Waters 996 diode-array detector. The following conditions were used: Nova-Pack C18 column (150 mm length × 3.9 mm i.d.), mobile phase of acetonitrile + water (35% + 65% by volume) with 0.1% acetic acid at a flow rate of 1 mL min^{-1}, 25 mL injection volume, and UV detection at 294 nm.

2.9. Statistical analyses

Statistical analyses were carried out using the SPSS package (11.5) for Windows. The data obtained were subjected to a one-way ANOVA. Pairwise multiple comparisons were performed using the Duncan test. Differences between results were considered statistically significant at a $p < 0.05$ level of probability. Pearson's correlation coefficient was used to test for relationships between sorption coefficients and selected soil properties.

3. Results and Discussion

3.1. Sorption-desorption studies

The sorption isotherms of metribuzin in the laboratory and field amended and unamended soils are shown in Figure 1. The values of the correlation coefficient (Table 2) for all treatments were very high ($R^2 \geq 0.929$), indicating that the Freundlich adsorption equation satisfactorily explained the metribuzin sorption results. The values of n_f in the original soils ranged from 0.647 to 0.758 (Table 2), indicating an L-type curve (Giles et al., 1960) which suggests a decrease in specific sorption sites as the concentrations of herbicide in solution increased. The K_d values for herbicide sorption on the original soils ranged between 0.46 L kg^{-1} for S2 and 0.59 L kg^{-1} for S3. These values are consistent with earlier findings by (Majumdar and Singh, 2007; Singh. 2008) who reported values of 0.28 L kg^{-1} and 0.38 L kg^{-1} in sandy loam and sandy soils, respectively. The relatively low K_d values found in the present four Mediterranean agricultural soils suggest that they have a very weak binding capacity for metribuzin, and that movement of this chemical should be significantly greater than in other agricultural soils that have been studied (López-Piñeiro et al., 2013).

The DW amendment had a significant influence on the sorption of metribuzin (Table 2). The K_d values of the laboratory soils amended with DW were greater than those of unamended soils, although the magnitudes differed among the four soils. Thus, for 5% of DW additions, the K_d values for the herbicide increased by factors of 2.72 in S1, 3.15 in S2, 2.48 in S3, and 3.55 in S4. The increases in metribuzin sorption due to increases in TOC content of the soils has been observed previously by various workers. For example, Singh et al. (2008) found that metribuzin sorption increased by factors of 1.73 and 2.68 after biocompost application at the rates of 2.5% and 5%, respectively, in a sandy loam soil.

Also, Majumdar and Singh, (2007) reported that, in a sandy loam soil from India, metribuzin sorption increased from 0.28 L kg^{-1} to 1.04 L kg^{-1} or to 4.19 L kg^{-1} using a 5% application ratio of manure or fly ash amendments, respectively. Daniel et al. (2002) conducted a multiple regression analysis and showed that sorption of metribuzin was related to the organic carbon content of the soil. Even after a significant increase in the WSOC content of the soil solution in the DW-amended soils (Table 1), metribuzin sorption increased significantly (Table 2). This indicated that metribuzin was sorbed by the solid-phase organic matter (Majumdar and Singh, 2007; Singh. 2008).

The greatest metribuzin sorption rates were found in the field-amended soils (AOW) despite their lower TOC (Tables 1 and 2). Thus, compared with the unamended soil S4, the K_d increased 4.79-fold with 5% (S4ADW5) addition. These results showed that TOC may not be the only factor determining metribuzin sorption with the DW amendments, and that the sorption of metribuzin increases with the degree of humification. Indeed, K_d was positively and significantly ($p < 0.05$) correlated with HA ($r = 0.803$), and that this correlation was stronger than found with TOC ($r = 0.711$), confirming that the transformation of organic matter into humic substances was important for the sorption of metribuzin. The highest values of K_{OC} were for the amended field soils (Table 2), also suggesting that the TOC is not the most important determinant of metribuzin sorption. This is consistent with the results of (López-Piñeiro et al., 2013) who found that the sorption of metribuzin increases with the degree of humification in soils amended with OW.

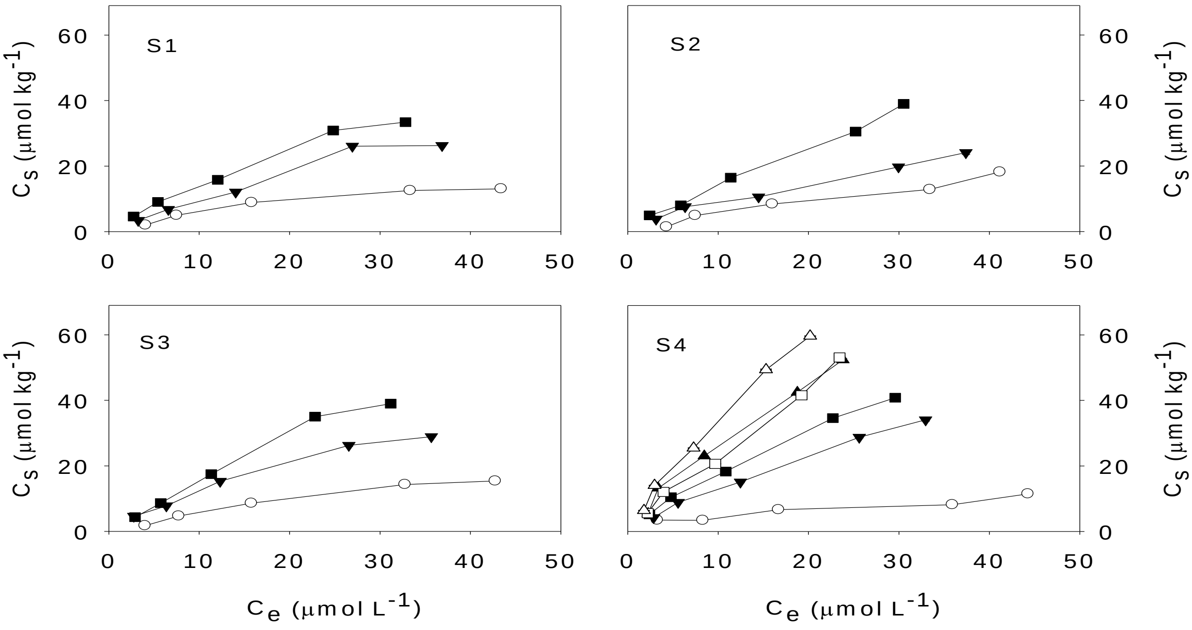

Figure 1. Effects of fresh, and aged de-oiled two-phase olive mill waste on the metribuzin sorption isotherms. Treatments are (○) unamended; (▼) DW2.5; (■) DW5; (▲) DW10; (□) ADW5; and (△) ADW10. Vertical bars represent one standard error of the mean which were lower than the symbols in all cases.

The hysteresis coefficients (H) indicated that adsorbed metribuzin was more readily desorbed in the amended than in the unamended soil (Table 2). This result could be understood as due to the high amount of WSOC present in the amended treatments. Previous herbicide studies have reported low hysteresis coefficients in OW and DW amended soils for S-metolachlor (Peña et al., 2013), metribuzin (López-Piñeiro et al., 2013), and simazine (Albarrán et al., 2004).

The increases in metribuzin sorption observed by (López-Piñeiro et al., 2013) were in all cases less than those in the present study despite OW having greater TOC than DW (535 vs 516 g kg^{-1}, respectively). This could be due to higher pH values of OW-amended soils, since the OW had pH 5.70 in contrast with the pH 5.30 of the DW. Soil pH affects sorption, which is increased by acidification of the soil (Weber et al., 2004). In the specific case of metribuzin, sorption is favoured by a decreased pH (Jacobsen et al., 2008) because of changes in the surface states of the components playing the role of adsorbents, particularly the organic matter (Oukali-Haouchine et al., 2013; Weber et al., 2004). Rigi et al. (2015) found that, in eight soils from different regions of Iran, metribuzin sorption was positively correlated with TOC and negatively correlated with pH, being the main factors influencing sorption of this herbicide.

Table 2. Effects of de-oiled two-phase olive mill waste addition on metribuzin sorption-desorption, half-life ($t_{1/2}$) in degradation studies, and percentages leached and extracted from the soils

	n_f	k_d (L kg^{-1})	k_{OC} (L kg^{-1})	R^2	H	$t_{1/2}$ (days)	R^2	Leached (%)	Extracted (%)
S1	0.758a	0.507a	52.3b	0.931	16.3c	22.9a	0.962	67.8a	9.70a
S1DW2.5	0.877a	0.943b	44.6a	0.986	7.24b	42.9b	0.804	58.8a	19.5b
S1DW5	0.808a	1.38c	41.9a	0.933	4.86a	59.2c	0.825	65.1a	20.0b
S2	0.720a	0.460a	68.9b	0.929	11.6b	35.8a	0.918	82.3b	12.4a
S2DW2.5	0.710a	0.911b	46.9a	0.984	6.42a	46.7b	0.944	77.5b	13.0a
S2DW5	0.828b	1.45c	46.3a	0.988	7.46a	51.1b	0.959	69.1a	26.7b
S3	0.709a	0.587a	44.0a	0.960	20.7b	29.1a	0.924	83.7b	16.2a
S3DW2.5	0.751a	1.19b	43.6a	0.990	4.82a	42.5b	0.976	59.2a	23.6b
S3DW5	0.953b	1.46c	36.8a	0.994	5.12a	45.7b	0.985	59.5a	38.6c
S4	0.647a	0.492a	49.7a	0.936	19.8d	20.0b	0.918	59.3c	33.0ab
S4DW2.5	0.883b	1.29b	51.8a	0.994	24.4e	24.0bc	0.909	34.9a	38.9bc
S4DW5	0.809b	1.75c	44.9a	0.996	8.48c	29.4c	0.969	45.9b	42.4bc
S4DW10	0.866b	2.56e	43.7a	0.957	5.52a	45.1d	0.957	35.4a	34.7abc
S4ADW5	0.898b	2.36d	115c	0.986	7.01b	7.27a	0.975	43.8b	28.0a
S4ADW10	0.878b	3.37f	98.5b	0.981	5.89a	3.93a	0.904	36.3a	44.1c

Values with the same letter within a column, for a given soil, are not significantly different at the $p < 0.05$ level of probability.

3.2. Degradation studies

The dissipation rates of metribuzin in unamended and amended soils are shown in Figure 2. The experimental data in all the treatments give good fits to first-order kinetics, with $R^2 \geq 0.804$ (Table 2). The order of metribuzin dissipation in the unamended soils was sandy loam soil (S2) < clay soil (S3) < loam soil (S1) = sandy clay loam soil (S4) (Table 2). The difference in the degradation rate of metribuzin in the original soils might be due to the differences in their TOC, clay contents, and pH (López-Piñeiro et al., 2013). Similar observations were found by (Kah and Brown, 2007; Ladlie et al., 1976) who reported positive correlations between metribuzin dissipation and soil properties such as pH, organic carbon content, and bioactivity. The $t_{1/2}$ values for metribuzin in the original soils ranged between 20.0 d for S4 and 35.8 d for S2. These values are coherent with earlier findings by (Undabeytia et al., 2011) who reported values ranging between 14.3 d and 31.2 d in different types of soils.

The addition of DW had a significant influence ($p < 0.05$) on the $t_{1/2}$ of metribuzin in all the soils, but this influence depended on the type of soil, on the amendment dose rate, and especially on the organic matter's maturity (Table 2). The $t_{1/2}$ values were significantly ($p < 0.05$) longer in the laboratory DW-amended soils than in the original soils and field DW-amended soils (Table 2; Figure 2). Thus, the addition of DW at the higher rate (5%) significantly increased the $t_{1/2}$ of metribuzin from 22.9 d in the unamended S1 soil to 59.2 d, from 35.8 d in the unamended S2 soil to 51.1 d, from 29.1 d in the unamended S3 soil to 45.7 d, and from 20 d in the unamended S4 soil to 29.4 d (Table 2). These increases in metribuzin's persistence agree with decreased microbial activity in these soils, as reflected in the lower values of their DH (Table 1; Figure 2). The findings are consistent with those of previous reports indicating that olive mill waste can depress soil microbial activity because of the action of toxic compounds such as phenols (Sampedro et al., 2009).

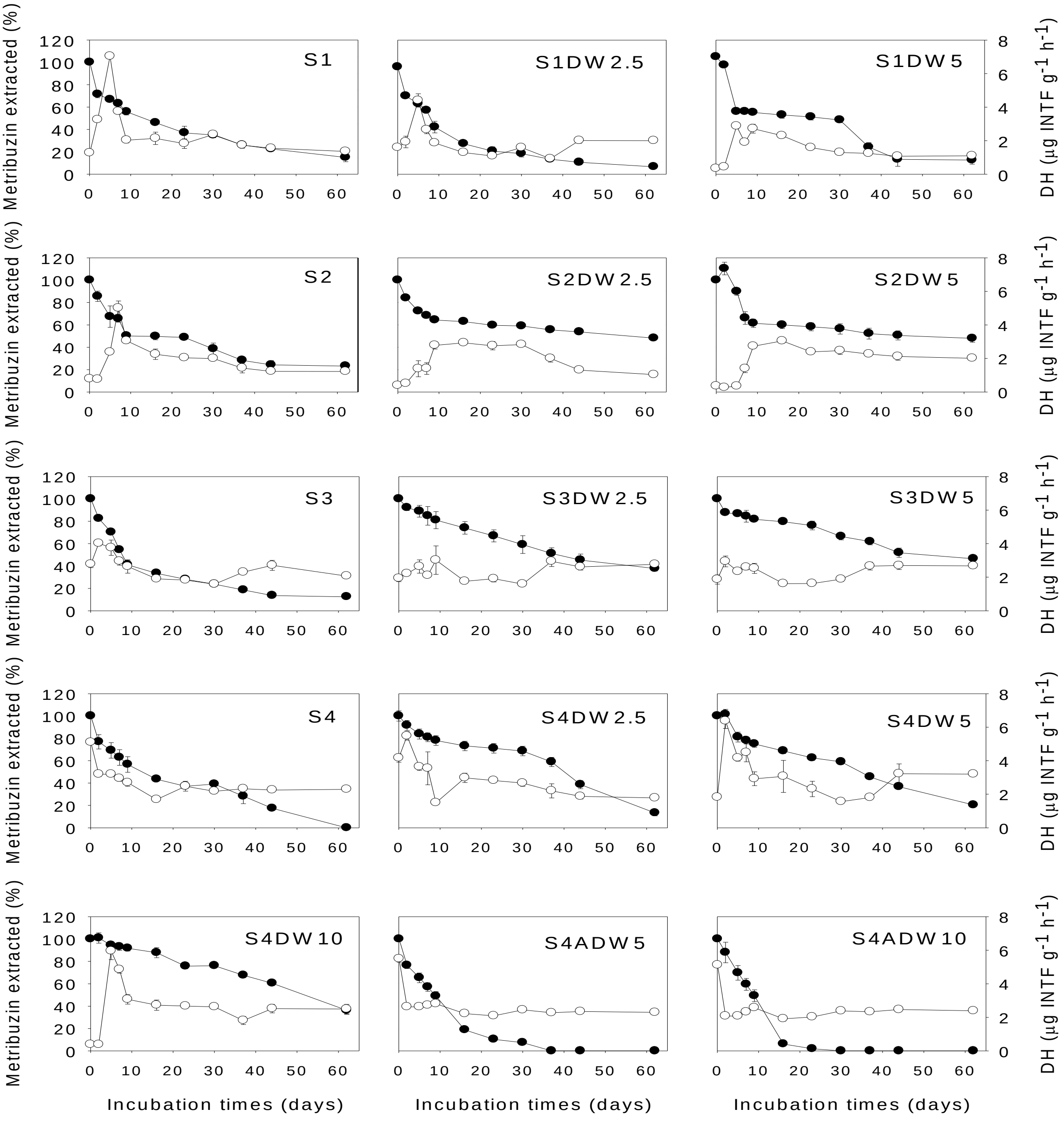

Figure 2. Effects of fresh and aged de-oiled two-phase olive mill waste on metribuzin dissipation (●) and dehydrogenase activity (○). Vertical bars represent one standard error of the mean that in some cases were lower than the symbols.

The processes of sorption and degradation have often been found to be correlated (Dyson et al., 2002). However, there was no clear relationship between these processes for metribuzin in our selected soils. Interestingly, although the field DW-amended soils presented the highest K_d values, their $t_{1/2}$ of metribuzin was significantly shorter than the original soil and the laboratory DW-amended soils (Table 2). The soils of the present study presented low sorption capacities so that it is difficult to distinguish the influence of this process on degradation, because organic matter has a positive effect on sorption and degradation by increasing the bioactivity of the soil (Maqueda et al., 2009). The microbial activity of the soils amended with aged DW, whose DH values measured throughout the degradation studies were similar to those of the laboratory DW-amended soils (Figure 2), could not be an explanation for the shorter metribuzin $t_{1/2}$. Possibly, the low metribuzin persistence observed in the field DW-amended soils may have been a result of the soil microorganisms using the pesticide preferentially as a carbon and energy source instead of the WSOC (López-Piñeiro et al., 2013) which was significantly reduced in these high humification index soils (Table 1). These results are consistent with those of (López-Piñeiro et al., 2013) who suggested that the transformation of the organic matter of the OW due to maturation processes (the aging effect) is likely to have been responsible for the observed shorter persistence of metribuzin.

3.3. Leaching studies

Figure 3 shows the cumulative breakthrough curves of metribuzin in the leaching studies for the unamended and amended soils. A mass balance calculation (Table 2) yielded metribuzin leaching values for the unamended soils that ranged from 59.3% to 83.7%, in the order S3 = S2 > S1 > S4. High leaching losses of metribuzin were reported by (Maqueda et al., 2008) who found values of the total leaching percentages ranging from 53.2 to 99% using new controlled-release formulations of metribuzin in a sandy soil. Singh, 2008 reported recovering about 93% of the initially applied metribuzin from the leachate in a sandy loam soil. These results showed the high risk of groundwater contamination represented by metribuzin in soils with low sorption capacities.

The application of DW significantly decreased the amount of metribuzin leached from the soil columns, independently of the type of soil (Table 2; Figure 2). In particular, the amounts of metribuzin in the leachates relative to the unamended soil were reduced by factors of 1.04, 1.19, 1.41, and 1.29 for S1DW5, S2DW5, S3DW5, and S4DW5, respectively, and 1.35 for S4ADW5.

The increase in sorption may explain the greater reduction of this herbicide in the leachate of the DW-amended soils (Table 2). Indeed, the amount of metribuzin leached was significantly ($p < 0.05$) negatively correlated with K_d ($r = - 0762$) and HA ($r = - 0.620$), indicating that the metribuzin leaching in DW-amended soils at least partially depends on the organic matter transformed into humic substances. Similar correlations were found by (López-Piñeiro et al., 2013) for metribuzin in OW-amended soils, with slightly greater decreases in percentages of metribuzin leached than obtained in the present study. In particular, (López-Piñeiro et al., 2013) reported that the amounts of metribuzin in the leachates relative to the unamended soil were reduced by factors of 1.23, 1.22, 1.47, and 1.40 for S1OW5, S2OW5, S3OW5, and S4OW5, respectively. The greater metribuzin persistence observed with the use of DW rather than OW could explain the slight differences in leaching found between the two amendments. Reliable model predictions of contaminant fate and transport in soils depend on adequate parameterization of relevant flow and transport processes. In this way, for herbicides, sorption distribution and degradation parameters have received the greatest attention (Roulier et al., 2006; Dusek et al., 2015).

Also, Singh, 2008 and Majumdar and Singh, 2007 showed that biocompost from a sugar distillery and animal manure, respectively, were fairly effective in reducing the downward mobility of metribuzin in packed soil columns of a sandy loam soil.

The amount of metribuzin extracted from the present soil columns ranged from 9.70% to 44.1% of the initially applied metribuzin (Table 2). The application in the laboratory of DW as organic amendment led to a significant ($p < 0.05$) increase in the amount of herbicide recovered, independently of the physicochemical characteristics of the soil to which it was applied. In particular, for 5% of DW addition, the amount of herbicide recovered increased by factors of 2.06, 2.15, 2.38, and 1.28 in S1, S2, S3, and S4, respectively. These findings are consistent with those described above for sorption and persistence (Si et al., 2011). Similar results have been described by Singh, 2008 and Majumdar and Singh, 2007 who found that biocompost and manure application significantly increased metribuzin retention in the soil in column leaching experiments. However, in the case of the S4ADW5 treatment, the amount of metribuzin recovered decreased by a factor of 1.18. The shorter metribuzin persistence shown by S4ADW5 relative to the original and the laboratory amended soils could be responsible for this behavior.

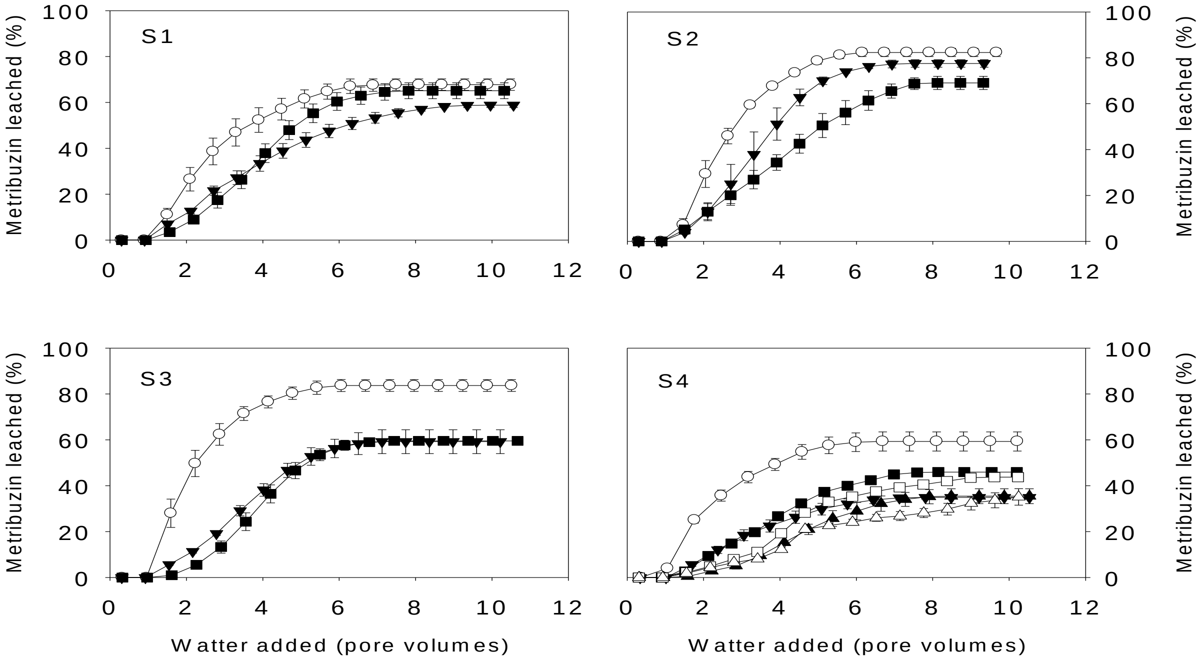

Figure 3. Effects of fresh, and aged de-oiled two-phase olive mill waste on the cumulative breakthrough curves of metribuzin. Treatments are (○) unamended; (▼) DW2.5; (■) DW5; (▲) DW10; (□) ADW5; and (△) ADW10. Vertical bars represent one standard error of the mean that in some cases were lower than the symbols.

4. Conclusions

The application of de-oiled two-phase olive mill waste (DW) to four typical Mediterranean agricultural soils greatly influenced the sorption-desorption, degradation, and leaching of the herbicide metribuzin. Amendments with fresh and field-aged DW both led to an increase in the metribuzin sorption capacity and a decrease in the amount of herbicide leached. This suggests that, independently of the degree of organic matter humification and of the physicochemical characteristics of the soil, application of DW may help reduce metribuzin leaching, and therefore may be an effective management practice to control the herbicide's leaching in low organic matter content soils. Whereas the fresh DW amendment increased the persistence of metribuzin, the field-aged treatments reduced it, and thus would contribute to its disappearance from the environment, but also potentially reduce the herbicide’s activity. Successive land applications of DW may well be environmentally and economically good practice, with a positive effect that could be especially significant in semiarid Mediterranean areas, whose agricultural soils are very poor in organic matter and are at a high risk of groundwater contamination from intensive annual pesticide applications.

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Conflict of interest

The authors declare that they have no conflict of interest.

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